PHYTOCHEMICAL EVALUATION OF *ARDRA* AND *SHUSHKA* DOSAGE FORMS AND RANDOMIZED CONTROLLED OPEN LABELLED CLINICAL TRIAL OF *ECLIPTA ALBA* HASSK. *(BHRINGARAJ) PANCHANGA* IN IRON DEFICIENCY ANAEMIA

A thesis submitted to

Tilak Maharashtra Vidyapeeth, Pune

For the degree of Doctor of Philosophy (Ph.D.)

In Dravyaguna Vidnyan

Under the Board of Ayurveda Studies

Submitted by

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Annexure IV

CERTIFICATE OF THE SUPERVISOR

It is certified that work entitled “Phytochemical evaluation of *ardra* and *shushka* dosage forms and randomized controlled open labeled clinical trial of *Eclipta alba* Hassk. *(Bhringaraj) panchanga* in iron deficiency anemia” is an original research work done by Dr. Sanjivani Samadhan Shekokar under my supervision for the degree of Doctor of Philosophy (Ph.D.) in Dravyaguna Vidnyan to be awarded by Tilak Maharashtra Vidyapeeth, Pune. To best of my knowledge this thesis

- embodies the work of candidate herself
- has duly been completed
- fulfils the requirement of the ordinance related to Ph. D. degree of the TMV
- up to the standard in respect of both content and language for being referred to the examiner.

Dr. Shraddha U. Nayak

MD (Ayu), Ph.D. (BHU)

Ph.D. Guide/ Supervisor

Annexure III

Tilak Maharashtra Vidyapeeth, Pune

Undertaking

I, Dr. Sanjivani Samadhan Shekokar, am the Ph. D. Scholar of the Tilak Maharashtra Vidyapeeth in Dravyaguna subject. Thesis entitled "Phytochemical evaluation of *ardra* and *shushka* dosage forms and randomized controlled open labeled clinical trial of *Eclipta alba* Hassk. (*Bhringaraj*) *panchanga* in iron deficiency anemia" under the supervision of Dr. Shraddha U. Nayak, solemnly affirm that the thesis submitted by me is my own work. I have not copied it from any source. I have gone through extensive review of literature of the related published/unpublished research works and the use of such references made has been acknowledged in my thesis. The title and the content of research are original. I understand that, in case of any complaint especially plagiarism, regarding my Ph.D. research from any party, I have to go through the enquiry procedure as decided by the Vidyapeeth at any point of time. I understand that, if my Ph.D. thesis (or part of it) is found duplicate at any point of time, my research degree will be withdrawn and, in such circumstances, I will be solely responsible and liable for any consequences arises thereby. I will not hold the TMV, Pune responsible and liable in any case.

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Dr. Sanjivani S. Shekokar

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ABBREVIATIONS

C. S. S.	-	Charak Samhita Sutrasthana
C.S.Chi.	-	Charak Samhita Chikitsasthana
S.S.Su.	-	Sushrut Samhita Sutrasthana
S.S.Ni.	-	Sushrut Samhita Nidansthana
A . H.	-	Ashtanga hridaya
A.H.S.	-	Ashtanga hridaya sutrasthana
A. S.	-	Ashtanga sangraha
A.S.Su.	-	Ashtang sangraha Sutrasthana
B. N.	-	Bhavaprakash nighantu
R. N.	-	Raj nighantu
D. N.	-	Dhanvantari nighantu
K. N.	-	Kaiiyadev nighantu
N. A.	-	Nighantu Adarsha
M.N.	-	Madanpal nighantu
N. R.	-	Nighantu Ratnakar
UV	-	Ultraviolet
GC	-	Gas chromatography
AAS	-	Atomic absorption spectrophotometry
HPTLC	-	High performance thin layer chromatography

LIST OF REAGENT AND SOLUTIONS

All reagents and solutions were of analytical grade.

1) Acetie acid, glacial – CH_3COOH – 60.05 (LR)

   Contains not less than 99.0 percent w/w of $C_2H_4O_2$ about 17.5 N strength.

2) Acetone – propan 2 – one $(CH_3)2$ CO = 58.08 Acetone of A.P.

3) Alcohol dilute

   60% Dilute 623 ml of alcohol to 1000 ml with water.

   Sp. Gravity at 15.56^o, 0.913 to 0.914

4) Ammonia liquor

   Sp. Gravity 0.91 (about 25% NH_3)

   NH_3 = 17.03

5) Ammonium oxalate

   $(CO_2\ NH_4)$ 2 H_2O = 142.11

   Colorless crystals

6) Barium chloride $Bacl_2$, $2H_2o$ = 244.27

   Description – colorless crystals

   Solubility – freely soluble in water

7) Calcium chloride – $Cacl_2$ – H_2o – 147.0

8) Chloral hydrate – $CCl_3\ CH(OH)_2$ – 165.40

   Description – colorless, transparent crystals, colors, pungent but not acid, taste, pungent and slightly bitter, volatilises slowly on exposure to air.

   Solubility – very soluble in water, freely soluble in alcohol, in chloroform and in solvent ether.

Chloral hydrate solution – dissolve 20 gm of chloral hydrate in 5 ml of water with warming and add 5 ml of glycerin.

9) Chloroform – $CHCl_3$ – 119.38

Description – colorless, volatile liquid; odour characteristic, taste, sweet and burning.

10) Copper sulphate – $CuSO_4$, $5H_2O$ – 249.68

Contains not less than 98.5 percent and not more than the equivalent of 101.0 percent of $CuSo_4$, 5 H_2o

Description – Blue triclinic prisms or a blue crystalline powder.

Solubility – soluble in water.

Very soluble in boiling water

11) Ether, diethyl ether – $(C_2H_5)O$ = 74.12

A volatile, highly flammable, colorless liquid, boiling point, about 34^0,

Wt. Per ml about 0.71 gm

12) Ethyl acetate – $CH_3\ CO_2\ C_2\ H_5$ = 88.11

Colorless liquid with a fruity odour, B. P. about 77^0, wt. Per ml about 0.90 kg.

13) Ferric chloride – anhydrous ferric chloride, $FeCl_3$ = 162.22

14) Ferrous sulphate – $FeSO_4$, $7H_2O$ = 278.0

Description – transparent, green crystals or a pale bluish green, crystalline powder odourless, taste, metallic and astringent

Solubility – Freely, soluble in water, practically insoluble in alcohol.

PH – Between 3.0 and 4.0 (determined in 5.0% w/v solution)

15) Hydrochloric acid – conc. HCl = 36.46

XN – solution of any normality XN may be prepared by diluting 84 x ml of hydrochloric acid to 1000 ml with water (0.5 N HCl used)

16) Indigo carmine (Indian ink)

$C_{16}\ H_8\ N_2\ Na_2O_8S_2$ = 466.4

17) Iodine I_2 = 253.8

Description – heavy, bluish black, brittle, rhombic prisms or plates with a metallic luster, choracteristic odour, volatile at ordinary temperature.

Iodine solution – on dissolving 2.0 gm of iodine and 3 gm of potassium iodine in water to produce 100 ml.

18) Lead acetate – sugar of lead ; $(CH_3CO_2)2$ Pb, $3H_2O$ = 379.33

Description – small, white, transparent, Monoclinic prisms, or heavy, crystalline masses, odour, acetous, taste, sweet and astringent. Efflorescent in warm air.

Solubility – freely soluble in water and glycerin.

19) Mercuric chloride – $HgCl_2$ – 271.50

Description – heavy, colorless or white, crystalline masses or a white crystalline powder.

Solubility – soluble in water, freely soluble in alcohol.

20) Methyl alcohol – methanol : CH_3OH = 32.04

Description – clear, colorless liquid with a characteristic odour.

Sp. Gravity – at 25^0 not more than 0.79%

21) Methylene blue – $C_{16}\ H_{18}\ ClN_3S$, $3H_2O$

Letramethylthionine chloride

Methylene blue solution – dissolve 0.18 gm of methylene blue in 100 ml water to 75 ml of this solution, add 5 ml of 0.1 N sodium hydroxide and 20 ml of water.

22) Ninhydrin reagent – 30 ml ninhydrin is dissolved in 10 ml n- butanol, followed by 0.3 ml 98% acetic acid.

23) Petroleum light – petroleum spirit – petroleum ether.

Description – colorless, very volatile, highly flammable liquid obtained from petroleum, consisting of a mixture of the lower members of paraffin series of hydrocarbons and complying with the following light petroleum (boiling range, 60^0 to 80^0) wt. Per ml. At 20^0, 0.670 to 0.690

24) Phloroglucinol – 1:3:5 – trihydroxybenzene, $C_6 H_3 (OH)_3$, $2H_2O$

Description : white or yellow crystals or a crystalline powder.

Solubility – slightly soluble in water soluble in alcohol and solvent ether.

Phloroglcinol solution : 1% w/v solution of phloroglucinol in alcohol (90%).

25) Potassium dichromate – $K_2Cr_2O_7$ = 294.18

Description – orange red crystals or a crystalline power.

Solubility – water soluble

Potassium dichromate solution – A 7.0 percent w/v solution of potassium dichromate in water

Potassium dichromate solution 0.1 N – 4.903 gm in 1000 ml.

26) Potassium ferricyanide – K_3 Fe $(CN)_6$ = 329.25

Description – ruby red crystals.

Solubility – very soluble in water.

Potassium ferricynide solution must be freshly prepared.

27) Potassium iodide KI = 166.00

Description – colorless, crystals or white powder, odourless, taste, saline of slightly bitter.

Solubility – very soluble in water of glycerin, soluble in alcohol.

KI solution – 10% w/v solution of kg in water

28) Conc. Nitric acid – contains 70.0 percent w/w of NNO_3 about 16 N in strength.

Description – clear, colorless, fuming liquid wt. Per ml – at 20^0, 1.41 to 1.42 gm.

Nitric acid, dilute – contains = 10% w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Nitric acid, XN – solution of any normality XN may be prepared by diluting 63x ml of nitric acid to 1000 ml with water.

29) Potassium Nitrate KNO_3 = 101.1

30) Potassium thiocyanate – KCNS = 97.18

Analaytical reagent grade.

31) purified / Distilled water – H_2O = 18.02.

Description – clear, colorless, liquid, odourless, tasteless.

Prepared from potable water by distillation, (ion exchange treatment, reverse osmosis or any suitable process).

pH – between 4.5 and 7.0 determined in solution prepared by adding 0.3 ml of a saturated solution of potassium chloride to 100 ml of the liquid being examined.

32) Reorcinol – benzene – 1, 3 di-ol, $C_6H_4(OH)_2$ = 110.1

Colorless crystals or crystalline powder, melting point about 111^o .

Resorcinol solution – shake 0.2 gm of resorcinol with 100 ml of toluene until saturated and decant.

33) Safranine – basic red 2 microscopical staining grade. A reddish brown powder. Safranine solution – saturated solution of safranine in ethanol (70%).

34) Silica gel – partially dehydrated, polymerized, colloidal silicic acid containing cabalt chloride as an indicator.

35) Sodium bicarbonate $NaHCO_3$ = 84.01

Description – white, crystalline powder or small, opaque, monoclinie crystals; colorless, taste, saline.

Solubility – freely soluble in water, practically insoluble in alcohol.

36) Sodium cobalt nitrite – $Na_3CO(NO_2)_6$ = 403.94

Description – an orange yellow powder.

Solubility – readily soluble in water forming orange red solution.

37) Sodium Hydroxide – NaOH – 40.00

Description- white sticks, pellets, fused masses, or scales; dry, hard brittle and showing a crystalline fracture, very deliquescent, strongly alkaline of corrosive.

Solubility – freely soluble in water and in alcohol.

Sodium Hydroxide XN – solution of any normality $_1$XN may be prepared by dissolving 40x g of sodium hydroxide in water and diluting to 1000 ml.

38) Sudan Red G – Sudan III; solvent red 23;

1 - (4 – phenyl - azophenylazo) – 2 – napthol;

$C_{22}H_{16}N_4O$ = 352.40

Description – reddish brown powder.

Solubility – insoluble in water;

Soluble in chloroform, in glacial acetic acid; moderately soluble in alcohol, in solvent ether, in Acetone.

39) Sulphuric acid – H_2So_4 = 98.08

When no molarity is indicated – AR grade containing about 98% w/w of sulphurie acid. An oily, corrosive liquid weighing about 1.84 gm/ml of about 18 M in strength.

When solution of molarity XM is required, it should be prepared by carefully adding 54X ml of sulphuric acid to an equal volume of water and diluting with water to 1000 ml.

40) Sulphuric acid, Dilute – contains approximately 10% w/w of H_2So_4. Dilute 57 ml of sulphiric, acid to 1000 ml with water.

41) Acetic anhydride : $(CH_3\ CO)_2O$ = 102.09

Contains not less than 95.0% of $C_4H_6O_3$.

Description – clear, colorless liquid with a pungent odour.

42) Ammonium thiocynate : NH_4SCN = 76.12

Description – colorless crystals.

Solubility – very soluble in water forming a clear solution, freely soluble in alcohol.

Ammonium thiocynate solution : 10% w/v in water.

43) Ammonium Molybdate : $(NH_4)6\ MO_7O_{24}, 4H_2O$ = 1235.86

Contains about 80 to 83% of MgO_3.

Description – white crystals or crystalline masses.

Sometimes with a yellowish or greenish solubility : in water.

44) Benzene : C_6H_6 = 78.11

Description – colorless, transparent liquid, flammable.

45) N – Butyl Alcohol : butyl alcohol; butan-1-01. CH_3 CH_2 CH_2oH = 74.12

Description – clear, colorless, liquid.

Solubility – freely soluble in water.

46) Cholesterol : $C_{27}H_{46}O$ = 386.64

Description : white of faintly yellow, almost odourless pearly leaflets or granules.

47) Formic acid : H. CO_2H = 46.03

Description – colorless liquid having a very pungent odour, highly corrosive solubility – missible with water and alcohol.

48) Hexane – n- Hexane – a fraction from petroleum containing about 90% of n – hexane.

Description – colorless, mobite, highly flammable liquid.

49) Picric acid – 2,4,6 – Trinitrophenol. Not lessthan 99% $C_6H_3N_3O_7$.

Description – bright yellow, crystalline, powder, odourless; taste, very bitter explodes on rapid heating.

Solubility – Sparingly soluble in water, freely soluble in alcohol.

Picric acid solution – 1.0% w/v solution of picnic acid in not water.

50) n – propyl alcohol – propyl alcohol; propan – 1 – 01.

CH_3 CH_2 CH_2 OH = 60.10

Description – clear, colorless liquid.

Solubility – miscible with water with alcohol.

51) Silica Gel G : A fine, white, homogeneous powder of an average particle size between 10 and 40 um containing about 13% w/w of calcium sulphate hemihydrate. ($CaSo_4$ ½ H_2O).

52) Toluene : C_6H_5 CH_3 = 92.14

Description – clear, colorless, flammable liquid.

Solubility – Insoluble in water, miscible with ethyl alcohol.

53) Dichloromethane : Methylene chloride CH_2Cl_2 = 84.93

Description – clear, colorless, mobile liquid.

Solubility – sparingly soluble in water, miscible with alcohol with solvent ether.

54) Phosphoric acid – H_3PO_4 = 98.00

Orthophosphoric acid; cone phosphoric acid.

Description – clear colorless syrupy liquid corrosive.

Solubility – Missible with water with alcohol.

55) Ammonium Sulphate : $(NH_4)_2SO_4$ = 132.13

Description – colorless crystals or white granules.

Solubility – very soluble in water, insoluble in alcohol.

56) Propanol : n-propyl alcohol - propan - 1 - OL

Introduction

Pharmacological properties of the plant drug are based on the different phytochemical constituents[1] present within these plants. The phytochemical constituents produce definite physiological or pharmacological actions on the human body.[2]

The chemical composition of the plant drug is dependent not only on species identity and harvest time, collection time, maturity of plant, but also on soil composition, altitude, actual climate, processing, and storage conditions. Moreover during extraction, as well as during the isolation processes, transformation, processing and degradation may cause changes in the phytochemical compounds.[3] *Ayurveda* has considered all these factors since ancient times and accordingly formularies has been established which are based on some basic principles for the treatment as well as formulation of medicine. Various principles for the use and preparations of medicine are stated by *Ayurveda*. One such *ayurvedic* formulary is *Sharangdhar Samhita* in which *Sharangdhar Acharya*[4] has described to use s*hushka dravya* (dry drug) which is freshly collected from plant source and recently dried (not to use old *dravya* as *shushka dravya*) in the preparation of medicine. Similarly, when there is reference of *ardra dravya* (fresh plant drug) always use freshly collected samples from plant source and it is also stated to use *ardra dravya* twice in quantity to that of *shushka dravya*. This is because there may be some difference in the properties or quantity of the phytochemical constituents present in the drug in *shushka* and *ardra avastha* (condition). Therefore, a study was planned to understand this concept to evaluate the difference in physico-chemical and phytochemical properties in *ardra* and *shushka dravya* with the help of various physico-chemical and phytochemical analytical tests followed by a clinical trial to evaluate the clinical efficacy of *ardra* and *shushka dravya* in iron deficiency anemia (IDA).

Anemia is a global public health problem affecting both developing and developed countries with major consequences on human health ultimately hampering their social as well as economic development. Anemia is also a serious health problem in India mostly affecting school children. High prevalence of anemia is also found still in adolescent age group, mostly girls and also in non-pregnant women.[5] The clinical features of anemia can be correlated with *pandu roga*. The term anemia in Greek language means *lack of blood* or *hemoglobin*. It is one of the most common disorders in the developing countries because of the poor nutritional status. It occurs because of the poor intake of the iron and folic acid rich foods. The disease is most commonly seen in children, adolescent girls, pregnant women and lactating mothers. It occurs at all stages of life cycle and is most prevalent type of nutritional anemia. Therefore, iron deficiency anemia was selected for the clinical trial with the evaluation of the phytochemical variations in *ardra* and *shushka avastha* (wet and dry dosage forms).

World Health Organization (WHO) estimated that the number of anemic people of all age groups worldwide is to be a staggering number of two billion and approximately 50 % of all anemias can be attributed to iron deficiency. So, WHO had globally urged to establish monitoring system for assessing the magnitudes and distribution of iron deficiency disorder.[6]

In 2002, iron deficiency anemia (IDA) was considered to be the most important contributing factors to the global burden of disease.[7] In developing countries this high rate has been related to insufficient iron intake, exacerbated by chronic intestinal blood losses due to parasitic and malarial infections. In developed countries it is more commonly due to insufficient iron intake. In India the prevalence rate according to WHO is 70-80 % in children, 70 % in pregnant women, 24 % in adult men and 40 % in all age groups, affecting the health, mental status, and working efficiency of human beings. WHO had put forth 12x12 initiatives in 2012, targeting "to achieve hemoglobin level of 12 gm % by the age of 12 years

by 2012."[8] Many iron-containing allopathic formulations[9] are available in the market for the treatment of IDA, such as ferrous fumarate, ferrous sulphate, ferrous glycine sulfonate, ferrous gluconate, ferrous ascorbate,[10.] etc. The long-term treatment with iron salts is associated with several side effects like heart burn, nausea, upper gastric discomfort, constipation and diarrhea. Recently it has been shown that oral iron generates damaging free radicals in the intestine.[11] An alternative approach of therapy is needed to enhance the absorption of dietary iron mostly rather than mere increase in iron intake or diet.[12]

An alternative approach of therapy is necessary to enhance the absorption of dietary iron and also to reduce the associated side effects.[13] So despite the availability of large number of iron preparations for correction of anemia and iron deficiency there is a need for a better haematinic preparation without side effects. Several drugs are described in *ayurvedic* lexicons indicated for treating *pandu* correlated as anemia, out of which *Bhrungaraj*, *Eclipta alba* was selected for treating anemia which can be correlated with *pandu*.

Eclipta alba is an annual herbaceous plant which easily propagates and is very commonly found. The plant has several pharmacological activities and indicated in *pandu*, *yakrut vyadhi*, *jwara*, etc. It is found to be a wonder drug for spleen and liver enlargement, catarrhal jaundice, hyperacidity, gastritis, dysentery, laxative. *Eclipta alba* has a long history of traditional medicinal uses in many countries especially in tropical and subtropical regions. Recent studies showed an anti-venom property and corrosion pickling inhibitor action on mild steel in hydrochloric acid. A wide range of chemical compounds including coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes and their glycosides have been isolated from this species. Extracts and metabolites from this plant have been known to possess pharmacological properties. High therapeutic and medicinal values are due to its chemical composition with wedelolactone, demethylwedelolactone, 14-hepatocosanol,

luteolin-7-0-glucoside, alkaloids and polypeptides as principle components.[14] Because of its varied medicinal values it has great commercial demand which calls for further investigation at the bimolecular level. For the same reason, this species needs prime attention for its future cultivation and conservation.[15] *Bhavaprakash Samhita*[16] has described that *Bhrungaraj* is especially effective in *yakrut vikruti*, *yakrut vruddhi*, *pandu, kamala*, *shotha* and other diseases related to *yakrut*. *Dhanvantari Nighantu*,[17] *Raj Nighantu*,[18] *Kaiyyadev Nighantu*[19] has also described that *Bhrungaraj* is indicated in *pandu, shotha, kamala* and other diseases. *Bhrungaraj* is described by *Brihatrayi* in the various *Ganas* and *Vargas*; it is an important herb in therapeutics. *Charaka* indicated for *raktapitta* while *Vaghbhata* advocated its consumption for one month to have *rasayana* effect. In *Raj Nighantu* blue variety is claimed to be the best *rasayana*. So *Bhrungaraj* was selected for the clinical trial in iron deficiency anemia which can be correlated with *pandu*. *Shushka* and *ardra* dosage forms of *Bhrungaraj* were clinically tested in patients of iron deficiency anemia for evaluation of the efficacy of *shushka* and *ardra* dosage forms through a clinical trial in iron deficiency anemia which could be correlated with *pandu*. Physicochemical and phytochemical evaluation of all different dosage forms of *Bhrungaraj* was also studied in this thesis.

Aim

To study the phytochemical and physicochemical properties of *ardra* and *shushka* dosage forms of *Bhrungaraj (Eclipta alba* Hassk.*) panchanga* with its clinical efficacy in iron deficiency anemia

Objectives

a) Primary

1. To study the phytochemical and physicochemical properties of *ardra* and *shushka* dosage forms of *Bhrungaraj* (*Eclipta alba* Hassk.*) panchanga*

2. To study their clinical efficacy in iron deficiency anemia

b) Secondary

To understand the difference in chemical constituents of *ardra* and *shushka* forms and thereby the difference if so clinically

Review of literature

Disease review

Historical review of anemia

Iron deficiency anemia is characterized by pallor, dyspnea and edema. References of iron deficiency anemia are found in about 1500 BC in the Papyrus Erbs which is an oldest extant manuscript of Egyptian manual of therapeutics.[20]

In 1619, Jean Varandal[21] labeled different groups of anemias as "chlorosis" or "green sickness".

In Ayurveda, *Acharaya Sushruta* had described anemia as a form of '*panduroga*' or jaundice.[22] *Acharya Charaka* had described different types of *pandu* according to *doshas* and another form *Mrudbhakshanajanya pandu* caused by the eating of clay[23] which was treated with iron rust pills (*mandur bhasma*).[24]

Wang Shu-Ho in 280 AD, had diagnosed the deficiency of blood by the knowledge of superficial and weak pulse.[25] This ancient disease was labelled as "ancylostomal anemia" by medical historians, which is a form of iron deficiency anemia. Sydenham [1624-1689] first identified 'iron' as specific remedy[26] for chlorosis. "Chlorosis" or "green sickness was well known to European physicians after the middle of sixteenth century. In 1665, a globule of fat of a definite outline, reddish in colour in a blood vessel was observed by Malphigi which he described as corals of red rosary, and these fat globules from blood were later named as 'erythrocyte' by Antony Leuwonhoeck.

John Lange described a concise clinical picture of hypochromic anemia, which was mentioned in a letter to his friend whose daughter had extreme pallor; he described the symptomatology as palpitations, dyspnea and swelling of the ankles. He used the classical name[27] "marbus vigineus."

During the duration of 100 years between 1830 and 1930, iron was used for treatment of chlorosis.[28] In the beginning of the twentieth century, it had been established that decrease in the iron content of blood was the characteristic feature of chlorosis and can be diagnosed by the presence of hypochromic erythrocytes. Most of the fundamental work on iron metabolism and iron deficiency was done during the twentieth century.

Modern review of disease

Definition of anemia

The term anemia refers to reduction in the concentration of hemoglobin in the peripheral blood below[29] the normal range for the given age and sex of the patient.

The term anemia is derived from two Greek words viz 'a' meaning 'without' and 'haima' meaning blood.

The World Health Organization defines anaemia[30] as

- Hb <13 g/dL in men over 15 years old
- Hb <12 g/dL in nonpregnant women over 15 years old
- Hb <12 g/dL in children aged 12-14 years

General signs and symptoms of anemia

The presenting features of anemia[31] are

- tiredness
- easy fatigability
- generalized muscular weakness
- lethargy and
- Headache

Signs include

- pallor
- hyperdynamic circulation
- attacks of faintness
- retinal hemorrhages

- menstrual disturbances
- mild proteinurea
- anorexia
- flatulence

Pathophysiology of anemia

Erythropoiesis[32]

The process of hematopoiesis takes place in the liver and spleen. Erythopoiesis and iron metabolism, intra cellular oxidation of the erythrocytes takes place mainly in the reticuloendothelial system and liver parenchymal cells, predominantly in the form of ferritin.

The mature erythrocyte is a non-nucleated circular, biconcave disc most suited to perform the function of gaseous exchange. It contains hemoglobin. It is a highly specialized, metabolically active cell which obtains energy by utilizing glucose by anaerobic pathways. It is provided with enzymes which are required for metabolism of glucose such as hexokinase, glucose-6-phosphate isomerise, phosphofructokinase, and aldolase. Those taking part in oxidative glycolysis are glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase, transketolase and transaldolase. This pathway is connected with glutathione metabolism through the enzymes glutathione reductase and glutathione peroxidase.

Hemoglobin is a conjugated protein synthesized inside the immature erythrocyte, consisting of the hememoiety (the red pigment) comprising iron and porphyrin and the protein moiety, globin. In an adult haemoglobin (HbA), the globin consists of two alpha chains each containing 141 amino acids and two beta chains each with 146 amino acids.

The rate and amount of production of the alpha, beta and gamma chains and their synthesis to form haemoglobin are controlled by specific genes. For the continuous production of red

cells and synthesis of hemoglobin several nutrients are required. These are mainly iron, proteins, vitamin B12, folic acid, pyridoxine, vitamin C, nicotinic acid, copper and cobalt.

Liver is the site of most of the storage of iron in human body. The principle function of liver is regulation of iron homeostasis by several regulatory mechanisms.[33] Liver control the production of iron regulatory genes, storage capacity and iron metabolism.

Dietry iron is absorbed in the form of ferrous state and presence of phosphates and phytates, absence of bile salts secreted by the liver and pancrese, all these factors [34] influences the iron absorption. The liver[35] also secretes moderate amounts of apotransferrin into the bile, which flows through the bile duct into the duodenum. Here, the apotransferrin binds with free iron and also with certain iron compounds, such as hemoglobin and myoglobin from the most important sources of iron in the diet. This combination is called transferrin. It, in turn, is attracted to and binds with receptors in the membranes of the intestinal epithelial cells. Then, by pinocytosis, the transferrin molecule, carrying its iron store[36] is absorbed into the epithelial cells and later released into the blood capillaries beneath these cells in the form of plasma transferrin.

Functions of hemoglobin

Hemoglobin is responsible for transport of oxygen from lungs to tissues and removal of carbondioxide from tissues and delivery to lungs. Rise in H^+ ion concentration and CO2 levels in the environment reduce the oxygen binding capacity of hemoglobin.

Classification of anemia[37]

A) Classification based on morphology

Based on MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration)

a) Normocytic normochromic anemia

(MCV= 80 to 100 fl and MCHC= 32 to 36 g/dl)

1. Acute blood loss

2. Hemolytic anemias

3. Erythrocyte mass deficit: chronic disease, toxic agent, malignancy, splenomegaly

b) Microcytic normochromic anemia

(MCV– 60 to 80 fl and MCHC– 32 to 36 g/dl)

1. Erythrocyte mass deficit: chronic disease, toxic agent, malignancy, splenomagaly, etc.

c) Microcytic hypochromic anemia

(MCV– 60 to 80 fl and MCHC– 20 to 30 g/dl)

1. Iron deficiency anemia

2. Chronic lead poisoning

3. Thalassemia syndromes

4. Miscellaneous– Sideroblastic anemia, idiopathic pulmonary hemosiderosis, familial hypochromic microcytic anemia.

d) Macrocytic anemia

(MCV– 101 to 160 fl and MCHC– 32 to 36 g/dl)

1. Megaloblastic anemia

2. Non-megaloblastic anemia

B) Classification based on etiology

1. Iron deficiency anemia
2. Folate deficiency anemia
3. Vit. B12 deficiency anemia
4. Pernicious anemia
5. Haemolytic anemia
6. Thalassemia
7. Sickle cell anemia, etc

Iron deficiency anemia

Nutritional anemia is the condition[38] in which hemoglobin concentration of a given individual is below the normal level due to deficiency of one or more nutrients needed for hemopoiesis

and hemoglobin can be increased by supplementation of the deficient nutrients. Iron is an important micronutrient, the deficiency of which results in iron deficiency anemia.

Liver is the site of most storage iron. Depletion of iron stores is preceded by impaired production of iron-containing proteins, the most prominent of which is hemoglobin. The two main phases of iron deficiency are

- Depletion of iron stores without anemia
- Depletion of iron stores with anemia

Iron replacement therapy cannot be commenced until the source of the iron shortfall is ascertained.

Iron requirements

Table1: Daily requirement of iron[39]

Daily requirement	Age
Infancy	1 mg/kg
1 – 10 yrs	8 – 12 mg/kg
10 – 18 yrs	15 mg/kg

Sources of iron: Dietary iron[40] is derived from two sources

1. Heme: (more absorbable), present in meat, fish and poultry
2. Non heme: (less absorbable), present in pulses, green leafy vegetables, dates, bajra, nuts, jaggery etc.

Absorption of iron from the intestinal tract

Iron is absorbed from all parts of the small intestine; mostly by the following mechanism. The liver secretes moderate amounts of apotransferrin into the bile, which flows through the bile duct into the duodenum.

Etiologic factors in iron deficiency

A) Increased physiologic demand

1] Menstruation

2] Pregnancy

3] Lactation

4] Growth

B) Decreased iron assimilation

1] Iron poor diet

2] Faulty diet habit

3] Iron mal-absorption

C) Blood loss

1] Gastrointestinal bleeding[41]

2] Menorrhagia

3] Infestation with parasites

4]Intravascular hemolysis and hemoglobinuria

5] Dialysis treatment of chronic renal failure

6] Bleeding from respiratory tract

Menstruation

Iron deficiency in post pubescent girls is most commonly caused by the loss of more iron through menstruation than can be supplied by the diet. Menstruation causes loss of average approximately 40 ml (20 mg iron) per period. The amount of blood loss varies.

Pregnancy

The women, who have not got sufficient iron reserve and are on unbalanced diet, are likely to develop anemia during pregnancy.

Iron poor diet and faulty diet habit

Diminished intake of iron resulting from poor socio-economic factors, loss of appetite and vomiting in pregnancy are responsible factors.

Iron malabsorption occurs due to

1. Malabsorption syndromes: sprue, nontropical sprue

2. Gastrointestinal surgery

Gastrointestinal bleeding causes[41]

1. Esophagus: Varices, hiatus hernia
2. Stomach: Varices, ulcer, carcinoma, gastritis, drug ingestion
3. Small intestine: Ulcer, Meckel's diverticulum, helminthiasis
4. Colon: Ulcerative colitis, amoebiasis
5. Rectum: Hemorrhoids, ulceration

Menorrhagia

Excessive bleeding caused by uterine fibroids, malignant neoplasms, dysfunctional uterine bleeding and use of an intrauterine device for contraception

Parasitic infestation[42]

Iron requirement increases in case of chronic bleeding caused by parasites such as ancylostoma, necator schistomsoma and possibly trichuris trichura. In the case of hookworm disease, blood loss varies from 2 to 100 ml per day according to severity of infestation.

Clinical features of iron deficiency anemia

Symptoms: In mild anemia, patients may be symptomless.

In moderate to severe deficiency– fatigue,[43] weakness, listlessness, irritability, anorexia, paraesthesia, dizziness, vertigo, palpitations, angina, dyspnoea on exertion.

Physical findings[44,45]

1. Pallor of the skin and mucous membrane
2. Changes occur in the nails, tongue, mouth esophagus and hair
3. Finger nails become thin, lusterless, and brittle and show longitudinal ridging and flattening and in more severe cases become concave or spoon-shaped which is known as koilonychia.

4. Changes in the tongue are atrophy of the papillae, resulting in a pale, smooth, atrophic, shiny or glazed tongue (atrophic glossitis) and angular stomatitis.
5. Plummer-vinson (Paterson-kelly) syndrome is occasionally seen.

Diagnosis

Investigations: Following are the investigations available for the detection of iron deficiency anemia[46]

(I) Calculation of erythrocyte indices

(II) Examination of a stained blood smear

(III) Analysis of red cell distribution width

(IV) Biochemical investigations

(V) Bone marrow aspiration /biopsy

Commonly recommended investigations[47]

(a) Hb concentration: In women, Hb level <11 gm/dl is taken as anemic in pregnant and Hb level <12 gm/dl in non-pregnant women.

(b) Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and packed cell volume

- Increased- In macrocytic anemia
- Decreased- in microcytic anemia

Mean corpuscular hemoglobin: MCH is a measure of the average haemoglobin content.

MCH = Hb g/L/Red cell count (10^{12}/L)

Mean corpuscular haemoglobin concentration

It is an average concentration of haemoglobin in a given red cell volume.

Normal range– 32 - 36 %

Decreased MCHC- in hypochromic anemia

Increased MCHC- in spherocytosis

Red cell distribution width (RDW)

It is a red cell measurement that quantitates red cell volume heterogenecity. Microcytosis is considered.

MCH: MCH is less than 27 pg is significant

MCHC: MCHC when less than 31 gm/dl suggest hypochromia

Reduced value for MCHC is due to reduced Hb % and haematocrit values

Biochemical Investigations

(a) Serum iron

(b) Serum ferritin

(c) Total iron binding capacity

(d) Transferrin saturation

(e) Free erythrocyte porphyrin (FEP)[47]

Assessment of the adequacy of iron from the blood to developing red cells in the bone marrow is to assess in terms of transferrin saturation.

(Serum iron/ TIBC) X 100 = % Transferrin saturation

Percentage saturation of transferrin- 16 % or less significant for iron deficiency anemia

A transferrin saturation of less than 7 % suggest iron deficiency anemia.

Serum ferritin

Serum ferritin concentration is directly related to iron stores. A serum ferritin concentration less than 10 - 12 micrograms/litre are diagnostic of iron deficiency.

Differential diagnosis

Differential diagnosis of microcytic hypochromic anemia include,

1. Iron deficiency anemia
2. Thalassemia
3. Anemia of chronic infection
4. Sideroblastic anemia
5. Lead poisoning

Grading of anemia

Anemia is often graded arbitrarily as “mild” “moderate” and “severe” based on Hb value. World Health Organization grades anemia[48,49] according to hemoglobin level (Table 2).

Table 2: Grading of anemia

Hb levels	Grades
Between 10 g/dl to 12 gm/dl	Mild
Hb between 7 g/dl to 10 g/dl	Moderate
Hb under 7 g/dl	Severe
Hb under 5 g/dl	Very severe

Table 3: Clinical grading of anemia

Clinical observations[50]	**Grades**
Pallor restricting itself to only conjunctiva and / or mucus membrane	Mild
Obvious skin pallor	Moderate
Palmar creases too are affected	Severe

Examinations of blood smear [51,52]

1. Red cells should be uniform in size and shape with an average diameter of 7.2 to 7.9 micrometers.
2. Hypochromia reflects poor hemoglobinisation and results in a very thin rim of haemoglobin or an increased area of centered pallor.
3. Platelet numbers and morphology are also evaluated. They appear as small blue cytoplasmic fragments with red to purple granules.
4. Platelets are usually 1-2 micormetres in diameter with wide variations in shape.

Management

Ferrous sulphate,[53] ferrous fumarate, ferrous gluconate, ferrous ascorbate, etc. are given in the form of elemental iron through oral and parentral route.

Adolescents need 60 mg of elemental iron per day in case of mild anemia and 120 mg/day in moderate and severe anemia.

Table 4: Management response to iron therapy

Recovery Time	Changes / Response
12–24 hr	Replacement of intracellular iron, subjective improvement, decreased irritability, increased apetite
36–48 hr	Initial bone marrow response- erythroid hyperplasia
48–72 hr	Reticulocytosis, peaking at 5–7 days
4–30 days	Increase in hemoglobin level
1–3 mth	Repletion of iron stores

Drug review

Vedic review

The references of *Bhrungaraj* are available in *Rug Veda* in the management of diseases like *Kushta* and *Palitya*. This indicates the utility of *Bhrungaraj* for cosmetic purpose since vedic period.[54]

Samhita review

Bhruhatrayi

Charaka samhita

Acharya Charaka has not included *Bhrungaraj* in any *Mahakashaya* but used *Bhrungaraj* in management of various diseases. *Bhrungaraj* is described to be used in *Kasamardadi yoga* for *kaphaja kasa chikitsa.*[55] *Bhrungaraj* is also mentioned as one of ingredient of *raktapittahara yoga*[56] in *raktapitta chikitsa*, *sahacharadi taila* and *mahanila taila*[57] in

prakruti vighata chikitsa as *krimihara dravya*[58] and also described as one of the ingredients of *vamak* and *virechaka kalpa*[59] in *kalpasthana*. Charakacharya has described the actions of *Bhrungaraj* as *keshya*, *dipana*, *virechaka* and *krimihara dravya*.

Table 5: Therapeutic references of *Bhrungaraj* in *Charak Samhita*

Sr No	**Reference**	***Adhikara***	***Rogaghnata***	***Yoga Nama*/ Formulation**
1.	*Cha. Su.*	-	-	-
2.	*Cha. Ni.*	-	-	-
3.	*Cha. Vi.*	-	-	-
4.	*Cha. Sha.*	-	-	-
5.	*Cha. In.*	-	-	-
6.	*Cha. Chi.* 4/68	*Raktapitta Chikitsa*	*Raktpittghna*	*Raktapittanashak Yoga* (page no.130)
7.	*Cha. Chi.* 18/117	*Kasa Chikitsa*	*Kasahara*	*Kasamardadi yoga* (page no. 444)
8.	*Cha. Chi.* 26/264	*Trimarmiya Chikitsa*	*Palitya Khalityaghna*	*Sahacharadi Taila* (page no.761)
9.	*Cha. Chi.* 26/267-268	*Trimarmiya Chikitsa*	*Palitya Khalityaghna*	*Bhrungaraj Taila* (page no. 761)
10.	*Cha. Chi.* 26/268-275	*Trimarmiya Chikitsa*	*Palitya Khalityaghna*	*Mahanila Taila* (page no. 762)
11.	*Cha. Kalpa-sthana* 1/25	*Madankalpa adhyaya*	*Vamak*	*Madanaphala prayog* (page no.798)

Shusrut samhita

Acharya Sushrut also had not included *Bhrungaraj* in any *gana* but described in the management of *Shiroroga* like *khalitya* and *palitya*. In *mishraka chikitsa adhyaya,* it is described as an ingredient of *nili taila* for management of *palitya.*[60] In *kalpasthana*, *Bhrungaraj swaras* is described to use in *palitya* with *valmikamruttika.*[61] It is described in *Jwara chikitsa* in *uttaradha*, in the preparation of *patoladi Siddha Ghrut*[62] and also *Bhrungaraj taila* in *shwasa.*[63]

Table 6: Therapeutic references of *Bhrungaraj* in *Sushruta Samhita*

Sr. No.	**Refrence**	***Adhikara***	***Rogaghnata***	***Yoga Nama*/ Formulation**
1.	*Sushrut Chikitsa sthana* 25/28	*Mishraka Chikitsa*	*Palityanahaka*	*Palitanashak Taila, nili tail*
2.	*Sushrut Uttarardha* 39/227-228	*Jwara pratishedha*	*Jwara*	*Patoladi Siddhaghrut*
3.	*Sushrut Uttarrdha* 51/30	*Shwasa pratishedha*	*Shwasa*	*Bhrungaraj siddha tail*
4.	*Sushrut Kalpathana* 8/54	*Kita kalpa*	*Palityanahaka*	*Bhrungaraj swaras*

Ashtanga Hrudaya

Acharya Vagbhata has mentioned *Bhrungaraj* in *uttarasthana* in *shiroroga chikitsa* and as a *rasayana dravya*[64] in *rasayana adhyaya*. It is also described to use in *shiroroga* in the form

of *nasyayoga*[65] and also for external application with *ayoraja*, *triphala* and *krushnamruttika* for the management of *palitya*. *Bhringaraj* is mentioned as *rasayana*, *balya*, *viryavardhak* and *ayushya* when given in the form of *swaras* for one month.[66]

Table 7: Therapeutic references of *Bhrungaraj* in *Astanga Hridaya*

Sr. No.	**Reference**	***Adhikara***	***Rogaghnata***	***Yoga Nama*/ Formulation**
1.	*Ashtanga Hrudaya, Uttarasthana* 39/163	*Rasayana*	*Rasayana*	*Bhrungaraj Swaras*
2.	*Ashtanga Hrudaya, Uttarasthana* 39/166	*Rasayana*	*Rasayana*	*Bhrungaraj Swaras*
3.	*Ashtanga Hrudaya, Uttarasthana* 39/175	*Rasayana*	*Rasayana*	*Bhrungaraj Swaras*
4.	*Ashtanga Hrudaya, Uttarasthana* 32/33	*Kshudraroga pratishedha*	*Nasya*	*Bhrungaraj Swaras*

Laghutrayi

Bhavaprakasha samhita

Acharaya Bhavamishra has described that *Bhrungaraj* is used in *Kshudra roga chikitsa* as an ingredient of *Triphaladi lepa,*[67] *Kashmaryadi taila*[68] and *Gunjadi taila*[69] in the management of *Khalitya* and *Palitya*.

And also, in *upadamsha*[70] *visarpa,*[71] *mukhamandika* in *baalarogaadhikara*[72] and as *rasayana.*[74]

Bhrungaraj swaras is adviced along with milk to be given as *nasya* in the management of *suryavarta,*[74] *shadbindu taila*[75] and *kumari taila*[76] in *shiro roga*.

Sharangadhar samhita

Bhrungaraj is mentioned as an ingredient of different preparations like *Sneha kalpana* and *kwatha kalpana*, like *triphala ghruta* in *netra roga,*[77] *nimbabija taila,*[78] *Bhrungaraj taila* for *akala palitya,*[79] *nilikadhya taila*[80] in *khalitya* and *palitya*, *bhruhat manjishtadi kwatha* in *kushta*[81] and *ayorajadi lepa*[82] in *palitya*. It is also described as a *bhavana dravya* for *shodhana* and *marana* of *shilajatu.*[83]

Other Samhitas

Bheshjya ratnavali

Bhrungaraj is mentioned in *kshudra roga adhikara* for *khalitya, palitya chikitsa*[84] and also explained *Bhrungarajadi churna* in *rasayana adhikara*[85] as *rasayana dravya*.

It is one of the ingredients of *triphala prabhruti lepa,*[86] *kesharanjaka yoga,*[87] *nimba taila*[88] and *Bhrungaraj taila.*[89]

Chakradutta

Chakrapani mentioned *Bhrungaraj* in *kshudra roga adhikara* for management of *kushta, amlapitta, swarabheda, jwara, darunaka, netra roga*[90] and as *rasayana*. *Bhrungaraj* is an ingredient of *shadbindu taila*[91] *bhrungarajadya taila* for *darunaka chikitsa, snuhyadi taila* in management of *khalitya and triphaladi lepa* as well in *palitya.*[92]

Table 8: Therapeutic References of *Bhringaraj* in *Laghutrayee*

Sr. No.	Reference	*Adhikara*	*Rogaghnata*	*Yoga Nama*/ Formulation
1	*Bhavaprakah Samhita*	*Kshudra roga chikitsa*	*Khalitya and Palitya chikitsa*	*Triphaladi lepa Kashmaryadi taila Gunjadi taila*
2	*Bhavaprakah Samhita*	*Balarogadhikara*	*Upadamsha, Visarpa, Mukhamandika*	*Bhrungaraj swaras*
3.	*Bhavaprakah Samhita*	*Shiro Rogadhikara*	*Shiro Roga*	*Shadbindutaila and Kumara taila*
4.	*Bhavaprakah Samhita*	*Shiro Rogadhikara*	*Suryavarta*	*Bhrungaraj swaras with milk*
5.	*Sharangadhara Samhita, Madhyam khanda*, 9/149	*Netraroga*	*Netraroga*	*Triphala ghrut*
6.	*Sharangadhara Samhita, Madhyam khanda*, 9/152, 9/159-160	*Shiro Rogadhikara*	*Akalapalitya*	*Nimbabija taila Bhrungaraj taila*
7.	*Sharangadhara Samhita, Madhyam khanda*, 9/155-158	*Sneha kalpana*	*Khalitya, Palitya*	*Nilikadhya Tailam*
8.	*Sharangadhara Samhita*		*Kushta*	*Bhruhat Manjishtadi Kwatha*
9.	*Sharangadhara Samhita*	*Shiro Rogadhikara*	*Palitya*	*Ayorajadilepa*
10.	*Madhav nidana*	-	-	-

Rasa ratna samuchaya

Bhrungaraj is used as one of the ingredient of *lokanath rasa* used in *jwara chikitsa,*[93] as an ingredient in *kushtha chikitsa* in *kushthari taila*[94] it is also used in *vatagajankusha rasa* which acts as *vata shamak.*[95]

Table 9: Therapeutic references of *Bhrungaraj* in other Samhitas

Sr. No	**Reference**	***Adhikara***	***Rogaghnata***	***Yoga Nama*/ Formulation**
1.	*Bheshjya ratnavali*	*Kshudraroga-adhikara*	*Khalitya* *Palityachikitsa*	
2.	*Bheshjya ratnavali*	*Rasayana-adhikara*	*Rasayana and Dantya*	*Bhrungarajdi churna*
3.	*Bheshjya ratnavali*			*Triphalaprabhruti lepa, Kalpana-Ranjaka yoga, nimba taila and Bhrungaraj taila*
4.	*Chakrapan idutta*	*Kshudraroga-adhikara*	*Kushta* *Amlapitta* *Swarabheda* *Jwara* *Darunaka* *netraroga rasayana*	
5.	*Chakrapan idutta*	*Shiroroga - adhikara*	*Darunaka* *Khalitya* *Palitya*	*Shadbindutail* *bh snuhyadi taila* *Brungarajadhyatail* *triphaladi lepa*
6.	*Rasa ratna samuchaya*		*Kesharanjaka* *Keshya* *Khalitya*	*Mahaneeli taila* *Snuhyadi taila*

Nighantu Kala

Dhanwantari nighantu[96]

It is explained under *karaveeradi varga* and was indicated in *shotha, pandu, visha and hrudroga.*

Shodala nighantu[97]

Acharya Shodala described *Bhrungaraj* in *Karaveeradi varga* and used in drug for the treatment of *upadamsha*.

Madanapala nighantu[98]

Mentioned *Brungaraj* in *abhayadi varga* and its *gunakarmas* are *kapha vatahara, dantya, rasayana, twachya* and indicated in *kushta, shiroroga and netraroga.*

Kaiyadeva nighantu[99]

Kaiyadeva had included *Bhrungaraj* in *Aushadi varga*. *Suryavarta* and *Bhrungahva* are synonyms. He explained three varieties of *Bhrungaraj* as *shweta, pita and nila* used like *shiroroga, akshiroga, kasa, shwasa, krimi, kushta, shotha, pandu* and as *rasayana*.

Bhavaprakasha nighantu[100]

It is mentioned under *guduchyadi varga* and its two varieties of *Bhrungaraj* are mentioned as *shweta and pita*. Bhavamishra mentioned *guna karma* of *Bhrungaraj* as *katu tikta, ushna, ruksha, keshya, twachya dantya and rasayana*. Indicated in *krimi, kasa, swasa, shotha, pandu, kushta and shiroroga chikitsa*.

Raj nighantu[101]

It is mentioned in *shatahvadi varga* with more than many synonyms of *Bhrungaraj* based on its clinical utility and morphological characters like *Markava, Bhrungaraj, Kesharanjana, Pitrupriya, Keshya, Kuntala Vardhana, Harivasa, Haripriya, Vandaniya, Mahanila, Nilipushpa, Shadahva, Shyamal,* etc and the propertiess like *chakshushya, shophahara, vishaghana, rasayana*.

Three varieties of *Bhrungaraj* are mentioned as *Shweta, Pita* and *Nila. Nila* variety is said to be best for its *Rasayana* property.

Shaligrama nighantu[102]

The author of *nighantu ratnakara* mentioned various synonyms of *Bhrungaraj* its two varieties as *shweta and nila*.

Nighantu adarsha[103]

In this latest nighantu written by Bapalal Vaidhya *nirukti* of synonyms *Bhungaraja, Markava, Kesharaja and Kesharanjan*

Namrupavigyana* of *Bhrungaraj

Taxonomical classification

Kingdom – Plantae[104]

Unranked – Angiosperms

Unranked – Eudicots

Unranked – Asterids

Order – Asterales

Family – Asteraceae

Genus – Eclipta

Species – Eclipta alba

Botanical name - *Eclipta alba* Hassk.

Table 10: Synonyms of *Bhrungaraj* according to different *Samhita* and *Nighantus*

Synonyms	*Ch. S*	*Su. S*	*A.H.*	*M.N*	*D.N.*	*R.N.*	*K.N.*	*B. P*	*G. N*	*Sh.N.*
Angaraka				+	+			+		
Bhrungaraj	+	+	+	+	+	++	+	+		+
Bhrunga			+					+		
Bhrungaro								+		
Bhrungarajh			+				+	+		
Markava			+	+	+	+	+	+		
Kesharaj			+				+	+		
Kesharanjan				+	+	+	+	+		
Bhrungahva				+	+	+	+	+		
Pitrupriya						+				
Keshya						+				
Kuntala						+				
Vardhana						+				
Harivasa						+				
Haripriya						+				
Devapriya						+				
Vandaniya						+				
Mahanila						+	+			
Nilipushpa						+	+			
Shadahva						+				
Shyamal						+				+
Pankajata							+			
Ravipriya							+			
Suryavarta							+			
Suryavallabha				+						
Bhrungaro								+		+
Bhrungarenu					+					
Bhrugaraka					+					
Bhrungara					+					
Bhekhraj				+			+			
Bhrungirajo				+						

Ayurvedic classification

Cause effect relationship	–	*Karya Dravya*
Living/Non-living	–	*Chetana Dravya*
Constitution	–	*Prithvi, Jala*
Origin	–	*Audbhida*
Usage	–	*Aushadha Dravya*
Morphology	–	Herb
Life span	–	Perenial
Rasa	–	*Tikta, katu*
Vipaka	–	*katu*
Veerya	–	*Ushna*
Action on *Dosha*	–	*Kapha Vatashamak*
Karma	–	*Rasayana, Kasaghna, Keshya jwaraghna, Vihaghna etc.*

Etymological derivativations of synonyms as explained in *nighantus*

Nirukti[105]

Bhrungaraj– *Bhrunga eva rajate 'Rajru diptau*'

It appears like Bhrunga and enhances the beauty of garden

Bhrungaraj– *Bhrungan ranjayati eti*

This flower attracts the humming bee and pleases the humming bee.

Markava*– Marayati Keshashaukalyam vinashayati*

It stops greying of hair.

Kesharaja*– Keshaha rajante anena keshyatvata eti*. It is bestowing the hair and enhances its beauty.

Kesharanjana – *Ranjayati eti ranjanaha, keshanam ranjanaha keshyatvata eti*

It is *keshya* and hence it colors the white hair and enhances its beauty.

Vernacular names

Synonyms– *Eclipta erecta, Eclipta prostate, Verbesina alba, Verbesina prostrate*

Sanskrit– *Bhrungaraj, Kesharaj, Markava, Kesharanjana, Kesharaj, Superna*, etc

English– Trailing Eclipta, False Daisy

Hindi– Bhangara, Bhangarayya, Bungrah, Mochkhand, Babri

Punjabi– Bhangara, dodhak, Babri

Marathi– Maka, Bhangra, Dodhak

Gujarat– Bhangaro

Bengali– Kesuriya, Kesuti, Kesuria

Tamil– Kaikeshi, Karisha-langanni

Telgu– Galagara, Gunta, Galijaeru

Assamese– Keshraj

Tamil- Karisalankanni

Arabic– Kadim-ul-bint, Radim-el-bint

Malyalam– Cajenneam, Kanni

Konkani- Mako, Kajalamavu

Pharmacodynamics

The properties of *Bhrungaraj*[16] are well illustrated by *Bhavaprakash* in *guduchyadi varga*.

Rasa

Table 11: Showing *Rasa* described in various *Nighantu*

No.	Rasa	Dh	Kn	Mn	Rj	Pr	Ab	Nr	Sh	Bp	Sl
1.	Tikta	+	+	+	+						
2.	Katu	+	+	+						+	

Virya

Table 12: Showing *Virya* described in various *Nighantu*

No.	Virya	Dh	Kn	Mn	Rj	Pr	Ab	Nr	Sh	Bp	Sl
1.	Ushna	+	+	+	+					+	

Vipaka

Table 13: Showing Vipak described in various Nighantu

Vipak	Dh	Kn	Mn	Rj	Pr	Ab	Nr	Sh	Bp	Sl
Katu	+	+	+	+	+	+	+	+	+	+

Guna

Table 14: Showing Guna described in various Nighantu

Guna	Dh	Ka	Mv	Rj	Pr	Ab	Nr	Sh	Bp	Sl
Ushna	+	+	+							
Ruksha		+	+							
Tikshna										

Doshaghnata

Table 15: Pharmacological actions of *Bhrungaraj*

Doshaghnata	**Dh**	**Kn**	**Mv**	**Rj**	**Pr**	**Ab**	**Nr**	**Sh**	**Bp**	**Sl**
Kaphaghna		+								
Vataghna		+								
Kaphavatakara			+							

Table16: Showing B*hrungaraj karmukata* in various *nighantu*

No	***Karma***	**Dh N.**	**Rj .N.**	**Sh. N.**	**Mn. N.**	**Ast . N.**	**K. N.**	**Bp. N.**	**Am .N**	**M. D**	**Sl**
1.	*Shothaghna*	+	+	+			+	+		+	
2.	*Vishaghna*	+	+								
3.	*Pandu*	+	+				+	+			
4.	*Keshya, netrya*		+				+	+			
5.	*Twachhya*	+			+		+	+			
6.	*Hrudrag*	+									
7.	*Krumighna*						+	+			
8.	*Shwasa*						+	+			
9.	*Kasa*						+	+			
10.	*Rasayan*		+		+		+	+			
11.	*Balya*							+			
12.	*Kushtaghna*				+		+	+			
13.	*Shirorog, dantya*				+		+	+			
14.	*Upadansha*			+							

Bhrungaraj vyadhi nashakatva

Table 17: Showing *Bhrungaraj vyadhi nasakatva* in various Nighantu

Sr.	***Vyadhi***	**Dh. N.**	**Rj. N.**	**Sh. N.**	**Mn .N.**	**Ast. N.**	**K. N.**	**Bp. N.**	**Am .N.**	**M. D.**	**Sl**
1.	*Pandu*	+					+				
2.	*Hrudroga*						+				
3.	*Shotha*		+				+				
4.	*Kushtha*				+		+				
5.	*Netraroga*				+		+				
6.	*Shiroroga*				+		+				
7.	*Dantaroga*				+		+				
8.	*Krumi*						+				
9.	*Shwasa*						+				
10.	*Kasa*						+				
11.	*Yakrut vikara*		+					+			
12.	*Rasayan*		+		+		+				
13.	*Upadanha*			+							

Modern review

Family Characteriterstics

Asteraceae family

Habit

Members[106] of the Asteraceae are mostly annual perennial herbaceous plants, shrubs, trees and climbers. They are generally easy to distinguish from other plants, mainly because of their characteristic inflorescence.

Roots and Stems– fibrous taproots, stem erect, prostrate or ascending, fleshy or woody

Leaves– alternate, opposite or whorled, simple deeply lobed

Flowers– sessile, actinomorphic pentamerous, regular, unisexual and epigynous

Calyx– bristle like

Fruit- one seeded

Habitat: It is a common weed occurring moist places found throughout India.

Habit: herbaceous annual, 30-50 cm high, erect or prostrate

Botanical description of *Eclipta alba*

Eclipta alba (L.) Hassk. (syn. *Eclipta prostrata* L.), commonly known as False Daisy, yerba de tago, and Bhringraj,[107] is a plant belonging to the family Asteraceae. Root well developed cylindrical, greyish.

This is an erect annual plant growing upto 30-50 cms in height, with flat or round; blackish chocolaty, much branched, pubescent stems. The leaves are opposite, serrate, 3-5 cms long and blackish green in colour. Flowers are small, penny sized, white, on a stalk. The fruits are many seeded and seeds are black, resembling cumin seeds. The plant flowers in September, fruits in November. Floral heads are 6-8 mm in diameter, solitary, white, achene compressed and narrowly winged.

Distribution: It is abundantly found in India, China, Brazil and United States.

Cultivation and propagation: Vegetative propogation by using terminal buds of length of 5 cm. It can be planted in well prepared beds as above seed beds or nursery bags. The plants will be ready for transplanting within 30 to 45 days.

Species: Shri. Bapalal Vaidya (author of *Nihantu ardarsh*)[108] has described three varities of Bhrungaraj– white, yellow and black

White variety is known as- *Eclipta alba*, yellow variety is known as *Wedelia calendulaceae*

Black variety (*Nila*) is rare and a red variety also appears which is also rare known as *Flavieria rependa*.

Parts used: Herb roots and leaves, *panchanga, beeja* all parts are used

Various chemical constituents[109] are seperated from *Bhrungaraj* and are being clinically tested for various hepatic disorders, etc.

Main active principles consist of coumestans like wedelolactone, desmethyl wedelolactone[110] furanocoumarins, eclalbatin oleanane and taraxastane glycosides[111]

Pharmacological activities

Chemical Constituents: The main chemical constituents of *Eclipta alba* as described[112] in table no. 18, are coumestan derivatives like wedololactone, dimethyl wedelolactone, desmethyl-wedelolactone-7 glucoside, ecliptal, ß-amyrin, luteolin-7-O-glucoside, hentriacontanol, heptacosanol, stigmasterol, Ecliptaalbasaponin I and II. It is used as an anticancer, antileprotic, analgesic, antioxidant, antimyotoxic, antihaemorrhagic, antihepatotoxic, antiviral, antibacterial, spasmogenic, hypotensive, ovicidal, promoter for blackening and hair growth promoter drug.[112]

Table 18: Chemical constituents in different parts of *Eclipta alba*

Sr. No.	Plant part	Chemical constituents present
1.	Leaves	Stigmasterol, a-terthienymethanol, Wedelolactone [1.6%], Desmethylwedelolactone, Desmethyl-wedelolactone-7-glucoside
2.	Roots	Hentriacontanol, Heptacosanol and Stigmasterol4, Ecliptal12-1
3.	Aerial parts	ß-amyrin and Luteolin-7-0-glucoside, Apigenin, Cinnaroside, Sulphur compounds
4.	Stems	Wedelolactone
5.	Seeds	Sterols
6.	Twigs of the plant	Unnamed alkaloid
7.	Whole plant	Large amounts of resin, Ecliptine, Reducing sugar, Nicotine, Stigmastero, Triterpene saponin, Eclalbatin togetherwith a-amyrin, Ursolic acid, Oleanolic acid.

Table 19: Biological activities of Chemical constituents[113] of *Eclipta alba*

Sr no.	Chemical constituents	Biological Activities
1	Wedelolactone	Antihepatotoxic, Selective5 – lipoxygenase inhibitor with an IC50 of 2.5μM
2.	Demethylwedelolactone	Antihepatotoxic, Antimyotoxic, Antihaemorrhagic
3.	Coumarin Compounds	Antinociceptive, Anti-inflammatory

Ecliptasaponin C, a new triterpenoid glucoside, daucosterol and stigmasterol-3-O-glucoside were isolated from Eclipta alba[35] and 3-beta-O-beta-D lucopyranosyl-19beta-hydroxy olean-12-ene-28-oic acid 28-O-beta-D-glucopyranoside, Compounds 2 and 3 were obtained from Eclipta.[114]

A large amount of resin and an alkaloidal principle ecliptine is obtained. Wedelolactone is found in yellow and white variety.

Mostly juice is advisable to use and not extract decoction or churna.

Biological activites of parts of *Eclipta alba*

Uses: Root is used in,

1) *Shotha, vrana, savarnikarana, kesha vyadhis, shleepada, granthi, shirashula,* greying of hair, etc.
2) It is also used as application in hepatic and splenic enlargements and in various chronic skin diseases.

3) It is also useful internally in *yakrut vyadhis, yakrut vruddhi,* spleenic enlargements, jaundice, udarashula, etc.
4) In krumi with castor oil, shwasa, kasa and in mutradaha.
5) Useful in serpent bite, scorpion bite, chronic glandular swellings and other skin diseases and alopecia, etc.
6) Leaf juice is used as hepatic tonic and deobstructunt.

Vagbhat uttartantra[115]

Bhrungaraj swaras if taken for one month and only milk is taken as diet during the consumption of this *swaras*, that person lives life for 100 years.

Recent researches on Bhrungaraj

- In a clinical trial, a bi-herbal ethanolic extract (BHEE) of combination of leaves of *Eclipta alba* and seeds of *Piper longum* was administered orally at a dose level of 50 mg/kg body weight once for 14 days, it was found that the biochemical parameters like total protein, total bilirubin, total cholesterol, triglycerides, and urea were restored to normal levels.[116]
- Methanolic extract of leaves and the chloroform extract of roots of *Eclipta alba,* it showed that the Lysosomal enzymes level showed very significant activation reduction in the lysosomal enzyme. It was observed that the triterpenoid eclabasaponin fraction from methanolic extract of leaves produced was responsible for significant increase (78.78 %) and the alkaloidal fraction (60.65 %) reduction of carbon tetra chloride induced increase in lysosomal enzyme in the blood.[117]
- Immunomodulatory action of *Eclipta alba* was observed by the protection of neuronal tissues. So, *Eclipta alba* proves to be a potential memory modulator[118]

- In an experimental study the immunomodulatory activity of methanol extracts of whole plant of *E. alba* (1.6 % wedelolactone) using carbon clearance, antibody titer and cyclophosphamide immunosuppression parameters was assessed, it showed significantly increased phagocytic index and antibody titer and the F ratios of the phagocytic index and WBC count were also significant.
- This proved that protection of neuronal tissues may be possibly due to the immunomodulatory action of *Eclipta alba* methanolic extract. Therefore, *Eclipta Alba*[119] can be used as a potential memory modulator.
- In another similar study, aqueous leaf extract *Eclipta alba* Hassk. was fed into a fish (tilapia, Oreochromis mossambicus) at 0, 0.01, 0.1 or 1% levels as a diet for 3 weeks. In this study, every week the parameters like- non-specific humoral (lysozyme, antiprotease and complement) and cellular (myeloperoxidase content, production of reactive oxygen and nitrogen species) responses and disease resistance against Aeromonas hydrophila were noted which resulted in increased activity of non-specific immune parameters. This result indicated that dietary intake of E. alba aqueous leaf extract may enhance the non-specific immune responses and disease resistance[120] of O. mossambicus against A. Hydrophila.
- Methanol extracts of whole plant of *E. alba* was used to assess the immunomodulatory activity by using carbon clearance, antibody titer and cyclophosphamide immunosuppression parameters, the study showed significant increase in phagocytic index and antibody titre and the F ratios of the phagocytic index[121] and WBC count were observed to be also significant.
- The herbal drug Tefroli, containing extracts of the plant in combination with others, when administered to the patient of viral hepatitis, produced improvement[122] and good results.

Material and methods

Materials

Materials for physicochemical study

Samples of *Bhrungaraj* were self-collected genuine sample gathered from its natural habitat from the campus of Government Ayurved College, Nanded and Nagina ghat area of Nanded. The sample was authenticated and kept in the Department of Botany, Nanded Education Society's (NES), Science College, Nanded as voucher specimen (voucher specimen no. SSS-001) for further reference.

Preparation of study drug samples for physicochemical study and clinical trial

Collection of sample

Samples were self-collected from the natural habitat in the campus of Government Ayurved College, Nanded and Nagina ghat area of Nanded.

Botanical identification

The plant drug collected was botanically identified with its morphological characters as described in flora books.[123] *Eclipta alba* Hassk. is a small herbaceous plant belonging to the Asteraceae family, prostrate and grow erect up to 50 cm. The plant was identified by the botanical characters as *Eclipta alba* species.[124]

Authentication of sample

The collected plant sample of *Eclipta alba* Hassk. was botanically authenticated by local botanists and confirmed as *Eclipta alba* Hassk.

Methodology

Organoleptic characters

The sample of *Bhrungaraj* was examined for its organoleptic characters. The details are described in observations and results.

Macroscopic Examination

Macroscopic examination of the entire plant of Eclipta alba was carried out to examine the different parts like root, stem, leaf, fruit, seed, flowers etc.

Microscopic examination

The microscopic examination of root, stem, leaf, etc was carried out and noted in the observation and results.[126]

Preparation of study drug samples for physicochemical study and clinical trial

The aim of this study was to evaluate the difference in physicochemical and phytochemical properties of *ardra* and *shushka* dosage forms of *Bhrungaraj panchanga* i.e. *swaras* (*ardra*/wet form), *anukalpa swaras* (*ardra*/wet form), *shushka churna* (dry form) and tablet forms (*shushka*/dry form) and its clinical efficacy in iron deficiency anaemia. For this purpose, the self-collected sample of *Bhrungaraj* was selected for study and different formulations were prepared as described above to evaluate the physicochemical and phytochemical properties and also to assess their clinical efficacy.

Preparation of different dosage forms

1. Preparation of *swaras*

The fresh plants free from any pest, disease or decay were uprooted entirely and collected. These plants were washed with water then were cut with knife to small pieces and then crushed to paste-like form. This paste-like form was tightly held in a clean piece of cloth and the juice was obtained after filtering through the cloth. This juice was used as *Bhrungaraj swaras* prepared according to the procedure described in *Sharangdhar samhita.* [126]

2. Preparation of *anukalpa swaras,* standard operating procedure as per *Sharangdhar* and *Charak samhita*

Dried plant drug was grinded to prepare coarse *churna* and 2560 gms (1 *adhak*) of this churna was soaked in water which was added twice in quantity to that of *churna* i.e. about 5120 ml (*dviguna*). The filtrate was kept overnight and was smashed with hands and later filtered with clean cloth. This filtrate was used as *anukalpa swaras* (*nishoshit swaras/ swaras* from *shushka dravya*) as described in *Sharangdhar samhita*[127] and *Charak samhita.*[128]

3. Preparation of *ghan vati* of *anukalpa swaras*

Formulation of *Bhrungaraj anukalpa swaras ghan vati* was done by approved drug manufacturing company Chaitanya Pharmaceuticals, Pvt. Ltd., Nashik. Preparation was done according to standard operating procedure (S.O.P.). The method described in *Siddha sarasangraha*[129] for *ghan vati* preparation (described during the preparation of *Guduchi ghan vati*) was followed.

For one-part churna four times of water was added to it. The mixture was boiled until 1/4th of the mixture was left. This was then cooled and filtered with clean cloth and then the filtrate was kept for boiling again until it became thick paste. This was cooled and passed to tablet making machine for preparation of tablets of 500 mg each. This tablet form was prepared from *shushka churna* of *Bhrungaraj* so were named as *anukalpa swaras ghan vati*. Tablets were obtained and dried. Packing of tablets was done under hygienic condition.

4. Preparation of dried form of s*waras* (spray dry *swaras ghan vati*)

As *swaras* was difficult to preserve for long time and difficult to prepare daily for large number of patients, the *swaras* was prepared in bulk quantity and converted to spray dried powder form with the help of spray drying methods from approved drug manufacturing pharma company Amruta Herbals, Pvt. Ltd., Indore, Madhya Pradesh. This spray dried powder was used to prepare ghanvati[130] for the clinical trial. This sample was also used for physicochemical study and clinical trial and named as s*pray dry swaras ghan vati.*

Standard Operating Procedure for preparation of Spray dry powder as per manual provided by Amruta Herbals pvt. Ltd.[130]

The procedure of spray drying was conducted in five steps such as,

a. Concentration of the swaras
b. Atomization of the swaras
c. Droplet- air contact for drying
d. Droplet drying
e. Separation of prepared particles

a. Concentration of the swaras

Around 50 litres of *Bhrungaraj swaras* was taken and was concentrated by heating before introducing it into the spray dryer. This concentration process with the help of heating led to

reduced amount of liquid that was to be evaporated in the spray dryer and thus the solid contents were increased which helped for faster drying and particle formation.

b. Atomization of the swaras

In this step the concentrated swaras was introduced into the spray dryer and evaporation was started using rotatory atomizer by exposing the concentrated swaras spray with the heated air.

c. Droplet– air contact for drying

After atomization the atomized liquid was exposed to hot air at vacuum due to which more than 95 % of evaporation process was completed along with evaporation of water droplets within less time amount.

d. Droplet drying

Droplet drying was done in two stages. In the first stage, the temperature was maintained equal to the wet-bulb temperature of the air. The remnant moisture in the particle drops was evaporated at a constant rate during this stage. In the second stage the complete moisture was evaporated and was maintained at saturated conditions to form dried shell at the surface. The rate of evaporation reduced rapidly at the end. The resultant particles were in the form of porous spheres which were irregularly shaped.

e. Separation of prepared particles

After complete drying of the droplets the obtained powdered particles were separated from the bottom of the instrument and the remaining particles were collected during the separation of the equipment with the help of cyclones, bag filters, and electrostatic precipitators. Wet scrubbers were used to purify and cool the air so that it could be released to atmosphere.

The Spray dry powder was prepared as per the above method and was obtained from Amruta Herbals, Pvt. Ltd., Indore, Madhya Pradesh.

The method described in *Siddha sarasangraha*[130] for *ghan vati* preparation (described during the preparation of *Guduchi ghan vati*) was followed.

This spray dry powdered churna was taken to prepare ghan vati. To one part of churna four times of water was added to it. The mixture was boiled until 1/4th of the mixture was left. This was then cooled and filtered with clean cloth and then the filtrate was kept for boiling again until it became thick paste and then it was cooled and passed to tablet making machine for preparation of tablets of 500 mg each. This spray dried powder was used to prepare *ghan vati* of 500 mg each as per the method of *ghan vati* preparation.[130] These tablets were prepared from spray dry *swaras* powder so used as spray dry *swaras ghan vati.*

Tablets were obtained and dried, packing of tablets was done under hygienic condition.

Materials

Formulations used for physicochemical and phytochemical studies

Swaras, spray dried *swaras ghan vati, anukalpa swaras*, *ghan vati of anukalpa swaras* were prepared by the methods mentioned earlier.

Formulations used for clinical trial

The comparison between the *ardra* and *shushka* dosage forms of *Bhrungaraj* were clinically evaluated by using *ardra* (spray dry powder *ghan vati*) and *shushka* (*anukalpa swaras ghan vati*) dosage forms of *Bhrungaraj* in iron deficiency anaemia.

Administration of drug

Three groups were divided as follows,

Group 1- patients were given *anukalpa swaras ghan vati* prepared from dry sample (*shushka* dosage form) in single dose.

Group 2- patients were given *ghan vati* prepared from spray dried powder of fresh *Bhrungaraj swaras* in double dose (*ardra* dosage form)

Group 3 (control group)- patients were given iron ascorbate, 100 mg BD.

Shushka form i.e. *ghan vati of Shushka churna* in group 1 was given in single dose, according to the reference of *Sharangdhar Samhita,*[4] which describes to use *ardra dravya* twice in quantity to that of *shushka dravya*. *Ardra* form (spray dry powder tablets) was given to Group 2 patients in double dose.

Before initialization of the trial, deworming of patients was done by giving Tablet Albendazole 400 mg stat. After that a gap of three days was kept and then the treatment was initiated.

Methodology

The study was open labelled, comparative, interventional randomized trial.

Methodology for clinical trial

Type of study: Interventional study

Study design: Randomized open labelled clinical controlled trial

Patient selection: Allocation randomized open labelled clinical controlled trial

End point classification: Clinical efficacy and phytochemical study

Intervention model: Parallel assignment

Phase of trial: Phase III

Place of work

Cases were enrolled to the outpatient department and were randomly allotted to the three groups.

Screening and recruitment of patients

Screening of female patients lying within the age group 11 to 19 years was done according to the complaints and sign and symptoms. Patients were examined clinically and then included in the study as per the inclusion and exclusion criteria. The selected patients were randomly allotted in the three groups and treatment was given till the study period of 6 weeks. The dropouts were not included in the statistical calculation of final data.

Method of randomization: Random allocation method

Ascent and consent

Written consent (for subjects above 18 years of age) and assent (for subjects below 18 years of age) was taken for each and every subject in the case paper format attached in the annexures, prepared as per Indian Council of Medical Research (ICMR) guidelines for biomedical research 2006.

Sample size estimation

Sample size estimation was done on the basis of statistical tests related to the prevalence rate of Iron deficiency anaemia in India.

$n= t^2 \times p\ (1-p)/m^2$

where, n- required sample, t– confidence level at 95% standard value, p- estimated prevalence, m– margin of error at 5% level, Standard value– 0.05

So, for this study, the sample size obtained was 128 in each group; approximately 130 patients were taken in each group.[131]

Dose calculation

Group 1– *Anukalpa swaras ghan vati* was given in single dose to that of spray dry *vati.* Normal dose of *churna* is 5 gm, so 5 gm *churna* was taken and water was added twice in quantity to it and *kwatha* was prepared and dried. The extract obtained was 0.5053 gm. Tablets of 500 mg were prepared and given in the dose of 1 tablet OD (single dose).

Group 2–Patients were given test drug of spray dried powder *ghan vati* prepared from fresh sample of *Bhrungaraj swaras* in double dose (weight of one tablet = 500mg).

For calculation of the dose, 100 ml of fresh *swaras* was dried and after drying powder extract was obtained which was calculated. From 100 ml *swaras*, 4.7096 gm of powder extract was obtained. So, for 1 ml, 0.047096 mg; for 40 ml, 0.047096 x 40 =1.88384 gm. Daily dose is 20 ml and twice is 40 ml. Daily dose = 40 ml/2 = 1.88384/2 = 0.9419 gm approximately 1 gm. To double the dose, the calculated dose was given B.D. i.e. 2 gm daily. Tablets of 500 mg were prepared from spray dry powder and were given as 2 tablets in BD dose.

Group 3– Patients were given iron ascorbate 100 mg BD.

***Bheshajjya sevan kala*:** 30 minutes before meals in all the groups

Anupana– luke warm water

Duration– 6 weeks[132]

Inclusion criteria

1. Hb %- 7 - 11 g/dl
2. Age group 11 – 19 years
3. Only female patients

Considering sample size, local colleges were visited and information about the study was given. Female students were selected and enrolled for the study. Hence, patients included in the present study are of age 16-19 years.

Exclusion criteria

1. Hb % below 6 g/dl
2. Known hyper sensitivity or intolerance to oral iron

Parameters for assessment

Clinical symptoms

Pallor of skin, tongue, nail beds, pallor of conjunctiva and pallor of palms

Gradation of symptoms

Objective parameters

Pallor[133,134,135]

No pallor	0
Pallor of conjunctiva	1

Pallor of conjunctiva, nails, tongue	2
Pallor of conjunctiva, nails, tongue, skin	3
Pallor of conjunctiva, nails, tongue, skin, palms and soles	4

Laboratory investigations

Haemoglobin (Hb %) by Automated Hematology Analyzer

Additional investigations:

Serum ferritin	MCV
Total iron binding capacity (TIBC)	MCHC
Serum transferrin	Blood smear examination
Serum iron	

Follow up

The treatment was continued for 6 weeks and follow up was taken every 2 weeks during treatment.

Investigations were carried out at the baseline i.e. 0^{th} day and at the end of the treatment i.e. 45^{th} day.

Statistical analysis

Data was presented in terms of mean, standard deviation, standard error and student t-test (paired t-test for before and after treatment comparison in each group) for quantitative parameters at the level of $p<0.001$ as highly significant or $p<0.005$ as significant and $p>0.005$ as insignificant.

Methodology for physicochemical studies

Different dosage forms used for physicochemical and phytochemical studies

1. *Swaras,*
2. Spray dried *swaras ghan vati,*
3. *Anukalpa swaras and*
4. *Ghan vati of anukalpa swaras*

Tests applied for the different dosage forms

Tests applied for solid form of drug

1. Physicochemical tests
2. Phytochemical tests
3. Chromatographic analysis
4. Spectrophotometry UV
5. Atomic absorption spectrophotometry

Tests applied for liquid form of drug

1. Specific gravity
2. Viscosity
3. Spectrophotometry
4. Chromatographic analysis
5. Atomic absorption spectrophotometry

Procedures of tests carried out for various forms

Physicochemical standardization tests

Determination of Foreign matter[136]

The drug used was studied for the presence of moulds, insects, animal faecal matter, fibres and other contaminations such as soil, stones and extraneous material which is considered as foreign matter and its percentage was calculated.

Procedure– The collected drug sample was spread on white paper and foreign matter was separated, weight was taken and the percentage of foreign matter present was calculated.

pH Value[137]

pH is useful for ensuring identity, stability and detect that the drug is free from the contamination of water soluble adulterants which are acidic or alkaline in nature as each and every drug possesses its own acidity or alkalinity measured in pH range at a specific concentration.

Determination of pH– 1 gm of powdered drug was taken in 100 ml of distilled water and extracted for 24 hours. The extract was taken for measuring the pH value prior to the various procedures. At first the pH meter electrode was soaked in the buffer of pH 7 for eight hours at room temperature and after that kept on standby mode so that it could be readily used for measurement. The reference electrode was filled with set solution and air bubbles were taken out. The electrode was also tested for buffer of pH 4 to check the accuracy. The prepared extract was taken and the pH was measured.

Determination of swelling index[138]

Swelling is the property of some medicinal plant materials mostly gums due to the presence of mucilage, pectin or hemicellulose. This is known as the swelling index which is calculated by taking 1 gm of plant material with 25 ml of water in a glass topped measuring cylinder having

internal diameter of about 16 mm and length 125 mm marked in 0.2 ml division from 0 to 25 ml upwards. The powder with water were shaken vigorously for every 10 minutes up to 1 hour and after 1 hour was kept in standing position for 3 hours. Then the volume of *churna* was calculated and difference calculated from initial height reading.

Determination of foaming index (F.I.)[139]

Foaming index is the foaming ability of an aqueous decoction of plant materials and their extracts due to the presence of saponin.

Procedure– 1 gm of powdered *churna* was taken in a conical flask of 500 ml and boiling water was added to it and boiling continued for 30 min. after cooling the solution was filtered in a 100 ml volumetric flask and was diluted by distilled water to make 100 ml volume in the 100 ml volumetric flask.

This solution was then taken in 10 stoppered test tubes as 1 ml, 2 ml, 3 ml, etc. up to 10 ml successively in each test tube. The 1 ml, 2 ml and all the test tubes were made 10 ml by adding distilled water and were closed with stoppers and shaken vigorously for 15 seconds and then allowed to stand. Foam appeared on the surface the height was measured. The calculations were done as follows

> The height of foam in every tube was more than 1 cm so foaming index (FI) was more than 100.

The foaming index was calculated as FI = 100/a

Where a = the volume in ml of decoction used for preparing the dilution in the tube, where foaming to a height of 1 cm was observed.

This test was performed in all samples separately and the results were calculated as follows,

The height of foam in each test tube was more than 1 cm i.e. FI

For samples *Bhrungaraj churna* and spray dry powder of *Bhrungaraj* was >100.

Determination of moisture content / loss on drying[140]

Instrument used- Halogen moisture analyzer (Mettler ToledoHR73 model)

This procedure was carried out to determine the amount of volatile matter i.e. water content of the drug. The halogen moisture analyzer works on thermo gravimetric principle. In the beginning of the procedure the weight of the sample is determined on the screen, then the integral halogen dryer unit dries the sample and the moisture vaporizes which is displayed on the screen continuously as moisture content along with weight of the sample.

Procedure– The moisture content analyzer was arranged in horizontal position with the help of level indicator and levelling screws. The instrument was set with date, time, temperature setting (50º C - 200º C) and exact weight of sample taken.

1) Previously the instrument was switched on using <<ON/OFF>> key.

2) The automatic chamber was opened to add the sample using <<Open/Close>> key.

3) The screen displayed the symbol of empty dish which indicated to add the *churna* so sample was added to the instrument and weight noted.

4) After pressing the start key the drying process was started.

5) The screen display indicated the drying process as ascending bubbles which was continued till the end of the process.

6) At the end of the process an audio signal sound was produced which indicated the completion of the process.

7) Drying temperature, initial weight of sample, weight after drying, total drying time and % of moisture content was displayed at the end.

Digital Colorimetry[141]

Colorimetry technique is used in the standardization of drugs for determination of the concentration of a compound in the given sample by measuring the colour intensity of solution and the absorbing power of the solution at specific wavelengths.

Apparatus

i) Digital colorimeter

ii) Test tubes for solvents

iii) Cuvette of ccolorimeter

Procedure

5 % of water extract of *Bhrungaraj* was prepared by taking 500 mg of *Bhrungaraj churna* in 10 ml distilled water and after filtering used for colorimetry analysis with reference to distilled water.

The colorimeter was set with distilled water in cuvette and wavelength was decided according to the colour of solution. The sample of *swaras* and *anukalpa swaras* were placed in cuvette and the readings were taken. All the readings were noted at 670 nm with reference to distilled water.

Determination of Extractives[142]

a) Determination of alcohol soluble extractive

Extracts were prepared by taking 5 gm of the *Bhrungaraj churna* in 100 ml of alcohol in a conical flask, closed with rubber cork. The flasks were placed in shaker machine for 24 hrs, shaking for six hours and allowed to stand for 18 hrs. The solvent was filtered with filter paper in petri dishes; filtrate was evaporated till complete drying at 105º C to constant weight. The weight of empty petri dish was deducted from weight of petri dish with extract, then weight of extract

was calculated and the percentage of alcohol soluble extractive to the air-dried drug was also calculated.

Above procedure was applied for the determination of extractive values using various solvents instead of alcohol.

b) Determination of water soluble extractives

c) Determination of methanol soluble extractives

d) Determination of petroleum ether soluble extractives

e) Determination of benzene soluble extractives

f) Determination of chloroform soluble extractives

g) Determination of ethanol soluble extractives

Determination of Ash[143]

The ash is determined by 3 methods. a) Total ash, b) Acid insoluble ash and c) Water soluble ash

a**) Determination of total ash value**

The total ash value was determined taking 1 g of powdered sample in a tarred platinum crucible and heated to a temperature not exceeding 450º C.

The crucible was cooled and weighed and difference of empty crucible and crucible with ash taken and % calculated. Three constant readings were taken and mean was calculated.

b) Determination of acid insoluble ash

The ash obtained in the above method was boiled in 25 ml of 0.1N HCl for 10-15 min, cooled and filtered on ash less filter paper. The matter insoluble in HCl was collected on ash-less filter paper and again kept in crucible for burning. This percentage of acid insoluble ash was calculated with reference to air-dried drug.

c) Determination of water soluble ash

The total ash obtained was boiled in 5 ml of water for 5 minutes and the solution was filtered on ash-less filter paper. The insoluble matter was collected on an ash-less filter paper and kept in crucible and ignited to constant weight at a temperature. Weight was taken for three constant readings and mean was calculated. After that the weight of the ash was taken separately and the difference in weight was calculated to determine the percentage of water soluble ash

Specific Gravity[144]

Procedure: Specific gravity was calculated with the help of "specific-gravity bottle" or "pycnometer" which holds a known volume of liquid at a specified temperature. The empty specific gravity bottle was weighed (weight determined as W), then it was filled with distilled water and again weight was taken (weight determined as W_1). The bottle was dried with filter paper and again filled with the *Bhrungaraj swaras* and weighed again (weight determined as W_2). The specific gravity of *Bhrungaraj swaras* was calculated as follows;

Specific gravity of *Bhrungaraj swaras* = $W_2 - W / W_1 - W$, where W_2 is weight of specific gravity bottle with *swaras*, W_1 is weight of specific gravity bottle with distilled water and W is weight of empty specific gravity bottle.

Same procedure was repeated for *anukalpa swaras.*

Viscosity[145]

Viscosity of the liquid is the property of resistance to flow.

Apparatus: Ostwald's viscometer, specific gravity bottle

Procedure: The Ostwald's viscometer was filled with *Bhrungaraj swaras* through the tube 'U' slightly above the mark 'S' with the help of a pipette. The tube was kept vertically still in the water bath at a temperature 40° C for 30 min. The volume level of the tube was adjusted such

that its lower meniscus touched the mark “G”. The *swaras* was sucked to the above the mark “E” and one end of the tube was closed tightly with finger. After that the finger pressure was released slowly so that the liquid level felled from mark “E” to “F” during which time required for the swaras to fall from “E” to “F” was noted. The procedure was repeated for three times and mean was taken.

The dynamic viscosity was calculated using the formula; Dynamic viscosity = kQt

where, k = constant determined by using distilled water which was 0.015 mm^2/sec.

Q = mass/volume i.e. gm/cc and

t = time in second for meniscus to fall from “E” to “F”

Relative density was calculated as mass/volume

Kinematic viscosity was calculated as,

Kinematic viscosity = dynamic viscosity/ Relative density.

Relative viscosity was calculated as,

Relative viscosity = Time in second for liquid to fall from “E” to “F” / Time in second for water to fall from “E” to “F”.

Relative Density

Pycnometer (specific gravity bottle) was used for the determination of the density of *swaras* and *anukalpa swaras.* The weight of the empty pycnometer was denoted as (W) was measured in comparison with distilled water (W1). The filled pycnometer with reference liquid (W2) having

well known density (pw) and filled pycnometer with the sample (W+S). The density of the sample (Ps) were calculated as follows,

Ps = (W +S) – (W/ W1+ W – W1W2)

Equipment

Ostwald viscometer, pycnometer, 20 cm, pipette, capillary funnel, thermostat, analytical balance

Steps

1. Temperature of the thermostat was set and distilled water was kept in the lower reservoir and viscometer was kept on the thermostat.

3. The weight of the empty pycnometer was measured and after that it was filled with distilled water.

4. After that the distilled water was removed and it was filled with sample solution and temperature was maintained.

5. The pycnometer was again filled with distilled water weight measured and again cleaned and filled with the sample solution.

6. Same process was repeated for 6-8 times weight was measured.

7. Relative density was then calculated using the above formula.

Spectrophotometry[146]

Procedure: The tendency of every drug to absorb light of different wavelength denoted as an absorbance spectrum of light is a specific characteristic of a compound. Thus, this spectrum is used to identify and quantitate the concentration of the chemical substance in Ayurvedic drug containing various chemical constituents. These chemical constituents absorb light of different wavelength when extracted in different solvents.

This spectral pattern is specific for that drug and solvent system. Thus, this spectrum is used to identify the drugs and adulteration.

Apparatus

1. UV visible double beam scanning spectrophotometer (Model– Schimadzu UV 160 A)
2. Samples of *Eclipta alba* Hassk.
3. Solvents– water and alcohol

Procedure

500 mg of the sample powder was taken in the two separate test tubes and 10 ml of water and ethanol were added to it; shaken well for 1 hr. and filtered, this particle free filtrate used for spectrophotometry analysis.

UV visible spectrophotometer was switched on, initialized and wavelength (800-400, 400-200 nm), scanning, speed; absorbance, etc. were set on the computer.

The cuvettes of the spectrophotometer were filled with the alcohol and termed as blank or reference cuvette and kept in the respective socket of spectrophotometer and the other cuvette was filled with the alcoholic extract and was placed in another socket in the sample compartment of the spectrophotometer. The scanning was started and the specific absorption spectrum was displayed on the monitor.

The numbers of peaks, their absorbance of respective wavelength were recorded. Same procedure was repeated for water extract.

The above test was performed at laboratory- Qualichem laboratories, Nagpur

Batch no. 1

Instrument type - UV-Vis Double Beam Spectrophotometer 6.75

Standards used - Wedelolactone, Ecliptaalbasaponin I and II.

Chromatographic analysis

Thin Layer Chromatography[147]

Procedure

Thin layer chromatography is used for the separation, identification and quality control of drug. It also determines small amount of impurities or adulterants if present.

Apparatus

Glass plates of uniform thickness, 15-20 cm long and wide, spreader, chromatographic chamber of glass with a tightly fitting lid having suitable size to accommodate the glass plates and capillary, ultraviolet light source emitting short 254 nm and long 366 nm wavelengths.

Preparation of samples

The extract of *Bhrungaraj* samples were prepared by taking 1.0 g of drug and 1-10 ml of solvent, extract was stirred for about 30 minutes and filtered.

Preparation of thin layers in plates

The plates were cleaned, rinsed thoroughly until the water dried off from the surface of the plates. Spreading method was used for coating of the plates.

Spreading Method

Silica Gel– G slurry was prepared for coating of the glass plates by adding distilled water. The prepared slurry was spread using the spreading device; with a layer of about 0.25 mm thickness.

Activation of the adsorbent material

The plates were dried in air for 30 minutes and then kept in oven at 110° C for 30 minutes again for activation and were allowed to cool.

Sample application

The samples were applied on the coated glass plates with the help of a micro capillary, (micropipette) and spots were placed onto the starting line not more than 4 mm in diameter and the spot were dried.

Development of chromatographic chamber

The chromatography chambers were activated by the saturation of fumes caused due to the solvent mixtures used as mobile phase.

Solvent system

The solvent system was evaluated on the basis of the nature of the components by trial and error. Different solvent systems were taken for the separation as described in the results.

Development Method of Chromatograms

Ascending technique was used for the chromatography and the solvent was allowed up to the height of about 15-18 cm of the vertically placed plate in the closed chromatography chamber. This process took place within 40-50 min after which plate was removed, the level of the flow of the solvent was marked with needle plate was allowed to dry at room temperature.

Detection of component

The observed spots were marked with needle in daylight and also under 254 nm and 366 nm wavelength of the ultraviolet light. The distance from the centre of each spot up to application level was measured and distance calculated. The spots which were not detected in UV light were

kept in iodine vapours chamber for 1-2 hrs and Rf values were calculated using the formula as follows,

Retention Factor (Rf) = Distance travelled by the solute from the original line/

Distance travelled by solvent from the original line.

The obtained spots and their Rf values are described in the results section.

Atomic absorption spectrophotometry[148]

Principle

Atoms, ions or ion complexes of an element get atomized to ground state in a flame and they absorb light at the characteristic wavelength of that element and the absorbance is directly proportional to the number of absorbing atoms. So, this principle was used in the atomic absorption spectrophotometry.

Procedure

Apparatus

Chemito AA203 Atomic Absorption Spectrophotometer

Atomic absorption spectrophotometer had the emission source that provided the characteristic spectral line of the elements – the hollow cathode discharge lamp, monochromator, nebulizer burner system for introduction of the sample solution into the flame and detector system.

Method

The samples were prepared as per the concentration range applicable to the instrument and the elements. Distilled water was used as reagent and also for calibration of the instrument in the same concentration. After calibrations of the instrument the standard solutions were introduced

into the flame and the steady readings were recorded, the apparatus was washed through with water after every trial.

The concentrations of the elements were determined from the calibration curve.

This test was performed at Laboratory- Qualichem laboratories, Nagpur.

Batch no. 1

High performance thin layer chromatography (HPTLC)[149]

Test performed at- Qualichem laboratories, Nagpur

Batch no. 4

Procedure

Preparation of Test Solution

The sample was prepared by adding 100 mg of powder in 10 ml of methanol, soaked for 15 minutes. Centrifugation was done and the filtrates or supernatants were used as the *sample solution*. Out of this filtrate 50 µl of samples were dissolved in 1 ml of toluene and were used as the *sample solution*.

Preparation of the standard solutions

Wedelolactone, Ecliptaalbasaponin-I and Ecliptaalbasaponin-II were used as marker compounds; these markers were dissolved in methanol at a concentration of 1 mg/ml. The extracts of *Bhrungaraj* prepared were shaken and sonicated in methanol at a concentration of 10 mg/ml.

Sample application and plate layout

Samples were applied as narrow bands 8-9 mm above the lower edge of the plate. The sample application or developing points were marked with a pencil before the development of chromatography.

Preconditioning of the plate

After the sample application the plate kept standing in a closed chamber containing saturated solution of magnesium chloride for preconditioning before keeping in the mobile phase.

Preparation of the developing chamber and development of the plate

A twin trough chamber was filled with sufficient volume of mobile phase solvent mixture so that it attains a height of 5 mm in both troughs and also the filter paper and the chamber was left for saturation. After 20 min the plate kept in vertical position in the front trough of the chamber such that coating layer was facing the filter paper. The plate was removed from the chamber until the mobile phase travelled the distance of 6 cm from application point and dried.

Derivatization Procedure

1 -2 ml of derivatization reagent was sprayed on the plate at a defined speed and time.

Visualization

The plates were visualized and chromatograms were observed and compared with monograph and standards used. Observation and evaluation was done under UV 254 nm, UV 366 nm light wave.

System Suitability

The *System suitability* requirements in the samples were satisfied as these were similar to the marker standard solutions used.

Evaluation and Acceptance Criteria

Chromatograms of the *sample solution* and *standard solution we*re compared considering the colour, intensity, separation and Rf values.

Documentation

The results were recorded, Rf values calculated for UV and white light and were noted.

High performance liquid chromatography (HPLC) [150]

Laboratory- Qualichem laboratories, Nagpur

Batch no. 1

HPLC Procedure

Type of Column used for HPLC was Luna 5 micrometer, NH2 100A

1. Power strips of the pump, column heater and detector were turned on.
2. Detector power was also switched on 15–20 minutes before running of the samples.
3. The flow rate of the pump was set to 00.
4. The attenuator of the detector was set to 8X.
5. The solvent select knob and the waste knob located on the pump module were also set.

System set-up was initialized

1. Mobile phase reservoir checked and LOAD/INJECT was set to LOAD position; injection pin was kept in injection port and was locked.
2. Reference outlet and column outlet drains were kept in disposal container and caps removed.
3. Reference cell was flushed with 5 ml of mobile phase with the help of filling syringe for priming of the reference cell to ensure no air bubbles were present and draw off valve was tightened.
4. Thread syringe was kept to left side bypass valve of the reference inlet.
5. Pressure of the system was set to 2000 PSI.
6. The bypass valve was then flipped to the right to forward mobile phase to the reference side with the flow rate of 0.4 ml/min.
7. Pump was switched on and mobile phase was drawn into syringe for one full cycle.

8. After that the bypass valve was set to left position for running the mobile phase through the detectors along with the sample and then the syringe was kept into disposal container.
9. The mobile phase flow rate was maintained at 1.8 ml/min.

Type of Column used: Luna 5-micron NH_2 100 angstrom

Mobile Phase used: Toluene: ethyl acetate: formic acid (11:6:1:0.1v/v/v) for wedelolactone,

Flow rate: 3 ml/min

Temperature: 40° C

Injection Volume: 10 microliters

Column Preparation

1. Mobile phase was allowed to pass through column outlet at a rate of 3 ml/min and then into the disposal container.
2. Column heater was turned on and temperature was set to 40° C until the temperature was steady.

Software set-up

1. After starting the computer, the "Systems" tab was opened, HPLC box was checked.
2. After that Acquisition pull-down menu was started and baseline monitoring mode was selected.
3. Baseline was observed and was set to – 200K to 2000K.
4. Then (red X circle) option was clicked and sample was allowed to run when "Waiting for injection" was displayed on the screen.

Sample injection

1. Load / inject valve was set in the load position with flow rate of 3ml/min.

2. 10 micro litres of sample was injected with the Hamilton syringe at the injection port when “Waiting for injection” was displayed on the screen.
3. Round silver port plug was removed and bottom lever was turned tightly.
4. Upper switch was set on “Inject” position and after 30 min. upper switch was turned to “LOAD” position, so data acquisition was automatically started.
5. After that the display was changed to “Running” sample.

Chromatograph data acquisition

1. The sample ran through the column and returned to the baseline during this process the software collected the complete data.
2. When the sample returned to baseline stop button was clicked.
3. After that ‘tab ->open->chromatograph’ file was opened and results option was clicked.
4. File was saved and results were printed.
5. Chromatogram was then closed.

Phytochemical analysis

Qualitative phytochemical analysis of the extracts was carried out to know the presence or absence of chemical constituents or active principles described in *Eclipta alba* Hassk. *panchanga*.

Various tests were carried out as follows,

1) **Test for tannins**[151]

The *Bhrungaraj* extracts (*swaras and anukalpa swaras*) were dissolved in 10 ml methanol and warmed. Tests for presence or absence of tannins were carried out as follows,

a) Ferric chloride test: Few drops of ferric chloride (FeCl3) solution were added to the above filtrates and occurrence of a green coloration indicated the presence of tannins.

b) Lead acetate test: 4-5 drops of lead acetate solution were added to the above filtrate and formation of precipitate indicated presence of tannins.

2) **Test for resins**[152]

2-3 ml of the extracts was dissolved in alcohol and few drops of distilled water was added to this solution. No occurrence of turbidity suggested the absence of resins.

3) **Test for saponins**[153]

Foam test: Small amount of sodium bicarbonate and little water was added to the above extracts and shaken vigorously. Occurrence of froth was observed which suggested the presence of saponin.

4) Test for glycosides/carbohydrates[154]

Benedict's reagent: Benedict's reagent in 5 ml quantity was added few drops of aqueous extract and was boiled. Formation of violet coloured precipitate indicated the presence of glycosides.

5) Test for proteins[155]

a) Biuret test: To the above prepared extract little water and 1 ml of 4% sodium hydroxide solution was added along with a drop of 1% solution of copper sulphate solution. Presence of yellow colour was obtained which indicated that proteins were present in the above extracts.

b) Xanthoprotein test: Small amount of extracts were taken and 2 ml of water followed by 0.5 ml of concentrated nitric acid was added to that solution. Yellow precipitate was observed which indicated the presence of proteins.

6) **Test for sterol**[156]

a) Salkowinskii's Reaction: *Bhrungaraj* extracts were taken in 2 ml of chloroform and 2 ml of sulphuric acid (H2SO4) was added along the sides of the test tube. The test tube was shaken for a few minutes. There was no red colour and green precipitate which suggested the absence of sterols in the extract.

b) Libermanns–Buchard reaction: The extracts were dissolved in 2 ml chloroform and to it a few drops of acetic anhydride were added and after that concentrated sulphuric acid was added along the side of the tube. The absence of red to blue precipitate indicated the absence of sterols.

7) **Test for amino acid**[157]

Ninhydride test (General test)

The extracts were taken in test tube and to it Ninhydride solution prepared in alcohol (0.1%) were added. The absence of violet or purple coloured precipitate indicated the absence of amino acid.

8) **Test for alkaloids**[158]

Test solution was prepared as follows,

The few ml of extract was taken separately in 5 ml of 1.5 % (v/v) HCl, filtered and used as test solution.

a) Wagner's Reagent

1.27 gm of iodine and 2 gm of potassium iodide were dissolved in 5 ml of water and was diluted to 100 ml. The test solutions prepared as stated above were added to this reagent. Occurrence of brown flocculent precipitate indicated the presence of alkaloids.

b) Dragendroff's reagent

Stock reagent- Dragendroff's reagent was prepared by mixing solution A i.e. 17 gm of Bismuth subnitrate + 200 gm tartaric acid + 800 ml distilled water and solution B i.e. 160 gm of potassium iodide + 400 ml distilled water, in 1:1 (v/v) proportion.

This stock reagent was taken in 50 ml quantity and 100 ml of tartaric acid was added to it and diluted with distilled water to make the volume of 500 ml.

This prepared reagent was sprayed on a piece of filter paper and dried. After drying dil. hydrochloric acid was applied on the paper with the help of a capillary tube. It was observed that orange red colour was formed on the paper which indicated the presence of alkaloids.

9) **Test for starch non-reducing polysaccharides**[159]

Iodine test

Aqueous extract was taken and to it few drops of iodine solution was added, the colour of the mixture turned blue which suggested the presence of starch.

Observations

Observation and Results

Organoleptic characters: The sample of *Bhrungaraj* was taken and was examined for all its organoleptic characters. The details are described in Table no. 20.

Table 20: Organoleptic characters of *Bhrungaraj*

Sr no.	Characters	Bhrungaraj powder	Spray dry powder
1	Color	Dark green	Dark green
2	Odor	Mild	Mild
3.	Taste	Sour and bitter	Sour and bitter
4.	Structure	Smooth	Smooth
5.	Color of water extract	Dark green	Dark green

Macroscopic Examination

1. Root: secondary branches arising from the main root of the plant, which were up to about 7 mm in diameter, cylindrical, grayish.[111]

2. Stem: Stem herbaceous, branched, occasionally rooting at nodes, cylindrical in shape or flat, outer surface, rough due to oppressed white hairs, node distinct, greenish and brownish in colour.

3. Leaf: Leaves were opposite, sessile or sub sessile, 2.2-8.5 cm long, 1.2-2.3 cm wide, usually oblong or lanceolate in shape, apex was sub-acute or acute, strigose with appressed hair on both surfaces

4. Fruit: Fruit having achenial cypsela, one seeded, cuneate, with a narrow wing, covered with warty excrescences and brown in colour.

5. Seed: only one seed present, 0.2-0.25 cm long, 0.1 cm wide, dark brown, hairy and non-endospermic

6. Flower: flowers solitary or two, together on unequal axillary peduncles; involucre bracts were about eight, ovate in shape, obtuse or acute, herbaceous, strigose with oppressed hairs; ray flowers ligulate, ligule small, spreading, scarcely as long as bracts, not toothed, white; disc flowers tubular, corolla often four toothed; pappus absent, except occasionally very minute teeth on the top of achene; stamen are five, filaments are epipetalous, free, anthers united into a tube with base obtuse; pistil bicarpellary; ovary inferior, unilocular with one basal ovule appressed hair on both surfaces

Microscopy Examination

The microscopic examination of *Bhrungaraj* entire fresh plant[121,122] show following structures- vascular bundle containing xylem phloem vessels, fibers, etc.

Figures: 1-10

Figure 1: Entire plant of *Bhrungaraj*

Figure 2: Inflorescence

Figure 3: Entire plant

Figure 4: Roots of *Eclipta*

Figure 5: *Eclipta* Flower

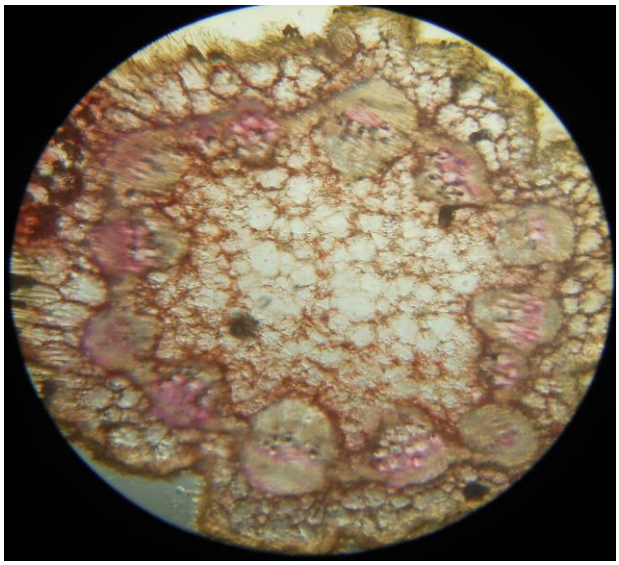

Figure 6: Root of *Eclipta* showing xylem and phloem

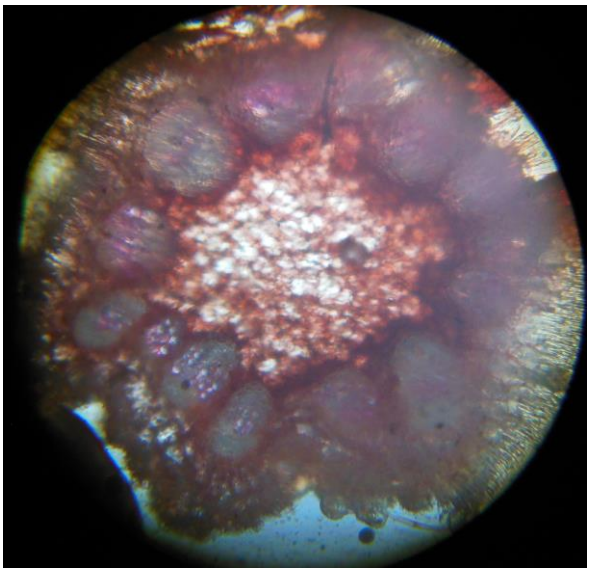

Figure 7: T. S. of stem

Figure 8: T. S. of leaf

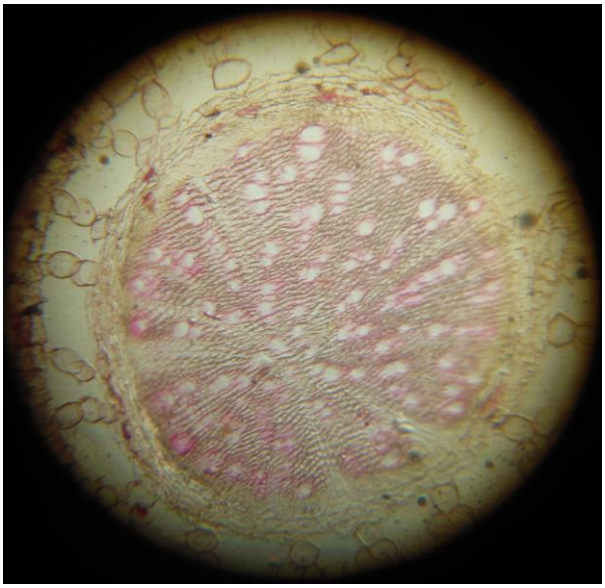

Figure 9: T. S. of leaf stem

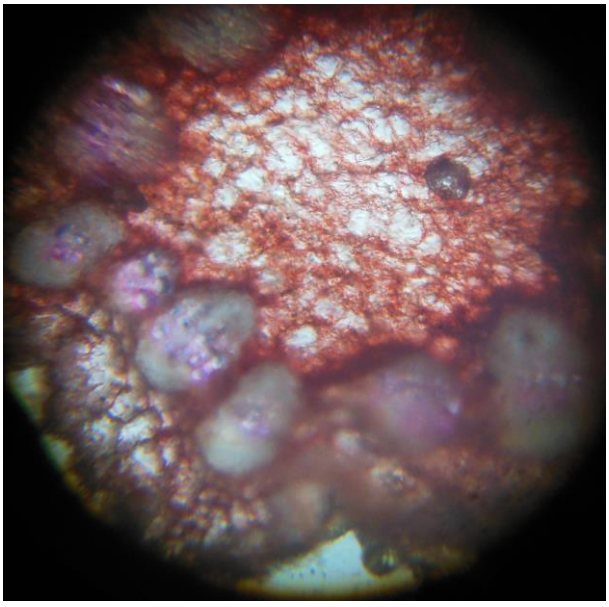

Figure 10: T. S. of stem

Physicochemical standardization

Test for determination of foreign matter

Table 21: Foreign matter

Samples	Weight of foreign matter collected	Percentage
Entire plant (dried)	54 gms	1.5 % W/W

pH values

Table 22: pH values

Samples	pH	pH after 24 hours
Bhrungaraj Churna	4.2	4.2
Bhrungaraj Swaras	4.1	4.1
Bhrungaraj Tablet	4.2	4.2
Bhrungaraj Anukalpa Swaras	4	4

Swelling index of *Bhrungaraj churna*

Table 23: Swelling index (in cms)

Observation	*Bhrungaraj Churna*	Spray Dry Drug
1.	1.1	1.1
2.	1.1	1.0
3.	1.0	1.1
Mean	1.03	1.03

Foaming index (F. I.)

Table 24: Foaming index

Sr. No.	*Swaras*	Spray Dry Drug	Tablet	*Churna*
Foaming Index	<100	<100	<100	<100

Moisture content

Table 25: Moisture content

Sr. No.	Sample	Initial Wt in gms	Wt. Afterwards (gms)	Loss (In Gm)	% M.C.
1.	***Bhrungaraj churna***	1.004	0.988	0.016	1.5936
2.	**Spray Dry Powder**	1.009	0.992	0.017	1.6848

Optical density at (670 nm) (Digital colorimetry)

Table 26: optical density

Sample	Optical density
Swaras (partial free)	0.41
Anukalpa Swaras (partial free)	0.25

Extractive values of *Bhrungaraj churna*

a) Alcohol soluble extractives (% w/w)

Table 27: Alcohol soluble extractives

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	9.6	11.9
2.	9.9	12.7
3.	9.7	12.9
Mean	9.8	12.5
SD	0.152753	0.5291

b) Water soluble extractives

Table 28: Water soluble extractive (% w/w)

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	26.00	26.4
2.	26.00	26.7
3.	26.00	26.9
Mean	26.00	26.6
SD	0.1632	0.2516

c) Methanol extractives (% w/w)

Table 29: Methanol soluble extractives

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	14.7	19.6
2.	14.9	19.4
3.	14.5	19.9
Mean	14.7	19.63
SD	0.1632	0.010

d) Petroleum ether extractives (fixed oil content)

Table 30: Petroleum ether soluble extractives (% w/w)

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	2.80	2.9
2.	2.80	2.8
3.	2.80	2.9
Mean	2.80	2.8666
SD	1.6165	0.0577

e) Benzene soluble extractives

Table 31: Benzene soluble extractives (% w/w)

Observation	*Bhrungaraj Churna*	*Bhrungaraj* spray dry drug
1.	13.1	13.6
2.	13.0	13.4
3.	13.0	13.9
Mean	13.0	13.6
SD	0.0577	0.2516

f) Chloroform soluble extractives

Table 32: Chloroform soluble extractives (% w/w)

Observation	*Bhrungaraj Churna*	*Bhrungaraj* spray dry drug
1.	10.80	11.2
2.	10.79	11.4
3.	10.80	11.6
Mean	10.79	11.4
SD	0.0057	0.2

g) Ethanol Extractive

Table 33: Ethanol soluble extractives (% w/w)

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	9.4	10.7
2.	9.7	10.4
3.	9.4	10.9
Mean	9.5	10.6
SD	0.1723	0.2516

Ash value

a) Total ash value

Table 34: Total ash value (% w/w)

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	14.2	4.2
2.	14.1	4.1
3.	14.9	4.9
Mean	14.4	4.4
SD	0.1723	0.4358

b) Acid insoluble ash

Table 35: Acid insoluble ash (% w/w)

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	2.1	1.8
2.	2.5	1.5
3.	4.6	1.9
Mean	3.06	1.7333
SD	1.3428	0.2081

c) Water soluble ash

Table 36: Water soluble ash (% w/w)

Observation	***Bhrungaraj Churna***	***Bhrungaraj* spray dry drug**
1.	12.3	3.1
2.	12.6	3.4
3.	12.3	3.1
Mean	12.4	3.2
SD	0.1732	0.1732

Specific gravity

a) Specific gravity of *swaras*

W_1 = weight of empty bottle

= 19.6558 gm

W_2 = weight of bottle with distilled water

= 72.0580 gm

W_3 = weight of swaras

= 72.9972 gm

Specific gravity of *Bhrungaraj swaras*= W_3-W_1/ W_2-W_1

= 72.9972-19.6558/72.0580 -19.6558

= 53.3414/52.4022

= 1.01792

b) Specific gravity of *anukalpa swaras*

W_1 = weight of empty bottle= 16.5853 gm

W_2 = weight of bottle with distilled water= 67.4648 gm

W_3 = weight bottle with Anukalpa swaras = 67.8334 gm

Specific gravity of Anukalpa swaras= W_3-W_1/ W_2-W_1

= 67.8334-67.4648/ 67.4648-16.5853

=51.2481/50.8795

=1.0072 gm

Relative density is also = 1.0072

Viscosity by Ostwald's viscometer

Viscosity of *anukalpa swaras*

Table 37: Viscosity of *anukalpa swaras*

Sr. no.	Viscosity	Mean value
1.	1 min 30 sec	1.41 min = 101 seconds
2.	1 min 29 sec	
3.	1 min 48 sec	
4.	1 min 57 sec	

Viscosity of *swaras*

Table 38: Viscosity of *swaras*

Sr. no.	Viscosity	Mean value
1.	1 min 20 sec	1 min 53 sec = 113 seconds
2.	1 min 12 sec	
3.	1 min 14 sec	

Dynamic viscosity

a) Dynamic viscosity of *swaras*

Dynamic viscosity = kqt

Where k= constant of instrument determined by using the reference liquid for viscometer = 0.015 mm^2/sec

q = mass/volume i.e. gm/cc obtained by multiplying the relative density of the liquid being examined by a factor 0.998203

t= time in seconds for meniscus to fall from E to F= 101 seconds

Dynamic viscosity= kqt, = 0.015x (1.01792 x 0.998203) x 101

=1.53937

Dynamic viscosity of Bhrungaraj fresh swaras=1.53937

b) Dynamic viscosity of *anukalpa swaras*

Dynamic viscosity = kqt

Where k= constant of instrument determined by using the reference liquid for viscometer = 0.015 mm^2/sec

q = mass/volume i.e. gm/cc obtained by multiplying the relative density of the liquid being examined by a factor 0.998203

t= time in seconds for meniscus to fall from E to F= 101 seconds

Dynamic viscosity= kqt,

= 0.015x (1.01792 x 0.998203) x 101

=1.4192

Dynamic viscosity of *Bhrungaraj anukalpa swaras*=1.4192

Kinematic viscosity

a) Kinematic viscosity of *swaras*

KV= dynamic viscosity/relative viscosity

= 1.53937/1.01792

= 1.51227 stokes

b) Kinematic viscosity of *anukalpa swaras*

KV= dynamic viscosity/relative viscosity

= 1.53937/1.01792

= 1.39425 stokes

Relative viscosity

a) Relative viscosity of *swaras*

RV = Time in seconds for liquid to fall from E to F/ Time in seconds for water to fall from E to F

Table 39: Relative viscosity of *swaras*

Sr.no.	Time in seconds for water	Time in seconds for *Bhrungaraj swaras*
1.	60 sec	90 sec
2.	61 sec	89 sec
3.	60 sec	108 sec
4.	60 sec	117 sec
Mean	60.25 sec	101 sec

RV = 101/60.25

= 1.67635

b) Relative viscosity of *anukalpa swaras*

RV = Time in seconds for liquid to fall from E to F/ Time in seconds for water to fall from E to F

Table 40: Relative Viscosity of *anukalpa swaras*

Sr. no.	Time in seconds for water	Time in seconds for *anukalpa swaras*
1.	60 sec	90 sec
2.	61 sec	87 sec
3.	60 sec	104 sec
4.	60 sec	103 sec
Mean	60.25 sec	96 sec

RV = 96/60.25

= 1.593361

Relative density

a) Relative density of *swaras*

Relative density = equal volume of liquid/ equal volume of water

= 53.3424/52.4015

= 1.01795

b) Relative density of *anukalpa swaras*

Relative density = equal volume of liquid/ equal volume of water

= 53.2168/52.4015

= 1.0155

Tests applied to *Bhrungaraj swaras*

Refractive index

a) Refractive index of fresh *swaras*

By Mettler Toledo–RE 40 D Refractometer: Method -2

Temperature- 30ºC Time-10 min

Table 41: Refractive index of *swaras*

Sr.no.	Refractive index	Mean value
1.	1.3441	1.3434
2.	1.3451	
3.	1.3410	

b) Refractive index of *anukalpa swaras*

Table 42: Refractive Index of *Anukalpa Swaras*

Sr. no.	Refractive Index	Mean value
1	1.3315	1.3312
2	1.3311	
3	1.3310	

Spectrophotometric analysis

Sample: Fresh juice

Reference: Alcohol

Wavelength: 200-400 nm

Table 43: Spectrophotometry analysis of fresh *swaras*

Table 44: Spectrophotometry analysis of *churna*

Table 45: Spectrophotometry analysis of spray dry *ghan vati*

Table 46: Spectrophotometry analysis of *anukalpa swaras ghan vati*

Tables 43-46 are included in annexures.

Thin layer chromatographic values of *Bhrungaraj churna*

Table 47: Petroleum ether extract

Mobile phase used- Benzene: Chloroform (1:1)

Rf values: S.F. = 15.8 cm

I/ Vis	Under UV light	In Iodine chamber
0.02	--	--

Table 48: Benzene extract

Chloroform: Ethanol ((9.5:0.5)

Rf values: S.F. = 15.8 cm

I/ Vis	Under UV light	In Iodine chamber
0.8	0.53	0.42

Table 49: Chloroform extract

Chloroform: Ethanol ((9.5:0.5)

Rf values: S.F. = 15.8 cm

I/ Vis	Under UV light	In Iodine chamber
0.8	0.53	0.4

Table 50: Ethanol extract

Chloroform: Ethanol ((8:2)

Rf values: S.F. = 15.8 cm

I/ Vis	Under UV light	In Iodine chamber
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Table 51: Water extract

Benzene: Acetic acid: water (4: 1.1:4.9)

Rf values: S.F. = 15.8 cm

I/ Vis	Under UV light	In Iodine chamber
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All visible spots were yellowish brown and spots visible in UV-blue and fluorescent color.

Atomic absorption spectrophotometry analysis

Table 52: Atomic absorption spectrophotometry

Sr.no.	Samples	Elements (concentration in ppm) Iron (Fe)
1.	Fresh juice (swaras)	108.0
2.	Dry powder (churna)	211.84
3.	Spray dry form (spray dry tablet)	370.137
4.	Tablet	3060.0

Content of wedelolactone by HPTLC

Markers used: Wedelolactone, Ecliptaalbasaponin I And Ecliptaalbasaponin II

Ecliptaalbasaponin I and Ecliptaalbasaponin II could not be detected in all the samples of fresh juice, spray dry tablet, *anukalpa swaras* and *anukalpa swaras ghan vati*.

Table 53: HPTLC analysis

Sr. no.	**Samples (formulations)**	**Content in % w/w**
1.	Fresh juice	0.005
2.	Dry powder	0.25
3.	Spray dry tablet	0.014
4.	Tablets	0.023

Content of wedelolactone by HPLC

Markers used: Wedelolactone, Ecliptaalbasaponin I and Ecliptaalbasaponin II

Ecliptaalbasaponin I and ecliptaalbasaponin II could not be detected in all the samples of fresh juice, spray dry tablet, anukalpa swaras and anukalpa swaras ghan vati.

Table 54: HPLC analysis

Sr. No	Samples (formulations)	Content In % W/W
1.	Fresh Juice	0.007
2.	Dry powder	0.25
3.	Spray dry tablet	0.014
4.	Tablet	0.017

Chemical tests

Table 55: Behaviour of powder on chemical tests

Sr.no.	Powder treated with	Observations
1	Normal light	Light greenish gray with roughness
2.	Phloroglucinol + HCL	Sclerenchyma fibers spiral and annular vessels of the veins turns to light pinkish color
3.	Iodine	Tissues turns to bluish coloration
4.	Acetic acid	Presence of prismatic as well as rosette crystals in abundance without any change
5.	Sulphuric acid (conc. H2SO4)	No effervescence but crystals dissolved
6.	Hydrochloric acid (HCL)	No effervescence but crystals dissolved

Phytochemical analysis

Table 56: Preliminary qualitative analysis of alcoholic extract of powder of *Bhringaraja* for the presence of various functional groups

Sr.	Reagent	Functional group	observation	Result
1	Alcohol	Resins	Turbidity	absent
2	Sodium bicarbonate	Saponin	Frothing	present
3	Biurets test	Proteins	Yellow ppt	present
6	Wagners reagent	Alkaloides	Brown ppt	present
7	Dragondroff's reagent	Alkaloids	Brown ppt	present
8	Salkowinskii reaction	Sterols	Green ppt	absent
9	Libermann's Buchard	Sterols	Green ppt	absent
10	Dil. $FeCl_3$ Test	Tannin	Blue ppt	present
11	Lead acetate test	Tannin	No ppt	present
12	Bendict's reagent	Glycosides	Violet color	present
13	Fehling's reagent	Glycosides	ppt formation	present
14	Neutral $FeCl_3$	phenols	Violet color	present

Qualitative color reactions of *Bhrungaraj* powder

Table 57: Colour reactions of *Bhrungaraj*

Sr.	Solvent / chemical	Observation	
		Before heating	**After heating**
1	Conc. HCl.	Green	Dark green
2	Dil. H_2SO_4 (50%)	Green	Blackish Green
3	Conc. H_2SO_4	Dark green	Chocolate
4	Lactic acid	Green	Light Brown
5	Conc. HNO_3	Burnt orange	Burnt orange
6	Acetic acid	Dark Green	Mars Green
7	Iodine soln.	Greenish transparent	Greenish brown
8	$FeCl_3$ (Aq).	Green	Emerald Green
9	KOH (5%)	Dark Green	No change
10	$FeSO_4$	Blackish brown	No change
11	Benzene	Green	Lettuce Green
12	Propanol	Brick red	No change
13	Water	Brown	Dark brown
14	Ethanol (95%)	Green	Pine green
15	Potassium iodide	Green	Dark Green

Observations and results

Observations

Effect of treatment on objective parameters

Paired t-test was applied to all the groups to assess before and after results. The test was found significant in all the groups in maximum parameters. The observations of the effect of treatments on haemoglobin (Hb%), TIBC (total iron binding capacity), serum ferritin and serum transferrin, serum iron, MCHC (mean corpuscular haemoglobin concentration) and MCV (mean corpuscular volume) were as follows,

Effect of treatment on Haemoglobin percentage

Table 58: Effect on haemoglobin

Groups	Before T/t	After T/t	Mean differences
Group 1	8.734615385	9.820769231	1.086153846
Group 2	8.754615385	9.410769	0.656154
Control	8.765384615	10.27769	1.512308

Statistical analysis

Data was presented in terms of mean, standard deviation, standard error and student t-test (paired t-test) was applied to the before and after results in each group. All tests were applied at the level of $p<0.001$ as highly significant or $p<0.005$ as significant and $p>0.005$ as insignificant.

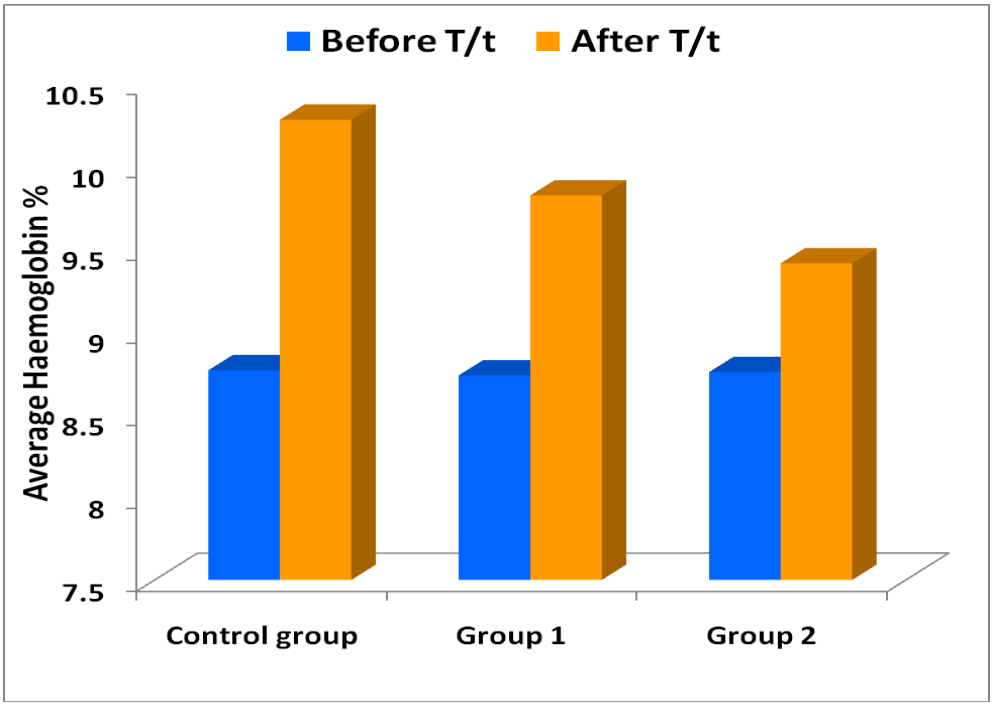
Table 59: Statistical calculation

Group	Variable	Mean Differences	SD	SE	t statistics	Degrees of freedom	p-value
Group1	Hb%	1.08615	0.1709	0.0149	t=43.59	129	<0.001
Group2	Hb%	0.6561	0.2860	0.0250	t=59.95	129	<0.001
Control	Hb%	1.5123	0.23130	0.0202	t=53.71	129	<0.001

Here, pairwise t- test results show, average haemoglobin level of control group has been significantly increased (Mean difference= 1.5123, SD= 0.231301949, t-statistics = 53.71, p-value = <0.001). The results are highly significant in control group.

For group I- pairwise t- test results, average haemoglobin level has been significantly increased (mean difference = 1.0861, SD= 0.170972, t-statistics= 43.59, p-value= <0.001). The results are highly significant.

For Group II- Pairwise t-test results show, Average haemoglobin level of Group II has been significantly increased. (Mean difference =0.6561, SD=0.286092, t-statistics=59.95, p-value= < 0.001). The results are significant.



Graph 1: showing changes in average haemoglobin %

Statistical test results show that t' statistics (t=1.5123, df = 129, p-value< 0.001), this show that there is a statistically significant increase of 1.5123 gm/dl after treatment in the control group. The mean differences are highest in control group than in group 1 and less in group 2.

Taken together, these results suggest that high levels of iron tablets really do have an effect on Hb. Specifically, the present study suggests that when there is high consumption of iron tables, there will be increase in Hb level. However, it should be noted that group 1 also appear to increase Hb level but less than control group.

Additional findings:

Effect of treatment on total iron binding capacity (TIBC)

Table 60: Effect on TIBC

Groups	Before T/t	After T/t	Mean differences
Group 1	532.7746	501.3944	-29
Group 2	522.565	503.8615	-18.7
Control	524.9769	478.7846	-46.1923

Table 61: Statistics of effect on TIBC

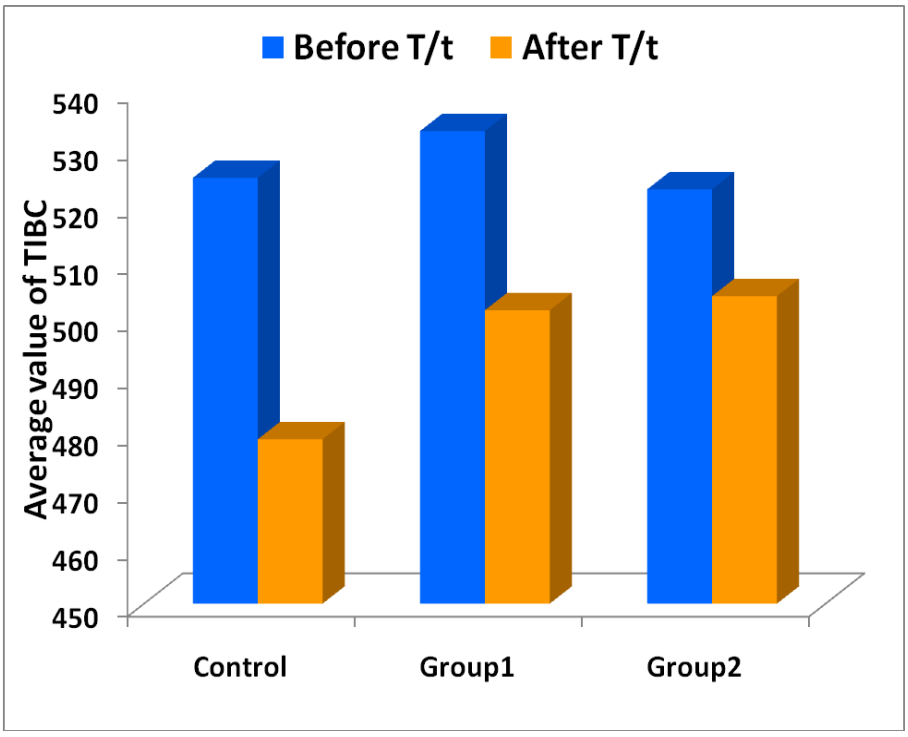
Group	Mean Differences	SD	SE	t statistics	Degrees of freedom	p-value
Group1	-29	8.7661	0.7689	t=-37.04	129	<0.001
Group2	-18.7	7.83449	0.687236	t=-26.65	129	<0.001
Control	-46.1923	8.535984	0.748771	t=-44.65	129	<0.001

Here, pair wise t- test results show, average TIBC of control groups has been significantly decreased (Mean difference= -46.1923 is negative, t-statistics = -44.65 also negative, p-value = <0.001).

Pair wise t- test results show, average TIBC of group 1 has been significantly decreased (Mean difference= -29 is negative, t-statistics = -37.04 also negative, p-value = <0.001).

Pair wise t- test results show, average TIBC of group 2 has been significantly decreased (Mean difference= -18.7 is negative, t-statistics = -26.65 also negative, p-value = <0.001).

The mean differences are highest in control group than in group 1 and less in group 2.



Graph 2: showing changes in average value of total iron binding capacity

Statistical test results show that t' statistics (t= values) are negative in all groups for (p-value< 0.001), this show that there is a statistically significant decrease after treatment in all groups, more decrease observed in control group with mean difference = -46.1923

Effect of treatment on serum ferritin

Table 62: Effect on serum ferritin

Groups	Before T/t	After T/t	Mean difference
Group 1	8.99253846	12.35046154	3.357923077
Group 2	9.003846	11.30054	2.296692
Control	9.03238462	17.4731538	8.448462

Table 63: Statistics of effect on serum ferritin

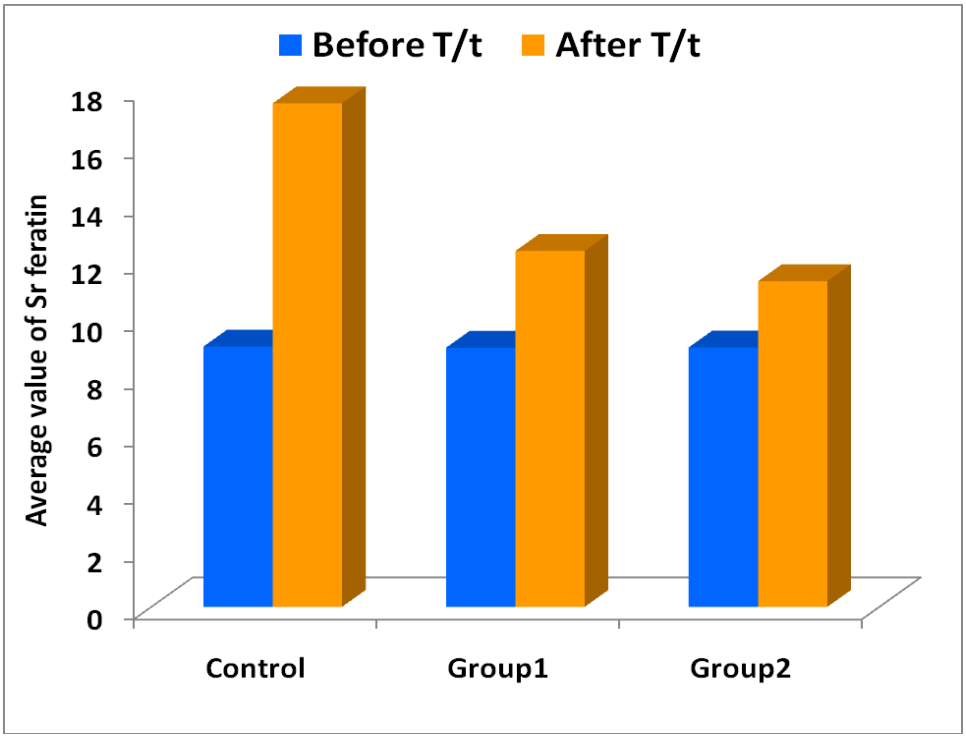
Group	Mean Differences	SD	SE	t statistics	p-value
Group 1	3.3579	0.9174	0.0804	t=42.91	<0.001
Group 2	2.2966	0.7223	0.0633	t=35.72	<0.001
Control	8.4484	0.9399	0.0824	t=107.7	<0.001

Here, Pair wise t- test results show, average serum ferritin levels of control group has been significantly increased (Mean difference= 8.4484, t-statistics = 107.7, p-value = <0.001). The results are highly significant in control group.

For group 1- Pair wise t- test results, average serum ferritin level has been significantly increased (Mean difference = 3.3579, t-statistics= 42.91, p-value= <0.001). The results are highly significant.

For group 2- Pair wise t-test results show, average serum ferratin level of group 2 has been significantly increased. (mean difference = 2.2966, t-statistics=35.72, p-value=< 0.001). The results are significant.

The mean differences are highest in control group than in group 1 and less in group 2.



Graph 3: showing changes in serum ferritin

Statistical test results show that the 't' statistics (t=107.7, df = 129, p-value< 0.001), this show that there is a statistically significant increase in serum ferritin levels of 8.4484 units after treatment in the control group. The mean differences are highest in control group than in group 1 and less in group 2.

Effect of treatment on serum transferrin

Table 64: Effect on serum transferrin

Group	Before T/t	After T/t	Mean difference
Group 1	9.3523	12.6706	3.3183
Group 2	9.3008	11.5836	2.2827
Control	9.3633	16.57	7.2066

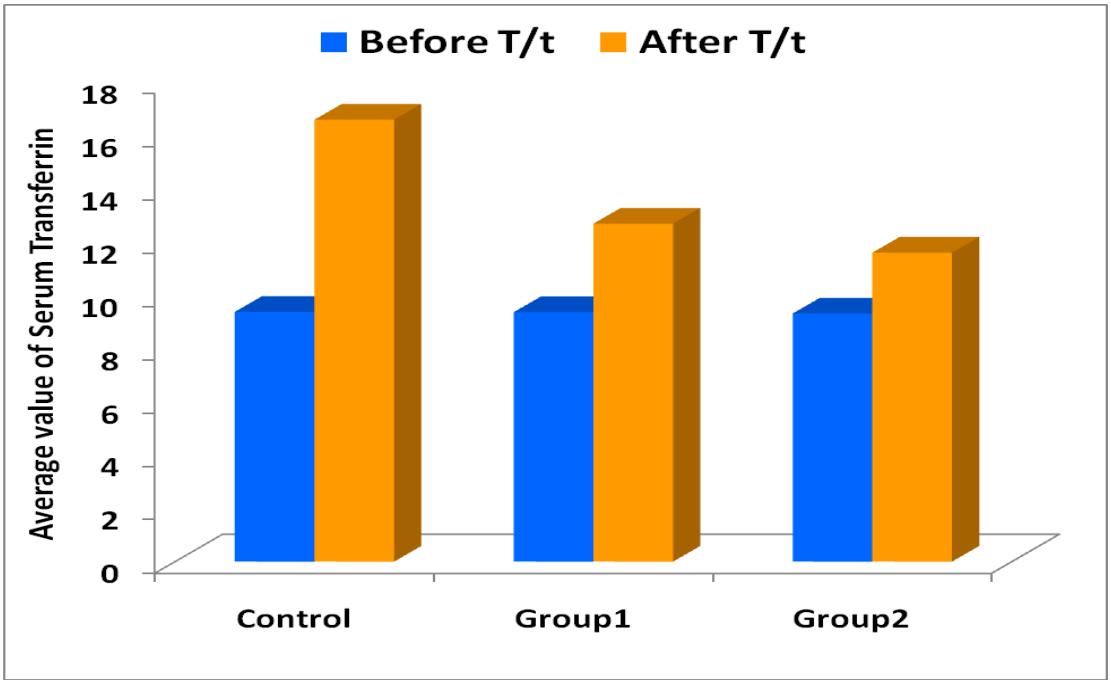
Table 65: Statistics of effect on serum transferrin

Group	Mean Differences	SD	SE	t statistics	p-value
Group 1	3.3183	0.7548	0.0662	t=50.15	<0.001
Group 2	2.2827	0.7396	0.068	t=35.9	<0.001
Control	7.2066	1.0435	0.0915	t=89.89	<0.001

Here, pair wise t- test results show, average serum transferrin levels of control group has been significantly increased (mean difference=7.206692, t-statistics = 89.89, p-value = <0.001). The results are highly significant in control group.

For group 1- Pair wise t- test results, average serum transferrin level has been significantly increased (mean difference = 3.318308, t-statistics= 50.15, p-value= <0.001). The results are highly significant.

For Group 2- Pair wise t-test results show, average serum transferrin level of group 2 has been significantly increased (mean difference =2.282769, t-statistics=35.9, p-value=< 0.001). The results are significant. Mean differences are highest in control group than in Group 1 and more less in Group 2.



Graph 4: showing changes in serum transferrin

Statistical test results show that the 't' statistics (t=89.89, df = 129, p-value< 0.001), this show that there is a statistically significant increase in serum transferrin levels of 7.206692 units after treatment in the control group.

Effect of treatment on serum iron

Table 66: Effect on serum iron

Groups	Before T/t	After T/t	Mean difference
Group 1	26.7230	46.0076	19.2846
Group 2	26.7615	35.9461	9.1846
Control	26.7384	51.5138	24.7769

Table 67: Statistics of effect on serum iron

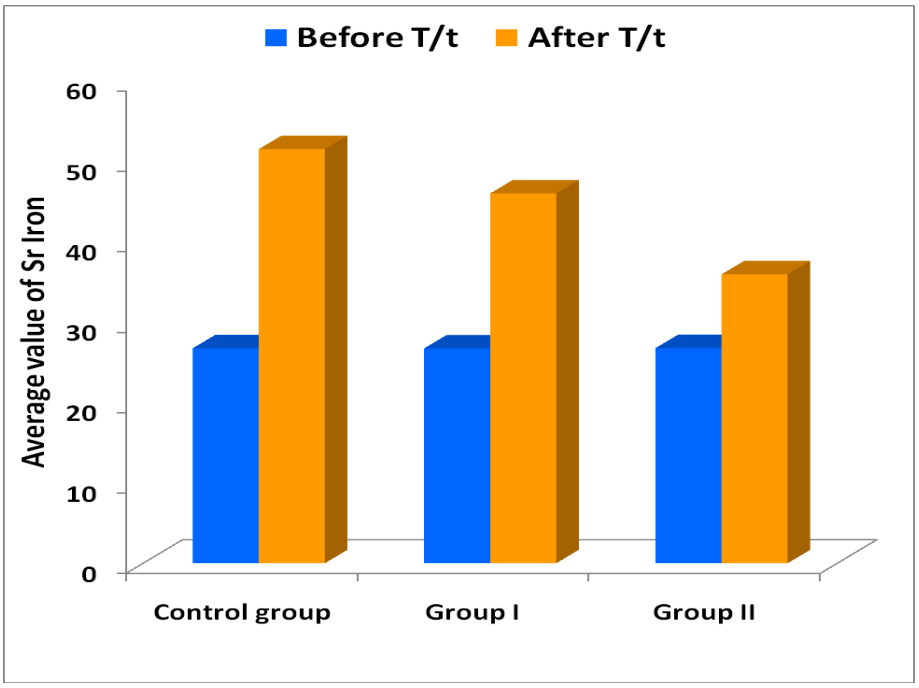
Group	Variable	Mean Differences	SD	SE	t statistics	p-value
Group1	Sr Iron	19.28462	2.874917	0.252186	74.59	<0.001
Group 2	Sr Iron	9.184615	0.1279711	0.0112255	27.61	<0.001
Control	Sr Iron	24.77692	2.354261	0.206514	119.9	<0.001

Here, Pair wise t- test results show, average serum iron levels of control group has been significantly increased (mean difference= 24.77692, t-statistics = 119.9, p-value = <0.001). The results are highly significant in control group.

For Group I- Pair wise t- test results, average serum iron level has been significantly increased (mean difference = 19.28462, t-statistics= 74.59, p-value= <0.001). The results are highly significant.

For Group II- Pair wise t-test results show, average serum iron level of Group II has been significantly increased. (mean difference =9.184615, t-statistics=27.61, p-value=< 001). The results are highly significant.

The mean differences are highest in control group than in Group 1 and more less in Group 2.



Graph 5: showing changes in serum iron

Statistical test results show that the 't' statistics (t= 119.9, df = 129, p-value < 0.001), this show that there is a statistically significant increase in serum iron levels of 24.77692 units after treatment in the control group.

Effect of treatment on mean corpuscular haemoglobin concentration (MCHC)

Table 68: Effect on MCHC

Groups	Before T/t	After T/t	Mean difference
Group 1	28.60923	30.4069	1.7976
Group 2	28.6346	29.8223	1.1876
Control	28.5984	31.1661	2.5676

Table 69: Statistics of effect on MCHC

Group	Mean Difference	SD	SE	t statistics	Degrees of freedom	p-value
Group 1	1.7976	0.6074	0.0532	32.81	129	<0.001
Group 2	1.1876	0.4425	0.0388	30.11	129	<0.001
Control	2.5676	0.6157	0.0540	42.7	129	<0.001

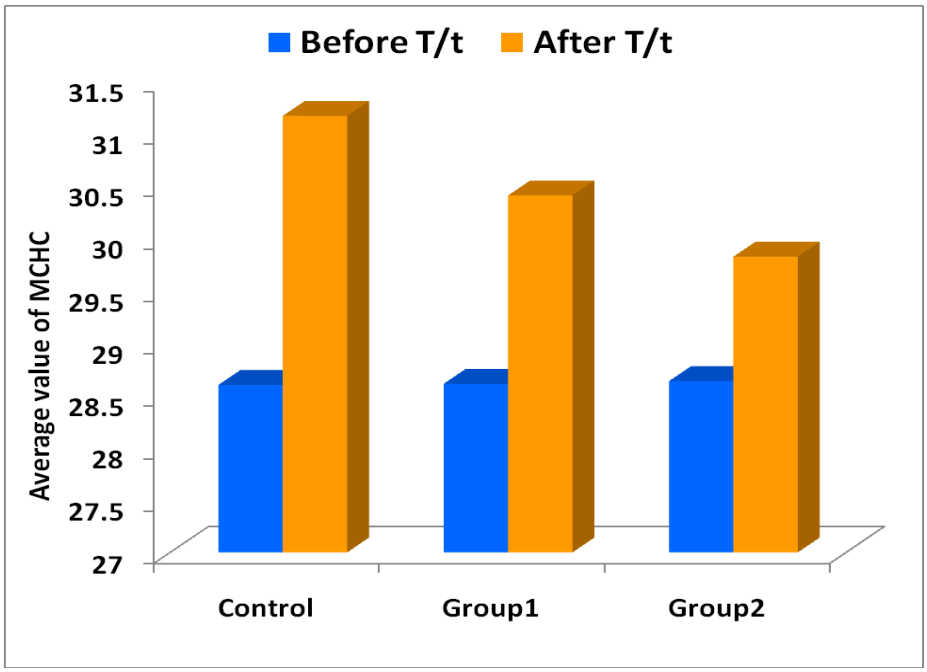
Here, pair wise t- test results show, average MCHC (mean corpuscular haemoglobin concentration) levels of control group has been significantly increased (mean difference=

2.5676, t-statistics = 42.7, p-value = <0.001). The results are highly significant in control group.

For Group I- Pair wise t- test results, average MCHC (mean corpuscular haemoglobin concentration) level has been significantly increased (mean difference = 1.797692, t-statistics= 32.81, p-value= <0.001). The results are highly significant.

For Group II- Pair wise t-test results show, average MCHC (mean corpuscular haemoglobin concentration) level of Group II has been significantly increased. (mean difference =1.187692, t-statistics=30.11, p-value=< 0.001). The results are significant.

The mean differences are highest in control group than in Group 1 and more less in Group 2.



Graph 6: showing changes in mean corpuscular haemoglobin concentration

Statistical test results show that the 't' statistics (t= 42.7, df = 129, p-value< 0.001), this show that there is a statistically significant increase in MCHC (mean corpuscular haemoglobin concentration) levels of 2.567692 units after treatment in the control group.

Table 70 and 71: Effect of treatment on MCV

Groups	Before T/t	After T/t	Mean difference
Group 1	60.4153	65.1546	4.7392
Group 2	60.4376	62.7761	2.3384
Control	60.4907	67.6930	7.2023

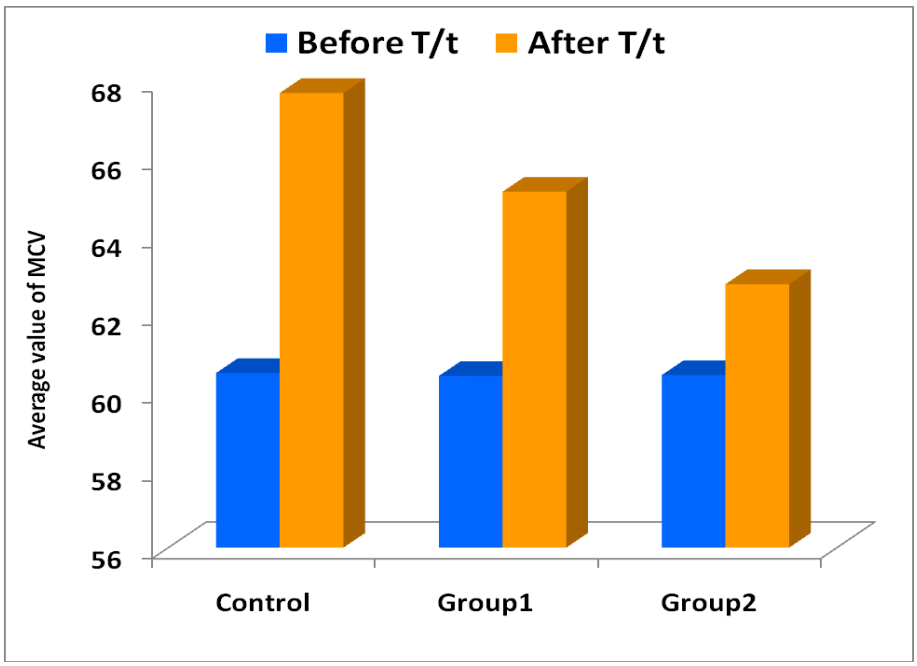
Group	Mean Differences	SD	SE	t statistics	Degrees of freedom	p-value
Group 1	4.7392	1.1723	0.1028	13.907	129	<0.001
Group 2	2.3384	0.8556	0.0750	12.403	129	<0.001
Control	7.2023	1.6228	0.1423	10.434	129	<0.001

Here, pair wise t- test results show, average MCV (mean corpuscular volume) levels of control group has been significantly increased (mean difference= 7.202308, t-statistics = 10.434, p-value = <0.001). The results are highly significant in control group.

For Group I- Pair wise t- test results, average MCV (mean corpuscular volume) level has been significantly increased (mean difference = 4.739231, t-statistics= 13.907, p-value= <0.001). The results are highly significant.

For Group II- Pair wise t-test results show, average MCV (mean corpuscular volume) level of Group II has been significantly increased. (mean difference =2.338462, t-statistics=12.403, p-value=< 0.001). The results are significant.

The mean differences are highest in control group than in Group 1 and more less in Group 2.



Graph 7: showing changes in mean corpuscular volume

Statistical test results show that the 't' statistics (t= 10.434, df = 129, p-value< 0.001), this show that there is a statistically significant increase in MCV (mean corpuscular volume) levels of 7.202308 units after treatment in the control group.

Effect of treatment on qualitative parameters

Table 72: Effect of treatment on pallor

Group	Mean score BT	Mean score AT	Mean Diff.	SD	SE	't' value	P value
Group 1	1.7692	0.9153	0.8538	0.4508	0.0395	21.593	P<0.001
Group 2	1.73846	1.2923	0.4461	0.4990	0.04377	10.194	P<0.001
Control	1.7692	0.5923	1.1769	0.4396	0.0385	30.524	P<0.001

Effects of treatments on pallor were significant in all groups. Mean difference was found more in the control group as compared to the other groups. More improvement was observed in control group than in group 1 and group 2.

Discussion

According to ancient research methodology before establishing any theory, *upanayana* (discussion) is the prior step to *nigamana* (conclusion). Discussion is a process of re-examining the knowledge to form a base for any conclusion. In spite of detailed classical study and experimentation in various ways, a theory is accepted only after the proper reasoning of observation. Hence discussion is a very crucial part of any scientific research.

According to Ayurveda, iron deficiency anaemia (IDA) can be correlated with *pandu vyadhi* because of the predominance of *pandutva* (paleness of skin) which is described in *Shabdakalpadrum* as '*peeta bhagardha ketaki dhuli sannibha*'.[160]

Iron deficiency anaemia

Iron deficiency anaemia is a condition characterized by a decrease in the concentration of blood haemoglobin due to nutritional deficiency of iron. The iron deficiency causes reduced work capacity in adults and affects the motor and mental development in children and adolescents persons[161].

There are few studies proving that iron deficiency[162] affects cognition in adolescent girls and causes fatigue in adult[163] women.

Iron deficiency anaemia is a global public health problem in developing and developed countries affecting more than one third of the world population of all age groups. The most affected among them are adolescent girls[164] due to the factors like poor nutrition, loss of blood during menstruation, etc. Adolescence is the determining period, crucial phase of growth in the life cycle of an individual, during which various important physical, psychological, and behavioural changes take place. Though the exact prevalence has not been determined, at least 65-75% adolescent girls in India are anaemic. [8,165]

Due to rapid growth, development of body built, health, mental status there is an increase in iron requirement in both adolescent boys and girls. Negligence during this developing period causes nutritional anaemia, which has been constantly ignored by public health programs.[166] Iron deficiency in adolescents is a complex disorder[167] and its effects on their physical endurance as well as cognitive performance which is serious fact of concern. Anaemia in adolescent age group is growing continuously due to lack of nutrition. Adolescents comprises about 22 % of indian population, a youth of over 225 million. Anaemia in adolescent girls has further implications during pregnancy period causing anaemia. Considering the severity of this disease, iron deficiency anaemia in adolescent age group was studied clinically in this thesis.

Selection of drug and formulations

Physicochemical and phytochemical study

Sharangadharacharya had described various concepts for the formulation of the medicines which are used as guidelines during the formulation of medication. In the drug formulation, *shushka* form[168] (dry but freshly collected and dried) should be taken in single quantity and while using the fresh drug or during the unavailability of the fresh drug, the green fresh plant drug should be taken twice in quantity to that of dry drug[11] as the dry drug is *guru* and *tikshna* due to the lack of moisture and increased concentration of chemical constituents[169] as described in *Sharangadhar Samhita-*

शुष्कम् नविन यत द्रव्यम योज्यम सकलर्मसु
आर्द्रम च द्विगुणम युन्ज्यादेष सर्वत्र निश्चय ॥१॥ शा. स. प्र. ख.१/४८

स्वरसस्य गुरुतवत्च पलमर्ध प्रयोजयेत्।
निशोशितम च अग्नि सिद्धम पलमर्ध रसम पिबेत॥ शा. स. प्र.ख.१/५

This concept is applied to all the medicinal plants except[12] few enlisted drugs as described below,

वासानिम्ब पटोलकेतकी बलाकुश्माण्ड केन्दिवरी
वर्शाभु कुटजश्चाश्वगन्धा सहिता पुतिगन्धा च अम्रुता।
नित्यशो ग्रह्यास्त्वक्शण्मेव न द्विगुणिता ये चेक्शु जाता घना॥ भे.र. २/२२–२३

गुडुचि कुटजो वासा कुष्माण्डश्च शतावरी।
अश्वगन्धा सहचरा शतपुष्पा प्रसारणी।
प्रयोक्तव्या सदैवार्द्रा द्विगुणा नैव कारयेत्॥ शा. स. प्र. ख.१/४५–४७

Bhavaprakash samhita[16] had described that *Bhrungaraj* is *tikta, katu* in *rasa, deepana, pachana, anulomana* and especially effective in *yakrut vikruti, yakrut vruddhi, pandu, kamala, shotha* and other diseases related to *yakruti*.

Dhanvantari nighantu,[17] *Raj nighantu,*[18] *Kaiyyadev nighantu*[19] has also described that Bhrungaraj is *tikta, katu* in *rasa, usna virya* and indicated in *pandu, shotha, kamala, khalitya, palitya* and other diseases.

Tikta rasa and *ruksha guna* of *Bhrungaraj* causes decline in the vitiated *pitta* and there by causes correction of the *pitta sthana vikruti* i.e. *yakrut* which is the cause of conditions like *pandu, kamala, shotha,* etc. So *Bhrungaraj* is selected for the study in iron deficiency anaemia which can be correlated with *pandu*.

Various dosage forms of *Bhrungaraj panchanga i.e. ardra* and *shushka* dosage forms were prepared. These were analysed by various physicochemical and phytochemical tests to know their constitution in co-relation with their clinical efficacy.

Ardra forms of *Bhrungaraj* were prepared as, a) fresh juice and b) spray dried powder of fresh juice.

Shushka forms of *Bhrungaraj* were prepared as, a) *anukalpa swaras* from *churna* of *shushka* drug and b) *ghan vati* of *anukalpa swaras*.

Physicochemical and phytochemical tests

Following physicochemical and phytochemical tests were applied to evaluate the difference in physicochemical and phytochemical properties of *ardra* and *shushka* dosage forms of *Bhrungaraj panchanga i.e. swaras* (*ardra*/wet form), *anukalpa swaras* (*ardra*/wet form), *shushka churna* (dry form) and tablet forms (*shushka*/dry form) and results were obtained as below.

1. **Test for determination of foreign matter**[136]

As the plant drug was self-collected the percentage of foreign matter (Table no. 21) in *Bhrungaraj churna* was 1.5 w/w that was not more than 2 percent, complying with Ayurvedic Pharmacopoeia of India (API) standards.

2. **Test for determination of pH value**[137]

The pH of all the samples (Table no. 22) was in the range of 4-4.5.

3. **Test for determination of swelling index**[138]

Swelling index of *Bhrungaraj churna* and spray dry drug was not significant as the drug was not having any mucilage content (Table no. 23).

4. **Test for determination of foaming index**[139]

Foam index, suggestive of saponin content in plant material was >100 in all samples and the saponin test (foam test) was found positive for all samples suggesting that *Bhrungaraj churna* was having some saponin content (Table no. 24).

5. **Test for determination of moisture content**[140]

Percentage of moisture content (Table no. 25) for *Bhrungaraj churna* was 1.5936 and that of spray dry drug was 1.6848. Both drugs were having moisture approximately similar but not more than 2 percent, which complied the API standards for *churna* of any drug.

6. Colorimetry (test for determination of optical density)[141]

Optical density was measured with the help of colorimeter,[21] which determined the concentration of a compound by measuring the colour intensity of solution in the form of light, absorbing power of the solution at a specific wavelength of the substance (Table no. 26).

The optical density of the fresh swaras was more (0.41) than the *anukalpa swaras* (0.25) indicating that the fresh juice was more concentrated than the *anukalpa swaras*.

7. Test for determination of extractive value[142]

Extractive values of *Bhrungaraj churna* and *Bhrungaraj* spray dry powder were evaluated for water, alcohol, methanol, chloroform, benzene and ethanol.

- The Alcohol soluble extractive value for *Bhrungaraj churna,* 9.8 %, was not less than 5 percent which complied the API standards and for the spray dry powder it was 12.5 (Table no. 27).
- The aqueous extractive value for *Bhrungaraj churna* was 26.00 % which was not less than 15 percent described in API, so it complied the API standards and for the spray dry powder it was 26.6 % (Table no. 28).
- Methanol soluble extractive value for *Bhrungaraj churna* was 14.7 % which was less than the spray dry powder, 19.63% (Table no. 29).
- Petroleum ether soluble extractive value (fixed oil content) for *Bhrungaraj churna* was 2.80 % which was more than the spray dry powder, 2.86 % (Table no. 30).
- Benzene soluble extractive value for *Bhrungaraj churna,* 13.0 %, was less than the spray dry powder, 13.6 % (Table no. 31).
- Chloroform soluble extractive value for *Bhrungaraj churna,* 10.80 %, was less than the spray dry powder, 11.4% (Table no. 32).
- The ethanol soluble extractive value for *Bhrungaraj churna* was 9.4 % that was more than the spray dry powder, 10.6 % (Table no. 33).

All values were taken as average mean of three readings.

From above readings, it was clear that solubility of *Bhrungaraj churna* and spray dry form was more in water, than in methanol and than in alcohol than other solvents used.

8. Test for determination of ash value[143]

Total ash value of *Bhrungaraj churna* was 14.4 and total ash value of *Bhrungaraj* spray dry powder was 4.4 which was not more than 22 percent, thus complied the API standards (Table no. 34).

Acid insoluble ash of *Bhrungaraj churna* was 3.6 which was not more than 11 percent and thus complied the API standards and acid insoluble ash value of *Bhrungaraj* spray dry powder was 1.7 (Table no. 35).

Water soluble ash of *Bhrungaraj churna* was 12.4 and total ash value of *Bhrungaraj* spray dry powder was 3.2 (Table no. 36).

It was observed that ash values of *Bhrungaraj churna* was more than the ash values of *Bhrungaraj* spray dry powder. All values of *churna* complied the API standards.

9. Test for determination of specific gravity[144]

Specific gravity of *swaras* was found more, 1.01, than the *anukalpa swaras,* 1.007.

10. Test for determination of viscosity[146]

Viscosity of liquids determine the consistency of that liquid, more viscous liquid is less absorbable or slowly absorbed while less viscosity helps easy transportation in body, easy solubility and faster absorption (Table no. 37 and 38).

The viscosity of fresh juice was more than the *anukalpa swaras*.

11. Test for determination of refractive index

Refractive index is suggestive of uniformity of solvent (oils, liquids, etc), thickness or thinness of the solvent and any mixture in the solvent changes the refractive index (Table no. 41 and 42). The *swaras* was freshly prepared, filtered and refractive index value was

calculated. Refractive index of fresh *swaras* was 1.3434 and that of *anukalpa swaras* was 1.33. Refractive index for both solvents was nearly same which showed their similar texture or thinness and homogeneity.

12. Spectrophotometry[146]

The spectrophotometric analysis was carried out for four samples, a) fresh *swaras*, b) *churna*, c) spray dried tablet and d) *anukalpa swaras ghan vati*.

Wavelength used was 200-400 nm and solvent used was alcohol. The spectra were taken in UV-Vis double beam spectrophotometer. The spectrophotometry analysis showed common peaks at 220 nm, 330 nm in all samples at absorbance of 1.750 and 1.000 respectively (Table no. 43 - 46).

13. Thin layer chromatography[147]

Thin layer chromatography analysis[24] was carried out using various mobile phases, the spots were observed in UV chamber and Rf (factor of retention) values were calculated.

a. Petroleum ether extract of *Bhrungaraj churna* and spray dry powder using mobile phase, benzene: chloroform (1:1) showed similar spots of Rf value, 0.02 in visible light (200-400 nm) (Table no. 47).

b. Benzene extract of *Bhrungaraj churna* and spray dry powder using mobile phase, chloroform: ethanol ((9.5:0.5) showed spots of Rf value, 0.8 (visible light), 0.53 (UV) and 0.42 (iodine chamber) (Table no. 48)**.**

c. Chloroform extract of *Bhrungaraj churna* and spray dry powder using mobile phase chloroform: ethanol (9.5:0.5) showed spots of Rf value, 0.8 (visible light), 0.53 (UV) and 0.4 (iodine chamber) (Table no. 49).

d. Ethanol extract of *Bhrungaraj churna* and spray dry powder using mobile phase, chloroform: ethanol (8:2) showed no spots in UV, visible light or iodine chamber (Table no. 50).

e. Water extract of *Bhrungaraj churna* and spray dry powder using mobile phase using benzene: acetic acid: water (4:1.1:4.9) showed no spots in UV, visible light or iodine chamber (Table no. 51).

Separation of constituents of *Eclipta alba* Hassk. was found maximum in chloroform and benzene extract using the mobile phase of chloroform: ethanol (9.5:0.5).

14. Atomic absorption spectrophotometry[148]

The atomic absorption spectrophotometry analysis was performed to know the iron content in various formulations of *Bhrungaraj* (Table no. 52). The observations showed following results, fresh *swaras*- 108.0 ppm elemental iron, *churna*- 211.84 ppm elemental iron, spray dried tablet- 370.137 ppm elemental iron, *anukalpa swaras ghan vati*- 3060 ppm elemental iron.

The iron content was 9-10 times more in *anukalpa swaras ghan vati* as compared to spray dry drug, *churna* and fresh *swaras of Bhrungaraj*. In the process of spray drying the fresh juice was exposed to high temperature and high pressure while passing through the chambers, exposure to heat might have affected the iron content in spray dry drug.

The fresh juice is dilute due to presence of water content while *Bhrungaraj anukalpa swaras ghan vati* was prepared by processing of adding water to *churna* and preparation of kwatha and this kwatha is then boiled until the preparation of paste like thick slurry form which was then passed to tablet making machine for tablet preparation. So, evaporation of moisture, might have increased the iron content in *anukalpa ghan vati* as compared to *churna*.

15. High performance thin layer chromatography[149]

High performance thin layer chromatography (HPTLC) was carried out taking all four formulations (Table no. 53) against above mentioned three markers. Observations were as follows, content of wedelolactone was only identified and the other markers used i.e. ecliptaalbasaponin I and ecliptaalbasaponin II were not detected in the HPTLC analysis. Percentage of wedelolactone was also maximum in *churna* and minimum in the fresh *swaras*.

16. High performance liquid chromatography[150]

High performance liquid chromatography (HPLC) was carried out for all four samples against three markers (Table no. 54). Wedelolactone content was nearly similar to that of the results obtained by HPTLC. The Ecliptaalbasaponin I and Ecliptaalbasaponin II could not be obtained in all four samples or formulations.

Wedelolactone is the important active coumestan derivative of *Eclipta alba*. Percentage of Wedelolactone was found more in the *Bhrungaraj churna* (0.25 % w/w), *anukalpa swaras ghan vati* (0.017%w/w) and comparatively less in spray dry powder *ghan vati* (0.014%w/w) and fresh juice (0.007%w/w).

The concentration of this constituent might be more in the dried state (*churna*) than in fresh juice and in spray dry powder *ghan vati* form. As per the reference of *Sharangdhar Samhita*[169] and *Bheshajyaratnavali*[170] dry drug is *guru in guna and tikshna* due to the lack of moisture and its *tikshna guna* may be due to the increased concentration of its active constituents in dry state.

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The stability of wedelolactone might be more in dried form than in the fresh or wet form, so this may be the reason for more percentage of wedelolactone in *churna* than in fresh juice. Moreover, the spray dry drug was prepared by passing the fresh juice through hot air under

pressure. While churna is prepared by shade drying of raw material and then grinding. The processing in hot air may have altered the wedelolactone content of the drug in spray dried form.

Clinical trial

Prevalence of anaemia is high in adolescent age group and it is more in adolescent females than in males due to i) less education, ii) unawareness about receiving extra iron containing diet, iii) work load or exertion, iv) nutritional deficiencies, v) menstrual blood loss. [5,171,172] The adolescent age group is the growing age group, both mental and physical growth are progressive[173] during this age. Onset of menstruation leads to blood loss this is also one of the reason for weakness and anaemia, especially iron deficiency anaemia.[174] So proper nutrition, balanced diet, iron rich diet is the requirement in this age group. For obtaining the large sample size of 130 patients in each group i.e. total 390 patients, local colleges were visited and information about the study was given. The maximum patients were obtained from these visited colleges and therefore patients were between age group 16 - 19 years.

Various iron regimens are routinely prescribed for iron deficiency anaemia which are associated with various complications like nausea, gastric discomfort, constipation, etc. Ayurvedic medication can be a better and safe alternative for the treatment. In this clinical study *Bhrungaraj panchanga* is used in *ardra* and *shushka* forms in the form of spray dry powder tablet and *anukalpa swaras ghan vati* in two groups against control of iron tablets.

Effect of Treatment on objective parameters

Effect of treatment on Haemoglobin %

(Table no. 58 and 59) (Graph no. 1)

The Group 1 (which was given *anukalpa swaras ghan vati*) pair wise t-test results showed significant increase in average haemoglobin level with mean difference of 1.0861, t-statistics =43.59, p-value <0.001.

Group 2 (which was given spray dried powder *ghan vati*) showed that average haemoglobin level had been significantly increased. (mean difference = 0.6561, t-statistics =59.95, p-value <0.001)

In the (control group) Group 3, pair wise t-test results showed that average haemoglobin level had been highly significantly increased. (mean difference = 1.5123, t-statistics = 53.71, p-value <0.001)

Taken together, these results suggest that high levels of iron consumption really do have an effect on haemoglobin percentage. However, it should be noted that increase in Hb level was also observed in group 1 but less than the control group.

Effect of treatment on Total iron binding capacity

(Table no. 60 and 61) (Graph no. 2)

In Group 1, pair wise t-test results showed that average TIBC level had been significantly decreased (mean difference= -29, t-statistics= 37.04, p-value <0.001).

Group 2 also showed that average TIBC level had been significantly decreased. (mean difference = -18.7, t-statistics= 26.65, p-value <0.001)

In the (control group) Group 3, pair wise t-test results showed that there was highly significant decreased in average TIBC level (mean difference= -46.1923, t-statistics=44.65, p-value <0.001).

In the present study it was observed that consumption of iron tables caused significant decrease in TIBC level. Decreased TIBC level was also observed in group 1 but less than control group. Thus, iron tablets were more effective in decreasing the total iron binding capacity as compared to other groups.

Effect of treatment on serum ferritin

(Table no. 62 and 63) (Graph no. 3)

In Group 1, pair wise t-test results showed that average serum ferritin level had been significantly increased with mean difference of 3.3579, t-statistics=42.91, p-value <0.001. In Group 2, average serum ferritin level had been significantly increased with mean difference = 2.2966, t-statistics=35.72, p-value <0.001. In the control group, pair wise t-test suggested that there was highly significant increase in average serum ferritin level with mean difference of 8.4484, t-statistics= 107.7, p-value <0.001.

From above results, the iron tablets had proven highly effective in increasing the serum ferritin levels, serum ferritin level was also increased in group 1 but less than control group.

Effect of treatment on serum transferrin

(Table no. 64 and 65) (Graph no. 4)

In Group 1, pair wise t-test results showed mean difference of 3.3183, t-statistics= 50.15 with p-value <0.001 which suggested significant increase in average serum transferrin level. In group 2, average serum transferrin level had been significantly increased with mean difference of 2.2827, t-statistics= 35.9, p-value <0.001. In control group, pair wise t-test results showed average serum transferrin level had been highly significantly increased with mean difference of 7.2066, t-statistics=89.89, p-value <0.001.

Treatment with iron tablets had highly significant effect in increasing the serum transferrin level, serum transferrin was also increased in group 1 but less as compared to the iron tablets and even lees in group II than in group I.

Effect of treatment on serum iron

(Table no. 66 and 67) (Graph no. 5)

The group 1, pair wise t-test results showed that average serum iron level had been significantly increased (mean difference=19.2846, t-statistics=74.59, p-value <0.001). Group 2 results showed average serum iron level had been significantly increased with mean difference of 9.1846, t-statistics=27.61, p-value <0.001. In the control group, pair wise t-test

results showed average serum iron level had been highly significantly increased with mean difference of 24.7769, t-statistics=119.9, p-value <0.001.

The control group showed highly significant increase in the serum iron levels, group1 also showed significant improvement in serum iron levels that was more than group 2.

Effect of treatment on mean corpuscular haemoglobin concentration

(Table no. 68 and 69) (Graph no. 6)

The Group 1, pair wise t-test results showed average MCHC level had been significantly increased with mean difference of 1.7976, t-statistics=32.81, p-value <0.001. In Group 2, average MCHC level had been significantly increased with mean difference of 1.1876, t-statistics= 30.11, p-value <0.001. In the control group pair wise t-test results showed highly significant increase in average MCHC level with mean difference of 2.5676, t-statistics = 42.7, p-value <0.001.

The MCHC levels were increased in control group with highly significant improvement and also significant in the group 1 and group 2, but mean difference was more in control group.

Effect of treatment on mean corpuscular volume

(Table no. 70 and 71) (Graph no. 7)

In Group 1, pair wise t-test results showed that average MCV level had been significantly increased with mean difference of 4.7392, t-statistics=13.907, p-value <0.001. Group 2 showed average MCV level was significantly increased with mean difference of 2.3384, t-12.403, statistics, p-value<0.001. In the control group, pair wise t-test results showed, average MCV level had been highly significantly increased with mean difference of 7.2023, t-statistics=10.434, p-value <0.001. There is significant increase in mean corpuscular volume in control group and also in group 1.

The overall effect of iron tablets proved highly significant improvement in all the haematological parameters in iron deficiency anaemia.

The Group 1 with *anukalpa swaras ghan vati*, also proved to be an effective treatment in iron deficiency anaemia with significant corrections in all haematological parameters, but less than the iron tablets.

The Group 2 with spray dry tablets also showed improvement in all haematological parameters, but as compared to the other groups the mean differences of all parameters was minimal.

Effect on qualitative parameters

Effect on Pallor

(Table no. 72)

The paired-t test was applied to gradations of pallor for comparing the observations before treatment and after treatment. It was observed that, in control group, t- value was 30.524 in control group, with mean difference of 1.1769. For group 1, t value was 21.59 with mean difference of 0.8538. For group 2, t-value was 10.19 with mean difference of 0.4461 and $p<0.001$. Mean difference was more in the control group as compared to other groups so the results were highly significant in control group for $P<0.001$, the effect of the treatments is statistically significant on pallor. So, pallor had association with the treatments in all groups. This suggests that there was overall reduction of pallor which was found more in control group than other groups.

Comparative effect of therapies

On comparing the subjective and objective characters of group 1, group 2 and control group, the mean differences of all the parameters were observed to be more significant in control group than the other two groups. But group 1 with *anukalpa swaras ghan vati* was also having statistically significant results. This proved that Group 1 treatment i.e. *anukalpa swaras ghan vati* also appeared to have significant results in iron deficiency anaemia but less

than the control group. Group 2 with spray dry powder tablet also showed statistically significant results but was minimum as compared to other groups.

Overall effect of the therapy

Group 1, few of the patients showed complete remission and improvement in subjective and objective parameters using *anukalpa swaras ghan vati*. Group 2, none of the patients showed complete remission but improvement in subjective and objective parameters. Control group, some of the patients showed complete remission and marked improvement in subjective and objective parameters.

Drug efficacy

The study proved significant hematinic potential of iron tablets in control group by showing statistically significant increase of 1.5123 gm/dl (mean value) in haemoglobin percent level, decrease in TIBC by 46 μg/dl, increase in serum iron levels by 24.77 μg/dl, increase in serum transferrin levels by 7.20 %, increase in serum ferritin levels by 8.44 μg/ml, increase in MCV levels by 7.20 fl, increase in MCHC levels by 2.56 gm/dl.

The study also proved the hematinic potential of *anukalpa swaras ghan vati* by showing statistically significant increase of 1.0861 gm/dl (mean value) in haemoglobin % level, decrease in TIBC by 29 μg/dl, increase in serum iron levels by 19.2846 μg/dl, increase in serum transferrin levels by 3.3183 %, increase in serum ferritin levels by 3.357 μg/mL, increase in MCV levels by 4.73 fl, increase in MCHC levels by 1.79 gm/dl in the patients of group 1 who were treated with *anukalpa swaras ghan vati* for the duration of 6 weeks.

The study again revealed that s*pray dry powder ghan vati* showed statistically significant increase of 0.6561 gm/dl (mean value) in haemoglobin % level, decrease in TIBC by 18.7 μg/dl, increase in serum iron levels by 9.18 μg/dl, increase in serum transferrin level by

2.2827 %, increase in serum ferritin levels by 2.29 µg/ml, increase in MCV levels by 2.33 fl, increase in MCHC levels by 1.18 gm/dl.

Conventional treatment of oral iron in control group proved highly significant improvements in all haematological profile of all the patients with some side effects in few patients like nausea, constipation, and vomiting, etc. The results were also found significant in group 1 with *anukalpa swaras ghan vati* in all the above haematological parameters. The results were also found significant in group 2 treated with *spray dry powder ghan vati* in the above all parameters. But mean differences of all the subjective and objective parameters after treatment were found more in iron tablets group (control), mean differences were comparatively less in *anukalpa swaras ghan vati* (group 1) than control and even least in the spray dry powder *ghan vati* (group 2).

For the formulation of medicine, *Sharangdhar Acharya* had suggested that *ardra* drug (wet drug) should be taken twice in quantity to that of *shushka* form (dry drug), to obtain the expected clinical efficacy. In the present study, the clinical efficacy of spray dry powder *ghan vati* (*ardra* form, which was given in double dose) and *anukalpa swaras ghan vati* (*shushka* form, which was given in single dose) should be equivalent according to the above concept. However, the obtained results showed that the clinical efficacy of *Bhrungaraj anukalpa swaras ghan vati* (*shushka* form) was more than the *Bhrungaraj swaras* spray dry powder *ghan vati* (*ardra* form) in treatment of iron deficiency anaemia.

The trail drugs both *Bhrungaraj anukalpa swaras ghan vati* and *Bhrungaraj swaras spray dry powder ghan vati* were subjected to analytical tests for atomic iron concentration carried out in atomic absorption spectrophotometer. It was revealed that iron concentration was more in percentage (3060 ppm) in the *anukalpa swaras ghan vati* (group 1), nearly more than 9-10 times, as compared to spray dry powder tablet ghan vati (370 ppm). So, more iron content in the *anukalpa swaras ghan vati* might be the main factor which had increased haemoglobin

levels in Group 1. Absorption of iron in this form could be more due to increased bioavailability resulting to improvement in hematinic values. Less elemental iron content in the spray dry drug may have caused less improvement in the Group 2. During the trial no side effects were observed in both Group I and Group II but in control group few side effects like nausea, gastric discomfort, constipation, in few subjects. This also proved that the drugs *Bhrungaraj anukalpa swaras ghan vati* and spray dry tablet are safe to use in iron deficiency anaemia.

Effect of dosage quantity of the drugs

Group 2 was given spray dry powder tablet in double dose as *ardra* dosage form according to the concept of *Acharya Sharangdhar*[4] and Group 1 was given anukalpa swaras ghan vati as *Shushka* dosage form in single dose.

Sharangdhar acharya had described to use *ardra* drug (fresh drug) twice in quantity to that of *shushka* drug (dried drug) always in any formulation except few enlisted drugs like *guduchi, kutaja, vasa, shatavari*, etc. Also, *anukalpa* s*waras* prepared from fresh plant is *guru* and *tikshna* in *guna,*[168] due to lack of moisture and increased concentration of the constituents.

However, results obtained in this trial showed that the *anukalpa swaras ghan vati* (*shushka* form) in single dose showed significant results as compared to the *ardra* form (spray dry powder *ghan vati*) in double dose. The possible reasons might be, during the spray drying procedure, fresh juice was exposed to heat and so the active constituents may have evaporated during the heating process. In *Bhavprakash Nighantu, Acharya* KC Chunekar[175] had described to use fresh swaras of *Bhrungaraj* and no heating to be done as the active chemical constituents may get evaporated due to exposure to heat.

Probable mode of action of the drug

Bhrungaraj is an important drug used in *yakrut vikara, pleeha vikara, agnimandya, kamala, pandu* like diseases. *Bhrungaraj* is *ruksha, laghu gunatmak, katu tikta rasatmak, katu vipaki* and *ushna viryatmak,* thus it is *kapha* and *vata shamak.* Due to *katu tikta rasa* and *ushna virya*, *Bhrungaraj* causes *agnideepan, amapachan* which increases absorption and digestion of food materials. It is thus *katu paushtika dravya* and thereby increases the appetite. Increased appetite and *amapachan* causes improved formation of all the *dhatus* and thus in turn, causes *rasa and rakta dhatu vardhan* which are the *utpatti sthanas* and *ashraya sthanas* of *pandu*. The *sapta dhatu vruddhi* takes place by *dhatu- updhatu poshana nyaya*. Thus, it acts as a *rasayana* (rejuvenating agent) and *balya* and proves to be a potent *rasayan dravya* enhancing the essence of all the *dhatus*. *Bhrungaraj* is *katu tikta* and *ushna* hence it acts as a *rakta prasadhan* and *rakta vardhak dravya.* It mainly acts on *yakruta* and acts as *pittasaraka* and as it is *yakrut uttejaka,* it excretes excessive *pitta* and causes *ama pachana*. It is *pitta rechaka* and thus reduces the excessive *pitta dosha* and helps to reduce *rasagata pitta* and helps to improve rakta dhatu pushti and helps to treat iron deficiency anaemia.

Present modality of drug

Literature review on *Eclipta alba* Hassk. suggest that it is highly effective in hepatic enlargement and hepatic disorders and had been proved to be potent hepatoprotective and anti-inflammatory and antihepatotoxic in various experimental studies. In an experimental study, alcoholic extract of whole plant *Eclipta alba* Hassk. showed a potent protective effect on experimental liver damage in rats and mice.[176] In another experimental study of hepatoprotective effect of the ethanol/water (1:1) extract of whole plant of *Eclipta alba* Hassk., conducted at subcellular levels in rats against CCl4-induced hepatotoxicity, *Eclipta alba* significantly counteracted CCl4-induced inhibition of the hepatic microsomal drug metabolizing enzymes. It also restored the loss of hepatic lysosomal acid phosphatase and alkaline phosphatase by CCl4. Thus, study elaborated that hepatoprotective activity of

Eclipta alba is mainly based on regulation of the levels of hepatic microsomal drug metabolizing enzymes.[177]

Antioxidant action

In an animal study, the alcoholic extract reduced serum hydroxyl radical (nmol/mg protein per minute) and serum lipid peroxide (nmol/mg protein) levels to some level as compared to untreated group. The 100 mg/kg dose of the extract of *Eclipta* significantly reduced carbonyl content of oxidatively modified proteins. The reduction in serum hydroxyl radical and serum lipid peroxide content proved its antioxidant activity.[178] Antioxidant activity of *Eclipta prostrata* was also determined by parameters such as ferric reducing antioxidant power (FRAP) radical scavenging activity, reducing activity, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay at various concentrations against α-tocopherol as reference drug[179]. The antioxidant activity of the hexane, ethyl acetate, ethanol and water extracts of *E. prostrata* were also determined by ferric thiocynate (FTC) method used to determine the amount of peroxide which decreased the concentration of peroxide thus proved the increased antioxidant activity.

In a study, wedelolactone proved to be a potent and selective 5-lipoxygenase inhibitor with an IC50 of 2.5 μM doses by its oxygen radical scavenging mechanism.[110] Coumestan constituents of the plant, wedelolactone and dimethyl wedelolactone were responsible for the potent antihepatotoxic activities in carbon tetrachloride, galactosamine and phalloidin induced liver damage in rats.[180] *Bhringaraj ghan vati* on the patients of *kostha-shakhasrita kamala* with special reference to hepatocellular jaundice.[181]

Probable mode of action of *Bhrungaraj* according to modern science

Wedelolactone is the major active coumestan derivative of *Eclipta alba* Hassk. which is responsible for its hepato-protective functions. Wagner et al[180] had confirmed in a study that the coumestan constituents of the plant wedelolactone and desmethylwedelolactone are responsible for the potent antihepatotoxic activities. The wedelolactone also acts as a potent and selective 5-lipoxygenase inhibitor which causes oxygen scavenging mechanism. Thus, free radical scavenging mechanism increases by wedelolactone thereby increases the antioxidant activity and proves as a rejuvenator drug.[111] The percentage of wedelolactone was found (0.007 % w/w) in fresh juice and (0.014%w/w) in spray dry powder *ghan vati* prepared from fresh juice. It was obtained in (0.25%w/w) in dry *Bhrungaraj churna* and (0.017%w/w) in *anukalpa swaras ghan vati* by HPLC as well as HPTLC analysis. Thus, the content of wedelolactone was found more in *anukalpa swaras ghan vati* as compared to spray dry tablet. So, the improved results in Group 1 with *anukalpa swaras ghan vati* might be due to the hepatoprotective action of wedelolactone. This may also be the factor for improvement in the blood formation process. Iron absorption and storage occurs in liver as this is the site of most of the storage of iron in human body. The principle function of liver is regulation of iron homeostasis by several regulatory mechanisms including iron regulatory genes, storage capacity and iron metabolism.[33] The process of haematopoiesis takes place in the liver and spleen. The liver also secretes moderate amounts of apotransferrin into the bile which binds with free iron and also with certain iron compounds, such as haemoglobin and myoglobin from the most important sources of iron in the diet forming the *transferrin* in blood. [43] Dietary iron is absorbed in the form of ferrous state and presence of phosphates, and phytates, absence of bile salts secreted by the liver and pancreas, all these factors influences the iron absorption.[34] So, liver play a vital role in the transferrin saturation also. Thus, the hepatoprotective effect of *Eclipta alba* Hassk. *panchanga* may be helpful for restoration as well as improvement of hepatic functions related to iron absorption and erythropoiesis

thereby acts as a hematinic agent improving and enhancing the blood formation process in the body. Moreover, the content of iron was found (108.0 ppm) in fresh juice and (370.137ppm) in spray dry powdered *ghan vati* prepared from fresh juice. Iron content was found to be (211.84ppm) in *dry churna* of *Bhrungaraj* and (3060.0 ppm) in *anukalpa swaras ghan vati* prepared from *churna* of *Bhrungaraj*. The content of iron was more than 10 times in *anukalpa swaras ghan vati* as compared to the spray dry *ghan vati*. The increased iron content in *anukalpa swaras ghan vati* may have been responsible for improved hematinic effect, improved blood formation in iron deficiency anaemia in group 1. Thus, the cumulative effects of all these factors and *agni deepana* and *yakrut uttejak* properties lead to correction of metabolism in iron deficiency anaemia, increase in bioavailability of iron and iron absorption, hepatoprotective action, and improved blood formation by interfering in the transferrin formation and regulation of iron metabolism, all these actions of *Bhrungaraj* are responsible for improvement in the subjective and objective haematological parameters in iron deficiency anaemia.

Summary

Present study, “phytochemical evaluation of *ardra* and *shushka* dosage forms and randomized controlled open labeled clinical trial of *Eclipta alba* Hassk. (*Bhrungaraj*) *panchanga* in iron deficiency anemia,” aims to undertake the conceptual study to evaluate the difference in phytochemical and physicochemical properties of *Bhrungaraj ardra* and *shushka* dosage forms according to the concept described in *Sharangdhar Samhita* to take *ardra* drug twice in quantity to that of *shushka* drug. The *ardra* and *shushka* dosage forms were prepared; spray dry powder tablet as *ardra* form and *anukalpa swaras ghan vati* as *shushka* dosage form.

The study has been presented in following manner,

1. Introduction
2. Review of literature
    - Disease review
    - Drug review
    - Historical review
3. Material and methods
4. Observations and result
5. Discussion and conclusion

The study was mainly classified in two parts,

A. Physicochemical and phytochemical study

Both the formulations or dosage forms were evaluated for their physicochemical and phytochemical studies using different tests like extractive values, ash values, specific gravity, HPLC, HPTLC (using marker like Wedelolactone, Ecliptaalbasaponin I and II) and atomic

absorption spectrum for the elemental iron content. Comparative analysis was carried out to study the difference or similarities if any.

The physicochemical studies revealed that there was little quantitative difference in HPLC and HPTLC analysis, but atomic absorption spectrophotometry analysis revealed that elemental iron content was found more in the *anukalpa swaras tablet* form used for group 1 than in the spray dry tablet used in group 2.

B. Clinical trial

Two dosage forms i.e. *ardra* and *shushka* formulations were also studied to evaluate their therapeutic efficacy in iron deficiency anemia.

Clinical study was conducted in three groups with 130 diagnosed female patients in each group suffering from iron deficiency anemia. Patients were selected following a screening of signs and symptoms, laboratory investigations. Patient's information was recorded in a specially designed case record form developed for this purpose.

The selected individuals were thoroughly examined for their complaints, signs and symptoms. Laboratory investigations were done and patients falling within the range of Hb % 7-11 gm/dl and only female patients were selected for the study due the increased prevalence in this age group. The selected patients were enrolled for study. After randomization of the sample, patient was allotted randomly to any of the three groups those were as follows,

Group 1- which was treated with *anukalpa swaras ghan vati* in single dose

Group 2- which was treated with spray dry powder tablet *ghan vati* in double dose, and

Group 3 (control group)- which was given conventional oral iron treatment

These individuals were evaluated by using parameters like- pallor of conjunctiva, nail bed, tongue, palms and objective parameter such as Hb %, total iron binding capacity, serum

transferrin, serum ferritin, serum iron, MCHC, MCV and PBS. All parameters were assessed before and after the treatment of 45 days.

The observations made on the 130 patients in each group in the present clinical study. Statistically highly significant increase in their Hb %, total iron binding capacity, serum iron, serum ferritin, serum transferrin, MCHC and MCV were observed in the control group that was given oral iron treatment.

The group 1- which was treated with *anukalpa swaras ghan vati* in single dose, showed statistically significant increase in the parameters like Hb %, total iron binding capacity, serum iron, serum ferritin, serum transferrin, MCHC and MCV and also in subjective parameters but were less in mean differences and standard deviation as compared to the control group.

While group 2- which was treated with spray dry powder tablet *ghan vati* in double dose, was also having statistically significant increase in the parameters like Hb %, total iron binding capacity, serum iron, serum ferritin, serum transferrin, MCHC and MCV levels but were less in mean differences and standard deviation as compared to the control group and even less than the group 1 which was given *anukalpa swaras ghan vati*.

The both dosage forms should have equivalent clinical efficacy but the study revealed that the *shushka* form i.e. *anukalpa swaras ghan vati* form in single dose was having comparatively significant effect in the trial than spray dried form i.e the *ardra* form in double dose.

Thus, the drug *Bhrungaraj* is a potent drug and can be used safely in iron deficiency anemia without any side effects.

Conclusion

The following conclusions could be drawn from the present study.

1. *Shushka* form of *Bhrungaraj i.e. anukalpa swaras ghan vati* in single dose had more potent clinical efficacy as a hematinic drug in iron deficiency anemia as compared to spray dried *swaras* tablet i.e. the *ardra* form in double dose.
2. The action of *Bhrungaraj* could be because of the higher iron content which was detected by atomic absorption spectrophotometry in *anukalpa vati* than in spray dry drug. Also, the spray drying process could have altered the composition of the fresh *swaras* due to exposure to heat.

   Therefore, activity of *Bhrungaraj swaras* has to be further explored.

Limitations/recommendations

1. As *swaras* was difficult to prepare for large number of patients daily, spray dried *swaras* was used as an alternative to it.
2. But in the process of spray drying, fresh juice is exposed to high temperature/heat under high pressure to evaporate the moisture and to convert fresh juice in to powder form.
3. The evaporation and heating process could have caused evaporation of active chemical constituents which may have hampered the expected clinical results in the present study.
4. HPTLC analysis was carried out by dissolving formulations like spray dry tablet, *anukalpa swaras* tablet, *churna* and *swaras*, processed filtered and made into different solvent in different solvents like alcohol, methanol, etc. So, concentration of the chemical constituents may differ due to different solubility in these formulations leading to differences in results in physicochemical studies and clinical trials.
5. The *Bhrungaraj swaras* and spray dry *swaras* were exposed to heating in spray drying process. This process of exposure to heat could have caused variation in results.

Further Scope of the study

1. Large population sample has to be taken to evaluate the clinical results.
2. The study has to be conducted for longer span of time to assess the long-term effect of different dosage forms.
3. Three to four times quantity of swaras dosage i.e. spray dry tablet has to be tried which may obtain equivalent results in clinical trials for *ardra* (twice in quantity) and *shushka* dosage (once in quantity) forms as observed by *Sharangadharacharya*.
4. The present study had shown remarkable results; however, it was carried out for a short period. Therefore, it is recommended to carry out the study in large number of patients for longer duration.

REFERENCES

1. Mosihuzzaman M, Choudhary M I. Standardization of herbal medicine, IUPCA. 2008. Pure and Applied Chemistry; 80. 2195-30
2. Hill AF. Economic Botany, A textbook of useful plants and plantproducts. 2nd Edi. New York: McGraw-Hill Book Company Inc; 1952
3. Bucar et al. 2013; Jones and Kinghorn, Discovery and resupply of pharmacologically active plant-derived natural products: A review, Biotechnol 2015
4. Parashar R. *Sharangdhar Samhita,* Purva Khanda, 1/47. 3rd Edi. Nagpur:1984: Baidynath publication; p.16
5. Toteja GS, Singh P, Dhillon BS et al. Prevalence of anemia amongst pregnant women and adolescent girls in 16 districts of India. Food Nutr Bull. 2006; 27:311-316
6. Sohab S et al. Professional Journal of Medicine. Joint statement of WHO& United Nations Children's fund, focusing in IDA. 2004.Dec 2010, 17 (4), 686-690
7. Anemia, Iron deficiency anemia. Who publications. Worldwide prevalence of Anemia 1993-2005
8. Core program clusters family and community WHO on Iron Deficiency Anemia, 12 x 12 initiative program
9. Sharma et al. Indian Journal of clinical biochemistry, 22 (2). Scientific evaluation of some ayurvedic preparations for correction of Iron Deficiency Anemia, 2007. p. 123-128
10. Hillmans RS. The Pharmacological Basis of Therapeutics "Hematopoietic Agents: Growth Factors Minerals and Vitamins" Goodman and Gillman's. 10th edition. New York: Hardman, JG and Limbard, LE (eds) McGraw –Hill; 2003. p.1487-17

11. Chatterjea MN, Shinde R, Textbook of Medical Biochemistry. Jaypee Brothers, New Delhi, 5th ed. 2002; p. 187-9

12. Sharma D et al. Indian Journal of physiology & pharmacology, vol.39, no.4, Correction of anemia& iron deficiency in vegetarians by administration of Ascorbic acid. 1995. p. 403-06.

13. Lund EK, Wharf SG, Fairweather- Tait SJ, Johnson IT. Oral ferrous sulphate supplements increase the free radical generating capacity of feces from healthy volunteers. Am J Clin Nutr. 1999 Feb; 69(2); 250-5

14. Mehra PN, Handa SS. Pharmacognosy of *Bhrungaraj* antihepatotoxic drug of Indian origin. Indian J Pharm. Volume 30, 1968, p 284

15. Neeraja P V, Margaret E. IJCPR, *Eclipta alba* (L.) Hassk: A Valuable Medicinal herb. November 2011-January 2012. 2(4). p. 188-197

16. Chunekar K C, Pandey G S. *Bhavprakash Nighantu*, *Guduchyadi Varga*, Shlokaa no. 240-41. Varanasi: Chaukhamba Vishva bharati Publication; 2002. p. 429

17. Sharma P V, Sharma G. Dhanvantari nighantu, *Karveeradi varga*, 4/ 10-11. 4th Edi. Ssss Varanasi: Chaukhamba orientalia publication; 2005. p .122-23

18. Tripathi I. *Raj Nighantu*, *Shatadvhadi varga*. 2nd Edi. Varanasi: Chaukhamda Krushnadasa academy; 2006. p. 89

19. Sharma P V, Sharma G. *Kaiyadeva nighantu*, *Oshadhi varga*, 1/ 1573-75. 1st Edi. Varanasi: Chaukhamba orientalia publication; 1979. p. 637

20. Williams WJ. Beutler E., Erslev A J and Lichtman M A. Haematology.3rd Edition. New York: Mc Graw Hill; 1983

21. Starcobinki J. Phsychological medicine. Chlorosis- the green sickness, 11.3.1981. p .459-68
22. ShashtriA. Sushrut Samhita, Ayurvedtatva Sandipika, Uttaratantra, Panduroga pratishedha Adhaya, 44/1-5 Varanasi: Chaukhamba Sankrit Santhan; 2006. p. 283
23. Pandit K, Chaturvedi Gorakhnath.Charak Samhita Uttaradha, Pandu Chikitsa, 16/2-3.Varanasi: Chaukhamba Bharti Academy; 1998. p. 486
24. Stephen, Chitura, Kothares. Charaka Samihita. Shri Gulabkuverda Publications; 1949
25. Wong, Chimin K & WO. History of Chinese Medicine. Tintsin Press Ltd Publications; 1932
26. Haden RL, MD, Jama. Historical aspects of Iron therapy in Anemia,111(12);1938. p. 1059-1061
27. Yogender P, Thakur B Singh, Rangaswamy R, Sreekantha. Int J Pharm Bio Sci, The Study Of Folic Acid And Vitamin B12 Levels In Anemia In Pregnancy, 5 (2);2014 April. (B) 41–47
28. Helen King. The diseases of virgins. London: Routledge Publication;2005. p. 1-17
29. *www.nhlbi.nih.gov*. "What Is Anemia? NHLBI, NIH". Retrieved 2016-01-31. *Anemia.* ISBN 978. *Stedman's medical dictionary (28th ed.). Philadelphia: Lippincott Williams & Wilkins; 2006*
30. The World Health Report: reducing risks, promoting healthy life, Geneva, Switzerland: World Health Organization; 2002
31. Haslett C, Chilvers E, Hunter J, Boon N. Davidson’s Principles & practice of Medicine, Diseases of Blood, Cha.11. Churchill Livingstone Publication; p .749
32. Kumar V, Abbas AK, Fausto N. Robbins & Contran Pathologic Basis of Disease, 7th Edition: Saunders; 2004

33. Erik R A, Yatrik MS, Iron homeostasis in liver, Comprehensive Physiology, .2013;3(1):315-330)
34. Donovan A, Lima SA, Pinkus JL, Pinkus Gs, Zon Li, Robine S, Andrews NS. The Iron exporter ferroportion/slc40a1 is essential for iron homeostasis. Sell Metab. 2005;1(3):191-200. (Pubmed)
35. Kerlin P, Reiner R, Davies M, Sage RE, Grantb AK, Iron deficiency anemia-a prospective study, Aust NZJ Med;9:402-7, 19791979
36. Lambert LA. Molecular evolution of transferin family and associated receptor. Biochimica et biophysica acta. 2012;1820(3):244-255 (Pubmed)
37. Lee GR, Foerster J, Lukens J, Paraskevas F, Greer J P, Rodgers G M. Wintrobe's Clinical Hematology, 10th edition. Williams & Wilkins; 1999
38. Rao BS, Narasinga. Prevention and Control OfAnemia In India Theory And Practice, NUTRITION FOUNDATION OF INDIA; April 1991.WHO Technical Report Series, No.40
39. *Nutritional anemias. Report of a WHO scientific group.* Geneva: World Health Organization;1968
40. Wessling- Resnick M, Iron In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Zlegler RG, eds. Modern Nutrition in Health and Disease. Baltimore, MD, Lippincott, 11th ed. Williams & Wilkins; 2014. p.176-88
41. Reyes López A, Gómez Camacho F, Gálvez Calderón C, Miño Fugarolas G., Rev Esp Enferm Dig, 1999 May, 91(5), 345-58). Iron-deficiency anemia due to chronic gastrointestinal bleeding. (https://www.ncbi.nlm.nih.gov/pubmed/10362876)

42. Fernandez O A, Baudet J S, Sanchez del Rio A. Clinical Gastroentrology and hepatology, Volume 4, Issue 7. Iron-Deficiency Anemia Caused by Hookworm Infestation. July 2006. p. 32
43. Elnicki DM, Shockcor WT, Brick JE, Beynon D. Am J Med. Evaluating the complaint of fatigue in primary care: diagnoses and outcomes.1992-93. p. 303-6
44. Sheth TN, Choudhry NK, Bowes M, Detsky AS. J Gen Intern Med, 12. The relation of conjunctival pallor to the presence of anemia. 1997. p. 102-6
45. Cook JD, Zanella A, Gridelli L, Berzuini A, Colottie MT, Mozzi F, Milani S, et al. Best Pract Res Clin Haematol. Diagnosis and management of iron-deficiency anemia. 2005. p. 319-32
46. Witte DA. Lab Med.Laboratory tests to confirm or exclude iron deficiency. 1985. p. 671
47. Sensitivity and predictive value of serum ferritin and free erythrocyte protoporphyrin for iron deficiency. J Lab Clin Med. 1989. p .73-8
48. Nutritional Anemia: Report of a WHOScientific Group. Tech Rep Ser. 405.World Health Organization;1968. P .1-40
49. Kariyeva G K, Magtymova A, Sharman A. Anemia http://dhsprogram.com/pubs/pdf/fr130/12chapter12.pdf
50. Kalantri A, Karambelkar M, Joshi R, Kalantri S, Jajoo U. German M Accuracy and Reliability of Pallor for Detecting Anemia: A Hospital-Based Diagnostic Accuracy Study, , Editor PLoS One;2010.5(1)
51. Sainani GS. API Text book of medicine. 6thedi.Mumbai: Association of physicians of India; 1999
52. Haslett C, Chilvers E, Hunter J, Boon N. Davidson's Principles& Practice of Medicine, chapter 11, Diseases of blood. 18thedi. Churchill Livingstone publication;1999. p. 738-60

53. Jimenez K, Kulnigg S Dabsch, Christoph G. Management of iron deficiency anemia.J Gastroenterology & hepatology 2015. http//www.ncbi.nlm.nih.gov
54. Sharma PV. Dravyaguna Vijnana. Cahukhambha Bharati Academy Varanasi; 2006. p .200
55. Agnivesha. Revised By Charak And Dridabala, Shastri K, Goraknath Hindi Commentary. 18/117. Varanasi: Chaukhambha bharati academy; Reprint 2009. p. 468
56. Agnivesha. Revised By Charak And Dridabala, Shastri K, Goraknath Hindi Commentary. Charaka Samhita- Vidyotinitika, Chikitsa Sthana,4/68. Varanasi: Chaukhambha bharati academy; Reprint 2009. p .151
57. Agnivesha. Revised By Charak And Dridabala, Shastri K, Goraknath Hindi Commentary. Charaka Samhita- Vidyotinitika, Chikitsa Sthana, 26/264-275.Varanasi: Chaukhambha bharati academy; Reprint 2009. p.762
58. Agnivesha. Revised By Charak And Dridabala, Shastri K, Goraknath Hindi Commentary. - Charaka Samhita- Vidyotinitika, Vimana Sthana Chap, 7 / 21. Varanasi: Chaukhambha bharati academy; Reprint 2009. p. 616
59. Agnivesha. Revised By Charak And Dridabala, Shastri K, Goraknath Hindi Commentary. Charaka Samhita- Vidyotinitika, Kalpa Sthana Chap:1/28. Varanasi: Chaukhambha bharati academy; Reprint 2009.p. 812
60. Shastri A. Sushrut Samhita, Chikitsa Sthana, 25/ 28. 6thEd. Varanasi: Chaukhambha Sanskrit Bhavan; 2003
61. Shastri A. Sushrutaacharya ‘‘Sushrutasamhita’’, Kalpa Sthana, 8/54. 6th Ed. Varanasi: Chaukhambha Sanskrit Bhavan; 2003
62. Shastri A. Sushruta acharya ‘‘Sushruta samhita’’, Uttara Sthana, 39/227.6th Ed. Varanasi: Chaukhambha Sanskrit Bhavan; 2003. p. 199

63. Shastri A. Sushruta acharya ‘‘Sushruta samhita,’’ Uttara Sthana- 51/31, 6th Edi. Varanasi: Chaukhambha Sanskrit Bhavan; 2003. p. 379
64. Atridevgupta Kaviraj. Vagbhataacharya, Ashtanga Hrudayam Vidhyodini Tika. Uttarasthana 24/37.Varanasi: Chaukhamba Samskruta Samsthana; 2005. p .537
65. Atridevgupta Kaviraj. Vagbhata acharya Ashtanga Hrudayam Vidhyodini Tika, Uttarasthana, 24/42. Varanasi: Chaukhamba Samskruta Samsthana; 2005. p. 538
66. Atridevgupta Kaviraj. Vagbhata acharya Ashtanga Hrudayam Vidhyodini Tika, Uttara Sthana Chap:24 Shloka 42, 39/162.Varanasi: Chaukhamba Samskruta Samsthana; 2005. p. 538
67. Mishrashastri B. Bhavprakash samhita, part II, shudraroga adhikara, 61/5. 9thedi. 2005.p.584
68. Mishrashastri B. Bhavprakash samhita, part II, shudraroga adhikara, 61/4. 9thedi. 2005.p.584
69. Mishrashastri B, Bhavprakash samhita, part II, shudraroga adhikara, 61/18. 9thedi. 2005. p. 585
70. Mishrashastri B, Bhavprakash samhita, part II, upadanhadhikara, 51/22. 9thedi, 2005. p. 510
71. Mishrashastri B, Bhavprakash samhita, part II, Visarpadhikara, 55/36, 9thedi. 2005.p. 552
72. Mishrashastri B, Bhavprakash samhita, part II, balrogadhikar, mukhamandika chikitsa,71/105. 9thedi. 2005.p. 803
73. Mishrashastri B.Bhavprakash samhita, part II, Rasayanadhikar, 73/10. 9thedi. 2005.p. 822
74. Mishrashastri B, Bhavprakash samhita, part II, shirorogadhikara, 62/ 42-48.9thedi. 2005.p.612
75. MishrashastriB. Bhavprakash samhita, part II, Shirorogadhikara, 62/36-38. 9thedi. 2005.p. 612
76. Mishrashastri B. Bhavprakash samhita, part II, shirorogadhikara, 62/18. 9thedi. 2005.p. 612

77. Tripathi B. Sharangdhara Samhita, Madhyam khanda, Sneha kalpana, Ghrut taila nirman vidhi, 9/65-69. Varanasi: Chaukahmba Sanskrut pratishthan; 2004. p. 331
78. Tripathi B. SharangdharaSamhita,Madhyam khanda, Sneha kalpana, Ghruttailanirmanvidhi, 9/152. Varanasi: Chaukahmba Sanskrut pratishthan; 2004. p .345
79. Tripathi B, Sharangadhara Samhita, Madhyam khanda, Sneha kalpana, Ghrut taila nirman vidhi, 9/159-160. Varanasi: Chaukahmba Sanskrut pratishthan; 2004. p. 347
80. Tripathi B, Sharangadhara Samhita, Madhyam khanda, Sneha kalpana, Ghrut taila nirman vidhi, 9/155-158. Varanasi: Chaukahmba Sanskrut pratishthan, Varanasi; 2004.p. 346
81. Tripathi B, Sharangadhara Samhita, Madhyamkhanda, kwatha kalpana, 2/140-145.Varanasi: Chaukahmba Sanskrut pratishthan;2004. p. 218
82. Tripathi B. Sharangadhara Samhita, Madhyam khanda, lepa kalpana, 11/27. Varanasi: Chaukahmba Sanskrut pratishthan; 2004.p.542
83. Tripathi B, Sharangadhara Samhita, uttarkhanda, dhatu shodhanamaran Kalpana vidhi, 11/ 94-95. Varanasi: Chaukahmba Sanskrut pratishthan; 2004.p.396
84. Shastri A. Bheshajyaratnavali, Kshudra rogadhikara, 60/ 69.11thedi. Varanasi: Chaukhamba Sanskrrit Sansthan; 1993.p. 664
85. Shastri B, Bheshajyaratnavali, Rasayanadhikara, 73/27-29. 11th edition Varanasi: Chaukhamba Sanskrrit Sansthan; 1993.p.767
86. Shastri A. Bheshajyaratnavali, Kshudrarogadhikara, 60/ 70. 11thedi. Varanasi: Chaukhamba Sanskrrit Sansthan; 1993.p.664
87. Shastri A. Bheshajyaratnavali, Kshudrarogadhikara, 60/ 71-73. 11thedi. Varanasi: Chaukhamba Sanskrrit Sansthan; 1993.p. 665

88. Shastri A, Bheshajyaratnavali, Kshudrarogadhikara, 60/ 79-80. 11thedi. Varanasi: Chaukhamba Sanskrrit Sansthan; 1993.p. 665
89. Shastri A, Bheshajyaratnavali, Kshudra rogadhikara, 60/ 82.11thedi. Varanasi: Chaukhamba Sanskrrit Sansthan; 1993.p.665
90. TripathiI, Chakradutta, netraroga Chikitsa, 59/161-163. Varanasi: Chaukhamba Sanskrrit Sansthan; 2015.p.364
91. Tripathi I, Chakradutta,Shiroroga Chikitsa, 60/31-33. Varanasi Chaukhamba Sanskrrit Sansthan; 2015.p .373
92. Tripathi I, Chakradutta, Kshudraroga chikitsa, 55/112. Varanasi: Chaukhamba Sanskrrit Sansthan; 2015. p. 321
93. Mishra S, Rasaratna Sammuchhaya, Jwara chikitsa adhaya, 12/81-82. 1stedi. Varanasi: Chaukhamba Orientalia publication; 2011.p.301
94. Mishra S, Rasaratna Sammuchhaya, Kushtha roga chikitsa, 20/195-196. 1stedi. Varanasi: Chaukhamba Orientalia publication; 2011.p.480
95. Mishra S, Rasaratna Sammuchhaya, Vata vyadhi chikitsa adhaya, 21/117-118. 1st Edi. Varanasi: Chaukhamba Orientalia publication; 2011.p. 492
96. Sharma PV, Sharma GN, Dhanvantari nighantu, Karveeradi varga, 4/ 10-11. 4th Edi. Varanasi: Chaukhamba orientalia publication; 2005.p. 122-23
97. Sharma PV, Shodhal nighantu, Karaveeradivarga,4/491. 1st Edi. Baroda: Oriental Institute; 1978.p.52
98. Upadhyaya PR, Madanpal nighantu, Haritakyadi varga, 1/ 37-38. Varanasi: Chaukhamba Sanskrit Sansthan;, 1990. p .43

99. Sharma PV, Sharma G. Kaiyadeva nighantu, Oshadhivarga, 1/ 1573-1575. 1st Edi. Varanasi: Chaukhamba orientalia publication; 1979.p.637

100. Chunekar KC, Pandey GS. Bhavprakash Nighantu, Guduchyadi varga, Shloka no. 240-245.Varanasi: Chaukhamba Vishvabharati Publication; 2002. p. 429.

101. Tripathi I. Raja nighantu, Shatavhadi varga, 4/138-141. 2nd Edi. Varanasi: Krushnadas academy; 1998. p. 89

102. Acharya S, Shaligram nighantu, Mumbai: Khemraj shrikrushnadas Publication; 1982. p. 127

103. Vaidya B, Nighantu Adarsha, Sahadevyadi Varga, Vol 1. 2nd Edi. Varanasi: Chaukhamba Bharati publication, 1998. p. 764-69

104. Taxonomical classification of Eclipta alba, Database of medicinal and aromatic plants in Rajasthsn, plant ID-0050, Birla institute of scientific research. (bioinfo.bisr.res.in)

105. Vaidya B, Nighantu Adarsha, SahadevyadiVarga, Vol 1. 2nd Edi. Varanasi: Chaukhamba Bharati publication; 1998. p. 764-65

106. Sharma PC, Yelne MB, Dennis TJ.Database on medicinal plants used in Ayurveda, Vol- 2. New Delhi: CCRAS; 2001. p.112

107. Khare CP. Encyclopedia of Indian Medicinal plants. New York: Springer-Verlag Berlin Heidelberg; 2004.p. 198

108. Vaidya B. Nighantu Adarsha, SahadevyadiVarga, Vol 1. 2nd Edi. Varanasi: Chaukhamba Bharati publication; 1998. p .765

109. Dixit SP, Achar MP. J. Sci. Res. Pl. Med., Vol-2. Study of *Bhringaraj* therapy in jaundice inchildren.1981. p. 96-100

110. Wagner H, Fessler B. Planta Med., Vol- 52. In vitro 5- Lipoxygenase inhibition by *Eclipta alba* extracts and the coumestan derivative wedelolactone. 1986. p. 374-77

111. Upadhyay RK, Pandey MB, Jha RN& Pandey VB, J. Asian. Nat. Prod. Res., Vol- 3. Eclalbatin a triterpene saponin from *Eclipta alba*. 2001. p. 213-17. (Jadhav et al, 2009, Khare, 2004) *Eclipta alba* (L.)

112. Jadhav VM et al. Journal of Pharmacy Research, 2(8). Eclipta alba Linn– ''Kesharaja'': A Review. 2009. p. 1236-41

113. Leal LK, Ferreira AA, Bezerra GA, Matos FJ, Viana GS.J. Ethnopharmacol, 2. Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin- a comparative study; 2000. p. 51-59

114. Rahman MS, Chowdhury R, Hasan CM, Rashid MR. The Dhaka university journal of pharmaceutical sciences, 4(5). Oleanane Glycosides from *Eclipta prostrate.* 2005

115. Garde GK. Sartha Vagbhatt, 39/163. 1st Edi. Anmol publication; 1994. p .555

116. Samudram P, Rajeshwari Hari, Vasuki R, Geetha, Sathiyamoorthi P. Hepatoprotective activity of Bi-herbal ethanolic extract on CCl4 induced hepatic damage in rats. AJBR. 2008; 2(2): 61-65)

117. Lal V.K, Amit Kumar, Prashant Kumar, Kuldeep Singh Yadav, Screening of Leaves and Roots of *Eclipta alba* for Hepatoprotective Activity, Arch. Appl. Sci. Res, 2010; 2(1): 86-94.)

118. Otilia Banji, David Banji, Annamalai Ar, Manavalan R,Investigation on the effect of *Eclipta alba* on animal models of learning and memory, Indian J physiolpharmacol, 2007; 51(3):274-78)

119. Ghosh M N. Fundamentals of experimental pharmacology. 2nd ed. Calcutta: scientific bookagency:1984.)

120. Roitt I, Grostoff J, Male D. Immunology. Mosby publication, London. 1998.16. Hudson L, Frank, Hay C. Practical immunology. 3rd ed. Black well publications. Oxford university press, London.1991

121. Jayathirthaa MG, Mishraa SH. Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and Centella asiatica. Phytomedicine, 2004; 11(4): 361-65)

122. Sankaran, J., An all India multicentric clinical survey on a herbal curetefroli for hepatitis, J. Natl. Integ. Med. Ass., Vol-26, 1984, 255-261.)

123. Sharma PC, Yelne MB, Dennis T J. Database on medicinal plants used in Ayurveda, Vol-2, botanical characters as Eclipta alba species. CCRAS, New Delhi; 2001.p.112

124. Sharma A et al, Journal of Pharmacy Research, Pharmacognostic and Phytochemical Evaluation of roots of Eclipta alba and prostrata, 2011, 4 (6), 1673-1674

125. Yadav P, Harisha CR, Prajapati PK. Journal of Current Pharmaceutical Research, Validation of pharmacopoeial characters of Bhringaraja (Eclipta alba Linn.)8 (1); 2011. p. 17-24

126. Parashar R. *Sharangadhar Samhita,* Madhyam Khanda, Pratham adhyaya - 1/2. 3rd Edi. Nagpur: Baidynath publication, Nagpur; 1984.p.125

127. Parashar R. *Sharangadhar Samhita,* Madhyam Khanda, Pratham adhyaya, 1/3-4. 3rd Edi. Nagpur: Baidynath publication, Nagpur; 1984.p.125

128. Shastri PK, Chaturvedi GN. Charak Samhita, Uttaradha, Chikitsa sthana, Rasayan adhyaya, 1/2/12.Varanasi: Chaukhamba Bharti Academy; 1998. p. 27

129. Vaidya Yadavji Trikamji Acharya, Siddha Yoga sangraha, Jwaradhikara, 6, Bidyanath Ayurved Bhavan, Nagpur, 9th edition, 1990.p. 4

130. Anandharamakrishnan C and Padma IS, Spray drying techniques for food ingredients encapsulation, 1st edition. Wiley J and Sons publication. 2015; IFT Press Book Spray drying techniques.p.1-36 and Amruta herbals Pvt. ltd. Booklet for procedure of spray drying technique

131. N.F.H.S. 3rd. International Institute for Population Science; 2006. National Family Health Survey for India conducted by Mumbai

132. Haslet C, et al. Principles &practice of Medicine, Davidson. Churchill Livingstone publication.19th edition.p.750-753

133. Kalantri A et al. Accuracy & Reliability of Pallor for Detecting Anemia: A hospital –based diagnostic accuracy study. Plos One. Dept. of Medicine, MGIMS, Wardha, Maharashtra

134. Deepika AK, Donga SB, Laxmipriya D. Efficacy of Punarnava mandur and Dhatri lauha in the management of Garbhini Pandu (anemia in pregnancy). Ayu2015; 36:397-403

135. Subir KK, Vyas SN, Chandola HM. Efficacy of Trikatrayadi lauha in Panduroga with reference to Iron Deficiency Anemia.Ayu2012: vol.33, 1: 62-66

136. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1sted. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007. Appendix 2,2.2.2, Foreign matter, p. 159

137. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government

of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007. Ph value, Appendix 3, 3.3, p.213

138. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-II, Vol. I, Chemical tests and assays. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007

139. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-II, Vol. I, Chemical tests and assays. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Foaming index, appendix 2, 2.2.7

140. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Moisture content, Appendix 2, 2.2.10, p. 161

141. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Colorimetry, appendix 2.2.6

142. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Extractive values, Appendix 2, 2.2.7, p.160

143. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II , Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government

of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, ash value, Appendix 2, 2.2.3, p.160

144. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, specific gravity, Appendix 3, 3.2, p.212

145. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, viscosity, Apendix3, 3.7, p. 220

146. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, spectrophotometry, appendix 2.2.5

147. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, chromatography, Appendix 2, 2.2.13, p. 164

148. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, AAS, Appendix 2, 2.3.7, p.178

149. Meier B and Spriano A. HPTLC, Standard operating procedures for HPTLC. Zurich University of Applied Sciences. International association for the advancement of High Performance Thin Layer Chromatography manual.p.1-2

150. Standard operating procedures for HPLC, (https://garner.chem.wsu.edu.>2013/10)

151. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I,Vol. II Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, TANIN, Apendix 5, 5.1.2, p. 239

152. Namjoshi AN. Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-II, Vol. I, Chemical tests and assays.1sted. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, RESIN

153. Namjoshi AN. Committee Chairman. The Ayurvedic Pharmacopoeia of India, Part-II, Vol. I, Chemical tests and assays. 1sted. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, SAPONIN

154. Namjoshi AN. Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-II, Vol. IChemical tests and assays.1sted. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, GLYCOSIDE

155. Namjoshi AN. Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government

of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Protein, appendix 2, 2.2.17, p.147

156. Namjoshi AN. Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-II, Vol. I Chemical tests and assays.1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Sterol

157. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-II, Vol. I, Chemical tests and assays.1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, amino acid

158. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, alkaloid, Appendix 2, 2.2.18, p. 147

159. Namjoshi AN, Committee Chairman. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. II, Tests and determinations. 1st ed. New Delhi: The Controller of Publication, Government of India, Ministry of Health and Family Welfare, Publication Department, ISM and H; 2007, Starch, Apendix 2, 2.2.14, p.146

160. Rajaradhakantadeva B., Shriharicharan V. Shabdakalpadrum, volume 3, Chakhamba Sanskrit series Varanasi, 3rd edition,1967, page 104

161. Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nutr 2001;131(2 suppl):676S-88S; discussion 688S-90S.)

162. Algarin C, Peirano P, Garrido M, Pizarro F, Lozoff B. Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. Pediatr Res 2003; 53:217-23

163. Verdon F, Burnand B, Stubi CL, Bonard C, Graff M, Michaud A, et al. Iron supplementation for unexplained fatigue in non-anemic women: double blind randomized placebo-controlled trial. BMJ 2003; 326:1124)

164. Kanani S (1995) Strategies for combating anemia in adolescent girls. Ind J Pediatrics 62; 375-77

165. Toteja GS, Singh P, Indian Council of Medical Research, New Delhi. 2003. National Workshop on Micronutrients, Background document, Micro-Nutrient profile of Indian Population

166. Chaudhary SM, Dhage VR. A study of anemia among adolescent females in the urban area of Nagpur. Indian J Community Med 2008; 33:4; p.243-5

167. Maeda M; Yamamoto M; Yamauchi K. Prevalence of anemia in Japanese adolescents: 30 years' experience in screening for anemia. Int J Hematol 1999; 69(2):75-80

168. Parashar R. *Sharangadhar Samhita,* Madhyam Khanda, 1/5. 3rdEdi. Nagpur:1984: Baidynath publication; p.126

169. Parashar R. *Sharangadhar Samhita, Purva* Khanda, 1/45-47. 3rdEdi. Nagpur:1984: Baidynath publication.p. 16

170. Sen GD, Edited by Mishra S., Manaparibhasha, Bheshajyaratnavaali, 2005, Chaukhamba Surbharti Publication, 1st edition, Cha. 2/ 22, 23, p. 23)

171. Benoist BD, McLean E, Egli I, Cogswell M,Worldwide prevalence of anemia. WHO Global Databae on Anemia. Geneva: World Health Organization; 1993-2005p.7

172. Khan Sk, Vyas SN, ChandolaHM.: Efficacy of Trikatraydi Lauha in Panduroga with reference to Iron Deficiency anemia. Ayu. Vol 33, Issue 1, p. 62-66

173. Kulkarni MV, Durge PM, Kasturwar MN. Prevalence of Anemia Among Adolescent Girls In An Urban Slum National Journal Of Community Medicine, 2012; Vol 3:1. p. 108-111

174. Gawarika R, Gawarika S, Mishra AK. Prevalence of anemia in adolescent girls belonging to different economic groups. Indian J Community Med.2006; 31:287-288

175. Chunekar K C, Pandey G S. *Bhavprakash Nighantu*, *Guduchyadi Varga, Vimarsha*. (commentry). Varanasi: Chaukhamba Vishva bharati Publication; 2002. p. 429-431

176. Singh B Saxena K, Chandan B, Agarwal S, Bhatia MS& Anand KK. Hepatoprotective effect of ethanolic extract of *Eclipta alba* on experimental liver damage, Phyto ther. Res., Vol-7. 1993; 154- 158

177. Saxena AK, Singh B, Anand KK, Hepatoprotective effects of Eclipta alba on subcellular levels in rats. Journal of ethnopharmacology. 1993; 40(3): 155-61

178. Bhaskar rao D, Ravi kiran C, Madhavi Y, Koteshwara RP, Raghava RT. Evaluation of antioxidant potential of clitoria ternate L. and Eclipta prostrata L. Indian journal of biochemistry and biophysics. 2009; 46: 247-52

179. Karthi kumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves of Eclipta prostrata (L). Sci. Res. Essays. 2007; 2(4): 101-04

180. Wagner, H., Geyer, B., Kiso, Y., Hikino, H.& Rao, G. S., Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulacea*, Planta Med., Vol-52;1986:370-374

181. Anon., Atrial of Bhringaraja Ghana satwa vati on the patients of Kostha -Shakharita Kamala (With special reference to hepatocellular jaundice), J. Natl. Integ. Med. Ass., Vol-24; 1982: 265-269

Sr. No.	Age (year)	HB0	HB1	HB Diff	TIBC0	TIBC1	TIBC Diff	SrFerratin0	SrFerratin1	Sr ferretin diff	SrTransferin0	SrTransferin1	SrTransferin_diff	SrIron0	SrIron1	SrIron_diff	MCHC0	MCHC1	MCHC_diff	MCV0	MCV1	MCV_diff	Blood_smear0	Blood_smear1	Pallor 0	Pallor 1	Pallor diff
1	18	8.4	9.8	1.4	541	501	-40	8.78	16.27	7.49	8.24	15.56	7.32	29	55	26	28.4	30.8	2.4	56.7	65.4	8.7	hypochromic microcytic	hypochromic microcytic	2	1	1
2	18	9.1	10.4	1.3	537	482	-55	9.26	18.1	8.84	9.06	16	6.94	30	57	27	29.2	31.9	2.7	55.4	61.1	5.7	hypochromic microcytic	hypochromic microcytic	1	0	1
3	19	9.5	10.9	1.4	532	487	-45	10.21	19.21	9	9.67	15.23	5.56	30	56	26	29.4	32.3	2.9	55.4	62.4	7	hypochromic microcytic	hypochromic microcytic	1	0	1
4	18	7.4	8.9	1.5	521	467	-54	6.97	15.23	8.26	7.46	16.46	9	20	45	25	26.8	29.5	2.7	53.3	59.9	6.6	hypochromic microcytic	hypochromic microcytic	3	1	2
5	17	9.8	11.4	1.6	549	501	-48	10.15	19.11	8.96	11.09	19.02	7.93	28	53	25	30.1	32.6	2.5	65.4	72.2	6.8	hypochromic microcytic	hypochromic microcytic	1	0	1
6	18	9.2	10.7	1.5	540	489	-51	9.34	18.62	9.28	9.26	15.91	6.65	29	54	25	28.9	31.2	2.3	62.7	67.2	4.5	hypochromic microcytic	hypochromic microcytic	1	0	1
7	19	10.3	11.5	1.2	490	441	-49	10.61	18.17	7.56	10.34	16.06	5.72	30	58	28	30.2	31.8	1.6	63.5	68.3	4.8	hypochromic microcytic	hypochromic microcytic	1	0	1
8	19	7.4	8.6	1.2	534	477	-57	7.86	17.69	9.83	8.92	15.29	6.37	20	46	26	28.1	30.9	2.8	56.1	61.6	5.5	hypochromic microcytic	hypochromic microcytic	3	2	1
9	18	8.6	9.7	1.1	540	487	-53	8.31	17.53	9.22	8.51	15.65	7.14	25	40	15	28.1	29.5	1.4	55.7	62.2	6.5	hypochromic microcytic	hypochromic microcytic	2	1	1
10	19	10.1	11.6	1.5	526	475	-51	10.78	19.92	9.14	11.35	20.59	9.24	32	59	27	29.8	32.4	2.6	55.1	63.1	8	hypochromic microcytic	hypochromic microcytic	1	0	1
11	19	7.6	8.9	1.3	539	492	-47	8.14	17.45	9.31	8.2	14.31	6.11	23	47	24	27.3	29.6	2.3	54.3	59.1	4.8	hypochromic microcytic	hypochromic microcytic	3	1	2
12	17	7.8	9.4	1.6	498	459	-39	8	16.46	8.46	8.96	15.26	6.3	28	52	24	27.2	30.5	3.3	53.7	60.9	7.2	hypochromic microcytic	hypochromic microcytic	3	1	2
13	17	9.4	10.7	1.3	509	465	-44	9.17	17.84	8.67	9.97	18.65	8.68	29	55	26	29.2	31.7	2.5	60.2	67.4	7.2	hypochromic microcytic	hypochromic microcytic	1	0	1
14	16	10.2	11.6	1.4	498	450	-48	11.24	17.79	6.55	11.09	17.1	6.01	35	63	28	30.1	32.6	2.5	65.7	75	9.3	hypochromic microcytic	hypochromic microcytic	1	0	1
15	18	9.3	10.6	1.3	563	517	-46	9.02	16.96	7.94	9.61	16.41	6.8	29	58	29	29.7	31.8	2.1	61.6	68.7	7.1	hypochromic microcytic	hypochromic microcytic	1	0	1
16	19	8.7	9.9	1.2	548	497	-51	8.22	16.28	8.06	9.34	15.27	5.93	29	51	22	28.1	31.1	3	55.4	59.5	4.1	hypochromic microcytic	hypochromic microcytic	2	1	1
17	19	9.1	10.8	1.7	523	482	-41	9.28	18.89	9.61	10.07	18.63	8.56	28	54	26	29.4	32.2	2.8	58.2	67.8	9.6	hypochromic microcytic	hypochromic microcytic	1	0	1
18	19	7.4	8.9	1.5	480	426	-54	6.71	13.52	6.81	8.4	15.04	6.64	17	41	24	27.8	29.9	2.1	53.4	59.1	5.7	hypochromic microcytic	hypochromic microcytic	3	2	1

19	19	10.2	11.5	1.3	565	506	-59	11.01	18.86	7.85	10.91	16.59	5.68	31	58	27	30.6	32.7	2.1	65.8	74	8.2	hypochromic microcytic	Normochromic normocytic	1	0	1
20	19	8.4	9.6	1.2	498	456	-42	8.42	16.23	7.81	9.03	15.98	6.95	24	52	28	27.1	30.2	3.1	55.4	62.7	7.3	hypochromic microcytic	hypochromic microcytic	2	1	1
21	18	9.2	10.9	1.7	564	513	-51	9.91	18.68	8.77	9.72	18.25	8.53	29	53	24	29.2	31.5	2.3	57.1	64.2	7.1	hypochromic microcytic	hypochromic microcytic	1	0	1
22	18	7.6	9.8	2.2	522	476	-46	7.16	15.71	8.55	7.65	14.72	7.07	22	47	25	27.1	30.5	3.4	52.2	60.4	8.2	hypochromic microcytic	hypochromic microcytic	3	1	2
23	17	8.1	9.7	1.6	577	522	-55	8.27	17.82	9.55	9.26	16.54	7.28	26	50	24	28.6	30.4	1.8	55.2	60.8	5.6	hypochromic microcytic	hypochromic microcytic	2	1	1
24	16	9.2	10.5	1.3	544	491	-53	9.39	18.27	8.88	10.47	16.99	6.52	29	55	26	29.1	31.8	2.7	58.1	65.9	7.8	hypochromic microcytic	hypochromic microcytic	1	0	1
25	19	9.5	11.3	1.8	533	481	-52	10.15	20.39	10.24	10.01	16.15	6.14	30	58	28	29.2	32.2	3	62.4	71.3	8.9	hypochromic microcytic	hypochromic microcytic	1	0	1
26	19	10	11.3	1.3	516	467	-49	11.42	19.52	8.1	10.3	16.82	6.52	31	57	26	30	32.2	2.2	64.2	72.1	7.9	hypochromic microcytic	hypochromic microcytic	1	0	1
27	18	8.5	9.8	1.3	507	456	-51	8.67	17.72	9.05	8.17	16.62	8.45	23	49	26	28.6	31.4	2.8	57.1	65.9	8.8	hypochromic microcytic	hypochromic microcytic	2	1	1
28	18	8.2	10.4	2.2	566	505	-61	8.94	16.79	7.85	9.67	16.71	7.04	27	51	24	28.2	30.7	2.5	55.2	61.1	5.9	hypochromic microcytic	hypochromic microcytic	2	0	2
29	19	9.4	10.7	1.3	536	485	-51	9.23	18.54	9.31	9.86	17.92	8.06	30	57	27	29.1	31.1	2	57.2	62.2	5	hypochromic microcytic	hypochromic microcytic	1	0	1
30	19	7.4	9.3	1.9	512	475	-37	7.92	16.2	8.28	8.04	15.44	7.4	18	46	28	27.6	30.2	2.6	52.4	58.1	5.7	hypochromic microcytic	hypochromic microcytic	3	1	2
31	19	8.6	10.4	1.8	486	435	-51	8.87	17.36	8.49	8.59	15.9	7.31	24	50	26	28.2	31.2	3	55.8	60.5	4.7	hypochromic microcytic	hypochromic microcytic	2	0	2
32	18	9.2	10.6	1.4	532	476	-56	9.46	17.59	8.13	9.96	15.96	6	29	53	24	28.2	31.1	2.9	55.4	62.8	7.4	hypochromic microcytic	hypochromic microcytic	1	0	1
33	17	7.5	9.3	1.8	541	486	-55	6.25	15.37	9.12	7.67	16.94	9.27	20	46	26	27.1	30.9	3.8	53.1	60.4	7.3	hypochromic microcytic	hypochromic microcytic	3	1	2
34	16	10.1	11.5	1.4	496	433	-63	11.93	18.62	6.69	10.64	17.75	7.11	31	59	28	30.1	32.2	2.1	64.2	71.7	7.5	hypochromic microcytic	hypochromic microcytic	1	0	1
35	19	9.6	11.2	1.6	467	414	-53	9.67	18.34	8.67	9.97	16.86	6.89	30	55	25	28.5	32.4	3.9	62.1	70.3	8.2	hypochromic microcytic	hypochromic microcytic	1	0	1
36	18	7.2	8.7	1.5	519	476	-43	7.39	15.46	8.07	7.51	14.92	7.41	21	44	23	26.4	28.6	2.2	55.8	60.1	4.3	hypochromic microcytic	hypochromic microcytic	3	2	1
37	19	9.5	11.3	1.8	527	488	-39	9.54	19.15	9.61	9.26	16.29	7.03	28	53	25	29.1	32.5	3.4	65.2	74.7	9.5	hypochromic microcytic	hypochromic microcytic	1	0	1
38	19	8.4	9.9	1.5	562	516	-46	8.61	17.68	9.07	8.45	15.56	7.11	25	48	23	28.2	30.2	2	56.2	61.7	5.5	hypochromic microcytic	hypochromic microcytic	2	1	1
39	19	7.3	8.7	1.4	499	438	-61	7.24	14.27	7.03	7.53	15.24	7.71	22	44	22	26.9	29.1	2.2	53.4	59.1	5.7	hypochromic microcytic	hypochromic microcytic	3	2	1

40	19	8.7	9.9	1.2	486	437	-49	8.56	16.53	7.97	8.64	15.63	6.99	28	49	21	28.1	30.1	2	56.4	61.7	5.3	hypochromic microcytic	hypochromic microcytic	2	1	1
41	17	10.1	11.7	1.6	509	462	-47	10.17	18.52	8.35	10.06	16.32	6.26	33	53	20	29.2	32.2	3	68.2	77	8.8	hypochromic microcytic	Normochromic Normocytic	1	0	1
42	18	7.2	8.9	1.7	565	503	-62	7.7	16.92	9.22	7.97	14.79	6.82	20	43	23	26.9	29.9	3	54.6	61.2	6.6	hypochromic microcytic	hypochromic microcytic	3	2	1
43	18	8.2	9.6	1.4	547	486	-61	8.6	17.67	9.07	8.06	15.26	7.2	28	52	24	28.4	30.6	2.2	58.6	67.3	8.7	hypochromic microcytic	hypochromic microcytic	2	1	1
44	19	10	11.4	1.4	521	476	-45	11.32	19.46	8.14	11.25	18	6.75	36	57	21	30.5	33.2	2.7	68.5	75.9	7.4	hypochromic microcytic	Normochromic Normocytic	1	0	1
45	19	10.2	11.7	1.5	515	464	-51	10.35	18.69	8.34	11.36	17.09	5.73	33	58	25	30.1	32.7	2.6	68	76.1	8.1	hypochromic microcytic	hypochromic microcytic	1	0	1
46	18	8.9	9.9	1	503	456	-47	8.02	16.75	8.73	8.11	16.21	8.1	24	45	21	28.4	30.5	2.1	58.1	63.4	5.3	hypochromic microcytic	hypochromic microcytic	2	1	1
47	18	7.8	9.4	1.6	566	504	-62	8.26	16.95	8.69	8.5	14.86	6.36	22	46	24	27.8	30.2	2.4	55.4	61.3	5.9	hypochromic microcytic	hypochromic microcytic	3	1	2
48	19	10.5	11.7	1.2	487	446	-41	11.21	20.76	9.55	11.68	18.72	7.04	31	51	20	30.1	33.1	3	67.3	76	8.7	hypochromic microcytic	hypochromic microcytic	0	0	0
49	17	8.2	9.6	1.4	554	513	-41	8.25	17.27	9.02	8.15	14.48	6.33	23	44	21	27.5	30.5	3	60.7	70.3	9.6	hypochromic microcytic	hypochromic microcytic	2	1	1
50	18	9.4	10.7	1.3	541	478	-63	9.27	18.63	9.36	9.13	16.25	7.12	29	53	24	29.1	31.2	2.1	64.1	70	5.9	hypochromic microcytic	hypochromic microcytic	1	0	1
51	16	10.1	11.7	1.6	537	486	-51	11.46	21	9.54	11.29	20.64	9.35	30	50	20	30.7	32.9	2.2	66.8	74.7	7.9	hypochromic microcytic	hypochromic microcytic	1	0	1
52	17	7.6	8.9	1.3	532	479	-53	8.75	16.64	7.89	7.78	15.35	7.57	22	46	24	27.1	29.8	2.7	53.7	59.5	5.8	hypochromic microcytic	hypochromic microcytic	3	2	1
53	16	9.7	11.4	1.7	521	477	-44	10.01	19.52	9.51	9.27	16.51	7.24	29	51	22	29.1	32.4	3.3	66.5	74.9	8.4	hypochromic microcytic	hypochromic microcytic	1	0	1
54	17	8.7	10.3	1.6	549	490	-59	8.93	17.47	8.54	8.65	14.24	5.59	28	54	26	28.8	31.5	2.7	58.6	63	4.4	hypochromic microcytic	hypochromic microcytic	2	1	1
55	18	7.2	9.5	2.3	561	515	-46	7.21	17.69	10.48	7.85	16.26	8.41	17	43	26	26.9	29.3	2.4	55.7	62.1	6.4	hypochromic microcytic	hypochromic microcytic	3	1	2
56	18	8.5	9.7	1.2	517	482	-35	8.55	15.32	6.77	9.36	15.47	6.11	25	47	22	28.4	30.6	2.2	59.1	68.2	9.1	hypochromic microcytic	hypochromic microcytic	2	1	1
57	19	9	10.4	1.4	536	478	-58	9.73	17.7	7.97	9.62	16.42	6.8	29	49	20	29.6	31.4	1.8	67.2	70.5	3.3	hypochromic microcytic	hypochromic microcytic	1	0	1
58	19	9.4	10.9	1.5	501	468	-33	10.13	19.41	9.28	9.91	18.11	8.2	30	50	20	29.7	31.2	1.5	58.2	66.8	8.6	hypochromic microcytic	hypochromic microcytic	1	0	1
59	19	8.2	9.8	1.6	546	494	-52	8.24	17.85	9.61	8.67	15.34	6.67	29	54	25	28.5	30.9	2.4	54.4	61.9	7.5	hypochromic microcytic	hypochromic microcytic	2	1	1
60	18	7.5	8.9	1.4	522	467	-55	7.75	15.46	7.71	8.1	15.25	7.15	21	43	22	27.5	30.5	3	55	60.8	5.8	hypochromic microcytic	hypochromic microcytic	3	2	1

61	17	9.8	10.7	0.9	514	458	-56	9.89	19.15	9.26	9.19	14.64	5.45	29	52	23	29.1	31.3	2.2	67.3	71.2	3.9	hypochromic microcytic	hypochromic microcytic	1	0	1
62	17	10.2	11.6	1.4	490	437	-53	10.44	17.86	7.42	10.35	18.27	7.92	33	56	23	30.6	32.8	2.2	67.2	76.2	9	hypochromic microcytic	hypochromic microcytic	1	0	1
63	19	8.6	10.5	1.9	549	495	-54	8.82	17.42	8.6	8.25	17.05	8.8	25	49	24	28.7	31.7	3	61.9	70.2	8.3	hypochromic microcytic	hypochromic microcytic	2	0	2
64	19	7.2	9.7	2.5	549	491	-58	7.68	17.49	9.81	7.77	15.97	8.2	20	46	26	26.4	30.4	4	53.7	60.9	7.2	hypochromic microcytic	hypochromic microcytic	3	1	2
65	18	8.7	10.2	1.5	531	489	-42	8.56	16.67	8.11	9.23	17.16	7.93	28	56	28	28.8	30.2	1.4	56.2	65	8.8	hypochromic microcytic	hypochromic microcytic	2	1	1
66	19	7.6	9.4	1.8	497	446	-51	7.23	14.79	7.56	8.19	18.64	10.45	22	46	24	27.7	30.4	2.7	54.1	62	7.9	hypochromic microcytic	hypochromic microcytic	3	1	2
67	19	10.4	11.7	1.3	501	453	-48	11.42	18.58	7.16	11.31	16.79	5.48	33	56	23	30.5	33.6	3.1	68.1	76.2	8.1	hypochromic microcytic	Normochromic Normocytic	0	0	0
68	19	8.5	9.8	1.3	553	501	-52	8.31	15.74	7.43	9.25	16.47	7.22	26	52	26	28.4	30.8	2.4	61.6	67.7	6.1	hypochromic microcytic	hypochromic microcytic	2	1	1
69	18	9.1	11	1.9	556	503	-53	9.97	18.15	8.18	9.86	18.36	8.5	29	54	25	29.8	31.8	2	65.2	71.6	6.4	hypochromic microcytic	hypochromic microcytic	1	0	1
70	18	7.4	8.7	1.3	546	499	-47	7.19	13.37	6.18	8.24	15.56	7.32	21	46	25	27.5	29.9	2.4	54.1	60.2	6.1	hypochromic microcytic	hypochromic microcytic	3	2	1
71	19	8.2	10.5	2.3	531	475	-56	8.25	18.26	10.01	9.36	16.67	7.31	26	52	26	28.8	31.8	3	58.2	68.1	9.9	hypochromic microcytic	hypochromic microcytic	2	0	2
72	18	10.3	11.7	1.4	499	459	-40	11.03	18.78	7.75	11.06	18.28	7.22	32	49	17	30.6	33.5	2.9	67.4	77.3	9.9	hypochromic microcytic	Normochromic Normocytic	1	0	1
73	18	10.2	11.7	1.5	486	427	-59	11.13	17.34	6.21	10.14	17.69	7.55	31	58	27	30.1	33.3	3.2	66.3	75.6	9.3	hypochromic microcytic	hypochromic microcytic	1	0	1
74	17	8.3	9.5	1.2	535	478	-57	8.67	15.86	7.19	8.27	16.61	8.34	25	54	29	28.9	30.2	1.3	63.8	68.3	4.5	hypochromic microcytic	hypochromic microcytic	2	1	1
75	17	7.4	8.9	1.5	537	481	-56	7.15	15.95	8.8	7.46	14.26	6.8	19	45	26	26.7	29.4	2.7	54.2	62.1	7.9	hypochromic microcytic	hypochromic microcytic	3	1	2
76	17	8.5	10.7	2.2	497	445	-52	8.54	16.57	8.03	9.64	16.75	7.11	24	50	26	28.2	31.2	3	65.1	70.7	5.6	hypochromic microcytic	hypochromic microcytic	2	0	2
77	17	8.1	9.7	1.6	551	501	-50	8.76	17.96	9.2	8.17	14.82	6.65	22	47	25	27.5	30.2	2.7	61.2	67	5.8	hypochromic microcytic	hypochromic microcytic	2	1	1
78	18	9.4	10.7	1.3	496	450	-46	9.35	18.35	9	10.25	16.39	6.14	30	58	28	29.2	31.5	2.3	65.3	72.3	7	hypochromic microcytic	hypochromic microcytic	1	0	1
79	19	8.7	10.2	1.5	517	467	-50	8.28	16.26	7.98	8.72	16.27	7.55	27	53	26	28.2	31.1	2.9	61.5	70.9	9.4	hypochromic microcytic	hypochromic microcytic	2	1	1
80	18	9.4	10.9	1.5	528	487	-41	9.04	17	7.96	10.76	18.64	7.88	29	53	24	29.9	31.6	1.7	66.7	72.3	5.6	hypochromic microcytic	hypochromic microcytic	1	0	1
81	17	7.2	8.9	1.7	531	476	-55	7.25	14.95	7.7	8.18	15.23	7.05	18	46	28	26.1	29.3	3.2	56.2	64.5	8.3	hypochromic microcytic	hypochromic microcytic	3	1	2

82	18	10	11.5	1.5	497	456	-41	10.5	18.53	8.03	11.29	17.26	5.97	37	66	29	29.6	33.1	3.5	68.3	76.7	8.4	hypochromic microcytic	hypochromic microcytic	1	0	1
83	19	7.9	9.2	1.3	557	524	-33	8.62	16.64	8.02	7.97	16.19	8.22	24	49	25	27.1	30.8	3.7	57.9	67.7	9.8	hypochromic microcytic	hypochromic microcytic	2	1	1
84	18	8.6	9.9	1.3	530	493	-37	8.21	16.71	8.5	9.45	17.42	7.97	27	53	26	28.1	31.1	3	55.4	63.2	7.8	hypochromic microcytic	hypochromic microcytic	2	1	1
85	19	9.7	10.9	1.2	490	460	-30	9.81	17.93	8.12	11.4	17.63	6.23	29	54	25	29.7	31.3	1.6	66.3	71.1	4.8	hypochromic microcytic	hypochromic microcytic	1	0	1
86	18	7.9	9.7	1.8	489	443	-46	8.65	16.24	7.59	8.9	16.54	7.64	23	47	24	28.1	30.5	2.4	59.7	67.7	8	hypochromic microcytic	hypochromic microcytic	3	1	2
87	18	8.2	9.8	1.6	474	435	-39	8.19	16.45	8.26	8.6	16.91	8.31	23	46	23	28.9	30.5	1.6	59.1	65.3	6.2	hypochromic microcytic	hypochromic microcytic	2	1	1
88	18	9.2	10.7	1.5	509	478	-31	9.9	18.14	8.24	10.6	17.68	7.08	30	57	27	29.4	31.6	2.2	63.8	71.2	7.4	hypochromic microcytic	hypochromic microcytic	2	0	2
89	17	10	11.1	1.1	498	459	-39	11.4	19.37	7.97	11.1	17.75	6.65	35	62	27	29.3	31.8	2.5	67.7	74	6.3	hypochromic microcytic	hypochromic microcytic	1	0	1
90	17	9.4	10.7	1.3	517	486	-31	9.92	18.41	8.49	10.8	18.16	7.36	29	54	25	29.7	31.8	2.1	66.4	72.2	5.8	hypochromic microcytic	hypochromic microcytic	1	0	1
91	18	8.6	10.3	1.7	548	518	-30	9.54	17.12	7.58	9.7	15.45	5.75	26	52	26	28.7	31.8	3.1	60.3	69.7	9.4	hypochromic microcytic	hypochromic microcytic	2	1	1
92	18	10.1	11.6	1.5	478	437	-41	10.65	18.62	7.97	11.29	18.26	6.97	32	58	26	30.5	33.8	3.3	67.5	75.8	8.3	hypochromic microcytic	Normochromic Normocytic	1	0	1
93	18	7.4	9	1.6	534	481	-53	7.61	14.29	6.68	7.52	14.87	7.35	19	46	27	26.5	30.6	4.1	59.7	68.6	8.9	hypochromic microcytic	hypochromic microcytic	3	1	2
94	17	9	10.7	1.7	501	457	-44	9.9	19.04	9.14	9.76	16.54	6.78	29	54	25	29.6	31.9	2.3	62.2	69.9	7.7	hypochromic microcytic	hypochromic microcytic	1	0	1
95	18	8.3	9.8	1.5	562	519	-43	8.4	17.1	8.7	9.25	17.17	7.92	26	51	25	28.2	30.7	2.5	59.3	66.2	6.9	hypochromic microcytic	hypochromic microcytic	2	1	1
96	18	7.4	9.1	1.7	499	467	-32	7.75	15.32	8.57	7.46	14.69	7.23	20	44	24	26.9	30.1	3.2	53.2	61.6	8.4	hypochromic microcytic	hypochromic microcytic	3	1	2
97	19	8.4	10.2	1.8	513	476	-37	8.04	16.26	8.22	9.78	19.29	9.51	27	50	23	27.9	31.9	4	62.4	68.3	5.9	hypochromic microcytic	hypochromic microcytic	2	1	1
98	19	10.1	11.8	1.7	487	439	-48	11.91	19.28	7.37	11.27	18.67	7.4	31	54	23	30.2	33.7	3.5	67.1	76.8	9.7	hypochromic microcytic	Normochromic Normocytic	1	0	1
99	19	7	8.6	1.6	587	538	-49	6.74	15.33	8.59	7.5	14.67	7.17	16	40	24	26.9	29.1	2.2	56.3	64.7	8.4	hypochromic microcytic	hypochromic microcytic	3	2	1
100	19	8.1	9.7	1.6	553	511	-42	8.65	15.96	7.31	8.56	16.46	7.9	25	49	24	27.4	30.6	3.2	56.9	63.6	6.7	hypochromic microcytic	hypochromic microcytic	2	1	1
101	17	9.9	11.2	1.3	499	468	-31	9.31	17.45	8.14	10.62	16.79	6.17	28	56	28	30.4	32	1.6	69.2	74.7	5.5	hypochromic microcytic	hypochromic microcytic	1	0	1
102	17	9.8	10.8	1	527	489	-38	9.91	17.49	7.58	10.96	15.7	4.74	30	59	29	29.2	31.4	2.2	67.8	72.2	4.4	hypochromic microcytic	hypochromic microcytic	1	0	1

103	18	8.9	10.9	2	503	462	-41	9.34	20.29	10.95	10.56	18.02	7.46	29	51	22	28.9	31.6	2.7	63.2	71.4	8.2	hypochromic microcytic	hypochromic microcytic	1	0	1
104	19	10.4	11.9	1.5	490	453	-37	10.74	20.23	9.49	10.95	17.45	6.5	33	61	28	30.2	33.7	3.5	67.7	76.2	8.5	hypochromic microcytic	Normochromic Normocytic	0	0	0
105	18	7.8	9.1	1.3	521	478	-43	6.53	15.62	9.09	7.73	14.36	6.63	21	46	25	27.1	30.7	3.6	60.4	69.6	9.2	hypochromic microcytic	hypochromic microcytic	3	1	2
106	17	8.6	9.8	1.2	560	529	-31	8.89	17.15	8.26	9.46	16.34	6.88	28	54	26	28.8	30.9	2.1	63.8	69.7	5.9	hypochromic microcytic	hypochromic microcytic	2	1	1
107	17	9.9	11.4	1.5	537	489	-48	10.45	21.27	10.82	10.28	19.14	8.86	30	55	25	30.1	32.2	2.1	68.1	75.5	7.4	hypochromic microcytic	hypochromic microcytic	1	0	1
108	17	8.3	9.6	1.3	489	453	-36	8.86	17.5	8.64	8.87	16.23	7.36	23	47	24	28.1	30.4	2.3	58.3	66.3	8	hypochromic microcytic	hypochromic microcytic	2	1	1
109	19	7.9	10.2	2.3	526	487	-39	7.65	16.84	9.19	8.6	17.62	9.02	24	48	24	27.2	30.5	3.3	61.1	70.6	9.5	hypochromic microcytic	hypochromic microcytic	3	1	2
110	19	9.4	10.8	1.4	471	429	-42	9.82	18.77	8.95	9.87	17.12	7.25	29	56	27	29.1	30.7	1.6	65.6	70.2	4.6	hypochromic microcytic	hypochromic microcytic	1	0	1
111	19	9.7	11.2	1.5	499	458	-41	9.96	18.63	8.67	10.34	16.14	5.8	30	56	26	29.4	32.6	3.2	66.2	74.1	7.9	hypochromic microcytic	hypochromic microcytic	1	0	1
112	17	7.1	8.3	1.2	563	517	-46	7.23	16.23	9	8.8	15.56	6.76	25	49	24	27.7	29.5	1.8	54.1	59.9	5.8	hypochromic microcytic	hypochromic microcytic	3	2	1
113	17	8.7	10.4	1.7	559	516	-43	8.56	17.25	8.69	9.8	16.72	6.92	25	48	23	27.3	30.1	2.8	59.6	68.7	9.1	hypochromic microcytic	hypochromic microcytic	2	1	1
114	18	10	11.6	1.6	503	455	-48	10.45	20.06	9.61	11	19.91	8.91	31	55	24	30.2	32.2	2	67.3	76.5	9.2	hypochromic microcytic	Normochromic Normocytic	1	0	1
115	18	8.3	9.7	1.4	544	497	-47	8.7	16.35	7.65	8.45	16.21	7.76	25	50	25	27.8	30.2	2.4	59.5	68.8	9.3	hypochromic microcytic	hypochromic microcytic	2	1	1
116	18	9.6	10.9	1.3	516	470	-46	9.34	16.56	7.22	10.52	17.21	6.69	30	55	25	29.6	31.2	1.6	66.2	72.5	6.3	hypochromic microcytic	hypochromic microcytic	1	0	1
117	17	8.4	10.3	1.9	498	449	-49	8.45	17.15	8.7	9.65	16.87	7.22	29	53	24	28.1	30.2	2.1	61.3	68.6	7.3	hypochromic microcytic	hypochromic microcytic	2	1	1
118	17	8.7	10.1	1.4	542	497	-45	8.98	16.67	7.69	10.4	16.16	5.76	29	53	24	28.9	30.5	1.6	63.5	68.3	4.8	hypochromic microcytic	hypochromic microcytic	2	1	1
119	17	9.7	11	1.3	506	474	-32	10.51	18.94	8.43	10	16.31	6.31	30	57	27	29.6	32.1	2.5	66.1	72.8	6.7	hypochromic microcytic	hypochromic microcytic	1	0	1
120	18	8.6	9.9	1.3	539	506	-33	8.45	16.02	7.57	10.21	15.58	5.37	27	50	23	28.6	30.1	1.5	57.4	66.6	9.2	hypochromic microcytic	hypochromic microcytic	2	1	1
121	19	9	10.6	1.6	547	509	-38	9.72	17.17	7.45	9.34	17.09	7.75	29	54	25	29.2	31.2	2	62.4	69.5	7.1	hypochromic microcytic	hypochromic microcytic	1	0	1
122	18	9.3	10.7	1.4	517	486	-31	9.83	17.61	7.78	9.9	16.5	6.6	26	48	22	29.2	31.4	2.2	64.2	70.3	6.1	hypochromic microcytic	hypochromic microcytic	1	0	1
123	19	8.7	10.3	1.6	536	490	-46	8.04	17.57	9.53	8.79	15.87	7.08	28	52	24	28.5	31.6	3.1	60.3	68.6	8.3	hypochromic microcytic	hypochromic microcytic	2	1	1

124	18	8.6	9.7	1.1	531	497	-34	8.58	17.04	8.46	8.43	14.3	5.87	29	54	25	28	30.2	2.2	59.2	68.3	9.1	hypochromic microcytic	hypochromic microcytic	2	1	1
125	19	8	9.6	1.6	553	510	-43	8.84	16.27	7.43	8.25	15.89	7.64	20	46	26	27.2	30.1	2.9	58.6	67.8	9.2	hypochromic microcytic	hypochromic microcytic	2	1	1
126	18	9.1	10.5	1.4	536	512	-24	9.16	19.34	10.18	9.73	16.46	6.73	29	53	24	28.9	31.1	2.2	64.7	69.6	4.9	hypochromic microcytic	hypochromic microcytic	1	0	1
127	19	7.4	9.2	1.8	562	524	-38	8.87	15.45	6.58	8.37	17.54	9.17	20	48	28	26.1	29.2	3.1	56.1	65.9	9.8	hypochromic microcytic	hypochromic microcytic	3	1	2
128	18	8.6	9.7	1.1	513	476	-37	8.46	17.93	9.47	9.5	15.92	6.42	28	54	26	28.4	30.2	1.8	61.3	67.2	5.9	hypochromic microcytic	hypochromic microcytic	2	1	1
129	19	7.6	9.4	1.8	549	507	-42	8.24	16.09	7.85	7.91	16.32	8.41	23	50	27	27.1	30.1	3	57.2	66.5	9.3	hypochromic microcytic	hypochromic microcytic	3	1	2
130	18	7.5	9.6	2.1	541	509	-32	7.02	17.51	10.49	8.47	18.85	10.38	23	48	25	27.2	30.9	3.7	58.2	65.1	6.9	hypochromic microcytic	hypochromic microcytic	3	1	2

Sr. No.	Age (year)	HB0	HB1	HB Diff	TIBC0	TIBC1	TIBC Diff	SrFerratin0	SrFerratin1	Sr ferratin diff	SrTransferin0	SrTransferin1	SrTransferin_diff	SrIron0	SrIron1	SrIron_diff	MCHC0	MCHC1	MCHC_diff	MCV0	MCV1	MCV_diff	Blood_smear0	Blood_smear1	Pallor 0	Pallor 1	Pallor diff
1	17	9.2	10.2	1	506	481	-25	9.24	12.57	3.33	9.45	11.35	1.9	29	45	16	29.7	30.6	0.9	60.2	63.9	3.7	hypochromic microcytic	hypochromic microcytic	2	1	1
2	17	9.5	10.4	0.9	521	484	-37	9.75	12.56	2.81	9.16	12.31	3.15	26	43	17	29	30.8	1.8	66.7	70.5	3.8	hypochromic microcytic	hypochromic microcytic	2	1	1
3	19	8.1	9.1	1	535	515	-20	8.02	10.71	2.69	8.34	12.21	3.87	22	39	17	28.5	30	1.5	55	59.7	4.7	hypochromic microcytic	hypochromic microcytic	3	2	1
4	19	7.9	8.7	0.8	541	528	-13	7.34	9.95	2.61	7.89	10.87	2.98	20	39	19	27.5	29.7	2.2	54.8	57.8	3	hypochromic microcytic	hypochromic microcytic	3	2	1
5	19	10.4	11.4	1	489	457	-32	11.6	14.79	3.23	10.37	14.52	4.15	33	56	23	30.2	32.4	2.2	70.4	75.9	5.5	hypochromic microcytic	Normochromic Normocytic	1	0	1
6	17	7.5	8.1	0.6	540	519	-21	8.13	10.76	2.63	7.54	10.69	3.15	24	38	14	28.1	28.5	0.4	55.2	58.5	3.3	hypochromic microcytic	hypochromic microcytic	3	2	1
7	19	8.9	10.1	1.2	518	491	-27	8.59	13.84	5.25	8.15	11.87	3.72	23	44	21	27.5	28.2	0.7	61.3	65.7	4.4	hypochromic microcytic	hypochromic microcytic	2	1	1
8	17	9.5	10.4	0.9	480	450	-30	9.76	12.72	2.96	10.23	12.96	2.73	21	40	19	29.8	30.6	0.8	63.7	66.9	3.2	hypochromic microcytic	hypochromic microcytic	2	1	1
9	17	7.6	8.7	1.1	540	521	-19	8.1	12.05	3.95	8.87	12.72	3.85	17	35	18	27.4	28.5	1.1	53.7	57.5	3.8	hypochromic microcytic	hypochromic microcytic	3	2	1
10	18	8.7	9.9	1.2	526	509	-17	9.45	12.62	3.17	8.7	11.75	3.05	29	46	17	29.1	30.8	1.7	59.1	63.7	4.6	hypochromic microcytic	hypochromic microcytic	2	1	1
11	18	7.7	8.6	0.9	489	451	-38	8.11	12.39	4.28	9.15	12.98	3.83	17	37	20	27.1	28.1	1	55.2	59.4	4.2	hypochromic microcytic	hypochromic microcytic	3	2	1
12	17	7.2	8.3	1.1	540	521	-19	6.48	10.76	4.28	7.3	11.43	4.13	28	43	15	27.4	28.1	0.7	55.1	59.8	4.7	hypochromic microcytic	hypochromic microcytic	3	2	1
13	18	8	9.4	1.4	536	504	-32	8.23	12.91	4.68	7.96	11.29	3.33	25	43	18	28.3	28.4	0.1	59.3	62.7	3.4	hypochromic microcytic	hypochromic microcytic	2	1	1
14	17	9.4	10.4	1	498	467	-31	9.21	12.34	3.13	9.12	12.72	3.6	29	49	20	29.8	31.9	2.1	63.1	69.9	6.8	hypochromic microcytic	hypochromic microcytic	1	1	0
15	16	8.6	9	0.4	533	523	-10	9.96	9.92	-0.04	8.54	10.74	2.2	26	41	15	28.2	29.7	1.5	58.1	60.7	2.6	hypochromic microcytic	hypochromic microcytic	2	1	1
16	18	10.1	11.3	1.2	504	482	-22	11.5	15.02	3.52	10.51	13.45	2.94	31	49	18	29.8	31.4	1.6	62.4	66.9	4.5	hypochromic microcytic	hypochromic microcytic	1	0	1
17	19	8.8	9.4	0.6	523	507	-16	8.64	11.73	3.09	8.72	11.65	2.93	29	48	19	28.7	30.5	1.8	57.3	61.1	3.8	hypochromic microcytic	hypochromic microcytic	2	1	1
18	18	9.4	10.3	0.9	521	501	-20	9.21	12.69	3.48	10.6	13.17	2.57	28	42	14	29.1	31	1.9	63.1	66.4	3.3	hypochromic microcytic	hypochromic microcytic	1	1	0
19	17	7.8	8.9	1.1	565	527	-38	8.17	11.93	3.76	8.13	11.91	3.78	21	38	17	28.6	29.9	1.3	56.4	60.7	4.3	hypochromic microcytic	hypochromic microcytic	3	2	1
20	17	9.5	10.6	1.1	498	464	-34	10.1	13.79	3.69	10.81	13.64	2.83	30	48	18	29.2	31.7	2.5	64.1	69.8	5.7	hypochromic microcytic	hypochromic microcytic	2	1	1
21	17	8.2	9.2	1	542	521	-21	8.34	11.85	3.51	9.2	12.04	2.84	27	44	17	28.1	29.8	1.7	58.8	62.2	3.4	hypochromic microcytic	hypochromic microcytic	2	1	1
22	16	9.1	9.9	0.8	522	504	-18	9.17	12.47	3.3	9.16	12.87	3.71	29	48	19	29.5	31.2	1.7	61.2	65.6	4.4	hypochromic microcytic	hypochromic microcytic	1	1	0
23	16	9.9	11	1.1	501	474	-27	11.6	14.71	3.13	10.06	14.23	4.17	31	49	18	29.2	31.1	1.9	68.4	71.1	2.7	hypochromic microcytic	hypochromic microcytic	1	0	1
24	18	8.4	9.2	0.8	544	521	-23	8.29	11.34	3.05	9.4	11.82	2.42	23	36	13	28.7	29.5	0.8	56.5	59.9	3.4	hypochromic microcytic	hypochromic microcytic	2	1	1
25	19	8.5	9.6	1.1	553	508	-45	8.12	12.57	4.45	8.57	11.12	2.55	27	47	20	29.2	30.7	1.5	59	65.7	6.7	hypochromic microcytic	hypochromic microcytic	2	1	1

26	17	8.9	10.3	1.4	516	476	-40	9.54	13.76	4.22	9.81	13.41	3.6	28	49	21	29.8	31.3	1.5	62.5	69.6	7.1	hypochromic microcytic	hypochromic microcytic	1	1	0
27	17	7.7	8.6	0.9	557	529	-28	7.41	10.78	3.37	8.05	11.79	3.74	19	36	17	27.7	30.2	2.5	56.9	60	3.1	hypochromic microcytic	hypochromic microcytic	3	2	1
28	16	8.1	9.2	1.1	566	531	-35	8.61	10.97	2.36	8.36	11.24	2.88	18	41	23	27.5	29.1	1.6	59.4	64.3	4.9	hypochromic microcytic	hypochromic microcytic	2	1	1
29	19	8.7	10	1.3	536	498	-38	7.96	11.95	3.99	8.42	11.96	3.54	25	43	18	28.6	31.3	2.7	60.2	67.6	7.4	hypochromic microcytic	hypochromic microcytic	2	1	1
30	18	8.9	10	1.1	512	485	-27	8.59	12.39	3.8	9.8	12.84	3.04	20	39	19	29.4	30.9	1.5	61.1	66.9	5.8	hypochromic microcytic	hypochromic microcytic	1	1	0
31	18	10.4	11.7	1.3	486	431	-55	11.7	14.79	3.12	11.85	14.67	2.82	31	56	25	30.4	32.2	1.8	69.8	76.3	6.5	hypochromic microcytic	Normochromic Normocytic	1	0	1
32	18	7.8	8.9	1.1	532	502	-30	7.82	11.57	3.75	9.73	12.58	2.85	27	45	18	28	29.5	1.5	57.1	60.2	3.1	hypochromic microcytic	hypochromic microcytic	3	2	1
33	18	9.1	10.3	1.2	521	491	-30	9.46	13.71	4.25	10.42	13.69	3.27	29	49	20	29.7	31.2	1.5	65.4	69.8	4.4	hypochromic microcytic	hypochromic microcytic	2	1	1
34	19	7.6	8.7	1.1	556	524	-32	7.91	10.99	3.08	8.4	11.76	3.36	23	42	19	27.4	29.1	1.7	56.3	59.7	3.4	hypochromic microcytic	hypochromic microcytic	3	2	1
35	17	7.3	8.4	1.1	547	522	-25	6.46	10.55	4.09	7.98	10.99	3.01	25	44	19	26.1	28.7	2.6	52.7	57.1	4.4	hypochromic microcytic	hypochromic microcytic	3	2	1
36	17	9.1	10	0.9	519	489	-30	9.23	11.65	2.42	9.78	12.76	2.98	28	49	21	29.5	30.9	1.4	63.2	68.5	5.3	hypochromic microcytic	hypochromic microcytic	1	1	0
37	16	7.1	8.3	1.2	568	534	-34	7.51	11.72	4.21	7.39	11.28	3.89	17	37	20	26.7	28.4	1.7	51.3	57.9	6.6	hypochromic microcytic	hypochromic microcytic	3	2	1
38	17	9.4	10.5	1.1	503	480	-23	10.4	13.55	3.14	9.73	12.39	2.66	29	48	19	29.3	30.6	1.3	65.8	70.1	4.3	hypochromic microcytic	hypochromic microcytic	2	1	1
39	18	8.3	9.5	1.2	562	523	-39	8.62	11.57	2.95	9.76	12.91	3.15	22	39	17	28.8	30.4	1.6	59.2	64.4	5.2	hypochromic microcytic	hypochromic microcytic	2	1	1
40	19	7.7	8.5	0.8	547	518	-29	7.21	10.16	2.95	8.37	10.69	2.32	18	36	18	27.9	29.3	1.4	55.2	60.4	5.2	hypochromic microcytic	hypochromic microcytic	3	2	1
41	17	8.1	9.3	1.2	539	500	-39	8.39	11.58	3.19	8.61	12.05	3.44	21	40	19	28.9	30.1	1.2	57.8	61.6	3.8	hypochromic microcytic	hypochromic microcytic	2	1	1
42	16	8.3	9.3	1	542	522	-20	8.92	11.64	2.72	9.11	12.14	3.03	24	45	21	28.1	30.6	2.5	57.5	61.4	3.9	hypochromic microcytic	hypochromic microcytic	2	1	1
43	19	7.4	8.7	1.3	547	501	-46	7.61	11.82	4.21	7.81	11.55	3.74	17	48	31	27.2	29.6	2.4	55.8	59.7	3.9	hypochromic microcytic	hypochromic microcytic	3	2	1
44	18	8.6	9.5	0.9	521	498	-23	9.04	12.37	3.33	9.82	12.48	2.66	28	47	19	28	29.8	1.8	58.6	63.3	4.7	hypochromic microcytic	hypochromic microcytic	2	1	1
45	17	9.4	10.6	1.2	515	484	-31	8.79	11.38	2.59	9.55	12.36	2.81	29	49	20	29.4	31.5	2.1	65.1	69.3	4.2	hypochromic microcytic	hypochromic microcytic	1	1	0
46	19	10.2	11.3	1.1	503	478	-25	11.9	14.46	2.57	9.73	13.97	4.24	28	48	20	30.4	31.8	1.4	66.3	71.7	5.4	hypochromic microcytic	hypochromic microcytic	1	0	1
47	19	9.4	10.2	0.8	487	465	-22	9.42	11.71	2.29	9.62	12.71	3.09	29	48	19	29.7	30.9	1.2	65.7	69.9	4.2	hypochromic microcytic	hypochromic microcytic	1	1	0
48	19	7.7	9	1.3	565	524	-41	7.26	11.51	4.25	8.55	12.57	4.02	18	37	19	27.4	29.5	2.1	56.3	62.6	6.3	hypochromic microcytic	hypochromic microcytic	3	2	1
49	19	9.4	10.5	1.1	524	502	-22	9.83	12.37	2.54	9.5	13.72	4.22	30	52	22	29.7	30.8	1.1	63.5	69.7	6.2	hypochromic microcytic	hypochromic microcytic	1	0	1
50	18	8.4	9.6	1.2	541	504	-37	8.41	12.29	3.88	9.82	12.91	3.09	29	46	17	28.3	29.9	1.6	59.3	64.4	5.1	hypochromic microcytic	hypochromic microcytic	2	1	1
51	18	9.1	10	0.9	517	495	-22	9.12	11.92	2.8	10.16	13.85	3.69	28	47	19	29.2	31.9	2.7	62	67.1	5.1	hypochromic microcytic	hypochromic microcytic	1	1	0
52	19	9.5	10.7	1.2	532	501	-31	9.47	12.88	3.41	9.92	13.21	3.29	31	48	17	29.4	31.7	2.3	65.9	69.6	3.7	hypochromic microcytic	hypochromic microcytic	1	0	1
53	17	7.4	8.9	1.5	521	479	-42	7.22	12.54	5.32	8.34	12.96	4.62	19	42	23	26.8	29.7	2.9	54.3	60.9	6.6	hypochromic microcytic	hypochromic microcytic	3	2	1
54	19	9.8	10.4	0.6	519	492	-27	10.6	12.74	2.11	10.14	13.26	3.12	29	48	19	29.6	30.8	1.2	64.7	69.1	4.4	hypochromic microcytic	hypochromic microcytic	1	1	0

55	19	8.5	9.3	0.8	523	494	-29	8.16	10.82	2.66	9.73	13.05	3.32	23	40	17	28.5	29.7	1.2	57.3	60.7	3.4	hypochromic microcytic	hypochromic microcytic	2	1	1
56	19	7.2	8.3	1.1	547	523	-24	6.52	10.97	4.45	8.16	11.95	3.79	21	38	17	26.8	28.5	1.7	52.2	57.3	5.1	hypochromic microcytic	hypochromic microcytic	3	2	1
57	19	8.1	9.5	1.4	536	487	-49	8.06	11.95	3.89	9.54	13.56	4.02	28	47	19	27.9	29.4	1.5	58.1	64.6	6.5	hypochromic microcytic	hypochromic microcytic	3	1	2
58	18	9.2	10.4	1.2	511	465	-46	9.51	11.96	2.45	9.76	13.31	3.55	29	49	20	29.5	31.7	2.2	64.6	68.7	4.1	hypochromic microcytic	hypochromic microcytic	1	0	1
59	18	9.7	10.6	0.9	507	486	-21	10.2	13.53	3.29	10.54	13.62	3.08	31	46	15	29.7	31.3	1.6	65.1	69.5	4.4	hypochromic microcytic	hypochromic microcytic	1	0	1
60	17	8.3	9.1	0.8	522	501	-21	8.85	11.19	2.34	9.28	12.75	3.47	28	49	21	27.5	29.1	1.6	59.8	65.6	5.8	hypochromic microcytic	hypochromic microcytic	2	1	1
61	18	7.6	8.9	1.3	564	524	-40	7.26	11.71	4.45	7.79	11.72	3.93	20	40	20	26.6	28.4	1.8	57.4	61.1	3.7	hypochromic microcytic	hypochromic microcytic	3	1	2
62	18	10.1	11.1	1	486	457	-29	9.78	12.56	2.78	10.59	13.45	2.86	31	49	18	30.1	31.7	1.6	68.7	73.5	4.8	hypochromic microcytic	hypochromic microcytic	1	0	1
63	19	10.7	11.6	0.9	475	462	-13	11.2	13.46	2.25	11.77	14.22	2.45	35	55	20	31.1	32.4	1.3	70.6	75.6	5	hypochromic microcytic	Normochromic Normocytic	0	0	0
64	18	8.9	10	1.1	549	514	-35	9.16	11.88	2.72	8.96	11.58	2.62	28	48	20	29.4	30.6	1.2	61.7	68	6.3	hypochromic microcytic	hypochromic microcytic	2	1	1
65	18	7.9	8.8	0.9	531	497	-34	7.77	10.89	3.12	8.45	11.83	3.38	25	40	15	27.8	29.9	2.1	55.1	58.6	3.5	hypochromic microcytic	hypochromic microcytic	2	1	1
66	19	8.2	9.4	1.2	497	456	-41	8.17	12.86	4.69	9.62	13.17	3.55	28	48	20	28.9	30.7	1.8	58.3	63.2	4.9	hypochromic microcytic	hypochromic microcytic	2	1	1
67	18	7.8	9	1.2	561	524	-37	8.46	11.97	3.51	8.73	12.58	3.85	27	45	18	27.8	29.9	2.1	56.4	62.1	5.7	hypochromic microcytic	hypochromic microcytic	3	1	2
68	19	10.8	11.7	0.9	463	447	-16	11.9	14.12	2.26	12.27	14.06	1.79	37	58	21	30.7	32.8	2.1	70.9	76.2	5.3	hypochromic microcytic	Normochromic Normocytic	0	0	0
69	17	8.6	9.9	1.3	556	531	-25	9.21	12.35	3.14	9.04	13.32	4.28	22	42	20	28.1	30.8	2.7	59.6	65.4	5.8	hypochromic microcytic	hypochromic microcytic	2	1	1
70	18	9.1	10.3	1.2	546	509	-37	9.35	12.57	3.22	9.8	13.84	4.04	28	46	18	29.1	31.1	2	64.8	68.9	4.1	hypochromic microcytic	hypochromic microcytic	1	1	0
71	18	7.1	8.5	1.4	531	489	-42	7.14	11.92	4.78	7.04	11.73	4.69	26	47	21	26.6	28.7	2.1	51.2	57.5	6.3	hypochromic microcytic	hypochromic microcytic	2	2	0
72	19	7.7	9.9	2.2	541	502	-39	7.25	12.79	5.54	8.21	13.86	5.65	27	46	19	27.9	30.3	2.4	60.4	66.4	6	hypochromic microcytic	hypochromic microcytic	3	1	2
73	18	8.3	9.4	1.1	549	521	-28	8.17	10.23	2.06	8.41	11.97	3.56	28	48	20	28.2	30.9	2.7	58.1	64.9	6.8	hypochromic microcytic	hypochromic microcytic	2	1	1
74	17	7.6	8.7	1.1	540	514	-26	8.36	11.47	3.11	8.15	11.37	3.22	27	45	18	27.3	29.7	2.4	55.4	59.4	4	hypochromic microcytic	hypochromic microcytic	3	2	1
75	18	9.1	9.9	0.8	490	466	-24	9.56	12.17	2.61	9.34	12.92	3.58	29	49	20	29.5	30.2	0.7	65.2	68	2.8	hypochromic microcytic	hypochromic microcytic	1	1	0
76	17	9.3	10.4	1.1	480	463	-17	9.21	11.38	2.17	9.26	12.99	3.73	30	49	19	29.4	30.7	1.3	66.9	70.8	3.9	hypochromic microcytic	hypochromic microcytic	1	1	0
77	17	7.8	9.2	1.4	540	504	-36	8.95	14.04	5.09	8.1	13.24	5.14	27	44	17	28.2	30.8	2.6	57.2	61.6	4.4	hypochromic microcytic	hypochromic microcytic	2	1	1
78	18	8.9	10.2	1.3	526	492	-34	8.72	12.82	4.1	9.16	13.53	4.37	28	47	19	29.1	31.5	2.4	63.4	69.7	6.3	hypochromic microcytic	hypochromic microcytic	2	0	2
79	18	7.4	8.6	1.2	528	486	-42	7.31	11.56	4.25	7.45	11.69	4.24	17	37	20	27.2	29.4	2.2	55.7	59	3.3	hypochromic microcytic	hypochromic microcytic	3	2	1
80	17	9.6	10.7	1.1	546	524	-22	10.1	12.68	2.61	10.61	13.76	3.15	30	48	18	28.9	30.6	1.7	63.4	68.6	5.2	hypochromic microcytic	hypochromic microcytic	1	0	1
81	19	8.1	9.4	1.3	487	456	-31	8.49	11.61	3.12	8.92	13.04	4.12	21	46	25	28.4	30.4	2	58.1	64.5	6.4	hypochromic microcytic	hypochromic microcytic	2	1	1
82	19	9.4	10.5	1.1	498	476	-22	10.8	12.36	1.61	10.23	13.61	3.38	29	49	20	29.4	30.5	1.1	65.6	68.2	2.6	hypochromic microcytic	hypochromic microcytic	1	0	1
83	19	8.5	10	1.5	554	513	-41	8.34	11.72	3.38	8.67	13.67	5	28	47	19	28.3	30.1	1.8	60.2	67.1	6.9	hypochromic microcytic	hypochromic microcytic	2	1	1

84	19	8.7	10.1	1.4	516	479	-37	8.93	12.23	3.3	9.52	14.41	4.89	21	44	23	28.1	30.9	2.8	63.3	69.4	6.1	hypochromic microcytic	hypochromic microcytic	2	1	1
85	19	7.9	9.1	1.2	507	469	-38	8.32	11.81	3.49	8.65	12.92	4.27	22	39	17	26.6	29.8	3.2	55.4	58.9	3.5	hypochromic microcytic	hypochromic microcytic	2	1	1
86	18	8.2	9.4	1.2	566	531	-35	8.78	11.57	2.79	9.62	13.56	3.94	24	44	20	28.5	30.1	1.6	57.6	63.8	6.2	hypochromic microcytic	hypochromic microcytic	2	1	1
87	17	8.7	9.9	1.2	536	498	-38	8.63	11.12	2.49	9.37	12.79	3.42	28	49	21	28.6	30.4	1.8	60.1	63.5	3.4	hypochromic microcytic	hypochromic microcytic	2	1	1
88	17	9.2	10.4	1.2	512	477	-35	9.41	12.69	3.28	10.11	13.45	3.34	29	48	19	29.2	31.5	2.3	63.1	68.8	5.7	hypochromic microcytic	hypochromic microcytic	1	0	1
89	17	10.4	11.5	1.1	486	456	-30	11.2	11.72	0.57	11.83	15.74	3.91	35	54	19	30.8	32.6	1.8	71.4	76.7	5.3	hypochromic microcytic	Normochromic Normocytic	0	0	0
90	19	8.4	9.3	0.9	532	507	-25	8.63	11.54	2.91	9.05	12.25	3.2	29	46	17	28.4	30.1	1.7	58.7	62.3	3.6	hypochromic microcytic	hypochromic microcytic	2	2	0
91	17	10.1	11.2	1.1	501	477	-24	10.3	14.27	3.96	10.41	12.47	2.06	32	52	20	30.6	31.5	0.9	62.4	67.2	4.8	hypochromic microcytic	hypochromic microcytic	1	0	1
92	17	7.8	9.3	1.5	506	476	-30	7.54	11.28	3.74	8.71	11.27	2.56	28	49	21	27.7	30.8	3.1	55.7	61.3	5.6	hypochromic microcytic	hypochromic microcytic	2	1	1
93	18	9.7	10.8	1.1	492	461	-31	9.94	13.35	3.41	10.63	12.76	2.13	30	48	18	29.9	31.5	1.6	64.1	68.9	4.8	hypochromic microcytic	hypochromic microcytic	1	0	0
94	17	9.2	10.6	1.4	534	502	-32	9.67	12.62	2.95	9.73	13.42	3.69	29	49	20	29.2	31.4	2.2	63.3	68.1	4.8	hypochromic microcytic	hypochromic microcytic	1	0	0
95	18	8.1	9.2	1.1	543	521	-22	8.35	11.44	3.09	9.89	12.43	2.54	24	41	17	28	30.2	2.2	56.3	61.1	4.8	hypochromic microcytic	hypochromic microcytic	2	1	1
96	18	9.8	10.8	1	505	468	-37	9.74	12.26	2.52	10.26	12.48	2.22	31	53	22	29.4	30.9	1.5	63.7	66.3	2.6	hypochromic microcytic	hypochromic microcytic	1	0	1
97	17	8.5	9.6	1.1	546	524	-22	8.63	11.26	2.63	9.81	12.55	2.74	25	38	13	28.6	30.8	2.2	59.2	65.7	6.5	hypochromic microcytic	hypochromic microcytic	2	1	1
98	18	9.8	10.6	0.8	521	505	-16	10.5	13.23	2.77	10.08	13.29	3.21	31	50	19	29.6	31.7	2.1	65.6	70.4	4.8	hypochromic microcytic	hypochromic microcytic	1	0	1
99	19	8.6	9.7	1.1	537	504	-33	8.51	11.85	3.34	8.79	11.34	2.55	29	49	20	27.4	30.2	2.8	59.1	65.6	6.5	hypochromic microcytic	hypochromic microcytic	1	1	0
100	17	9.3	10.4	1.1	523	504	-19	9.12	12.25	3.13	9.72	11.28	1.56	29	52	23	28.9	30.1	1.2	63.6	68.2	4.6	hypochromic microcytic	hypochromic microcytic	1	0	1
101	19	8.3	9.6	1.3	514	497	-17	8.54	11.56	3.02	9.67	11.56	1.89	28	55	27	27.9	30.6	2.7	58.8	62.2	3.4	hypochromic microcytic	hypochromic microcytic	2	1	1
102	18	8.9	9.2	0.3	501	488	-13	9.84	11.84	2	9.61	12.39	2.78	27	48	21	28.1	29.6	1.5	58.1	63.9	5.8	hypochromic microcytic	hypochromic microcytic	2	1	1
103	18	7.8	8.9	1.1	521	505	-16	7.31	11.29	3.98	7.69	11.17	3.48	27	45	18	27.4	29.6	2.2	52.2	55.5	3.3	hypochromic microcytic	hypochromic microcytic	3	2	1
104	17	9.7	10.8	1.1	500	468	-32	10.9	15.78	4.87	9.21	11.85	2.64	30	51	21	28.5	30.4	1.9	64.6	69.4	4.8	hypochromic microcytic	hypochromic microcytic	1	0	1
105	19	9.5	10.6	1.1	521	490	-31	9.13	13.58	4.45	9.65	12.31	2.66	30	48	18	29.2	31	1.8	55.7	60.3	4.6	hypochromic microcytic	hypochromic microcytic	1	0	1
106	17	9.4	10.1	0.7	537	524	-13	10	12.36	2.34	9.83	12.45	2.62	29	42	13	29.3	30.7	1.4	63.1	66.5	3.4	hypochromic microcytic	hypochromic microcytic	1	0	1
107	18	10.3	11.2	0.9	496	478	-18	11.3	16.45	5.11	11.28	14.72	3.44	33	55	22	30.9	31.8	0.9	67.1	72.9	5.8	hypochromic microcytic	hypochromic microcytic	0	0	0
108	18	9.4	10.5	1.1	512	488	-24	9.65	12.51	2.86	9.68	11.74	2.06	30	49	19	29.7	31.3	1.6	64.2	69.6	5.4	hypochromic microcytic	hypochromic microcytic	1	0	1
109	19	9.1	10.5	1.4	503	463	-40	8.97	14.62	5.65	9.71	12.57	2.86	29	49	20	29.2	30.8	1.6	63.4	68.1	4.7	hypochromic microcytic	hypochromic microcytic	1	0	1
110	17	7.9	8.9	1	531	502	-29	7.34	11.07	3.73	8.85	11.97	3.12	28	48	20	26.8	29.5	2.7	52.3	56.6	4.3	hypochromic microcytic	hypochromic microcytic	2	1	1
111	19	9.2	10.4	1.2	506	471	-35	9.7	13.85	4.15	9.9	13.43	3.53	29	35	6	28.1	29.9	1.8	65.4	69.2	3.8	hypochromic microcytic	hypochromic microcytic	1	0	1
112	18	8.1	9	0.9	459	446	-13	8.54	11.85	3.31	9.13	12.24	3.11	26	48	22	28.1	29.4	1.3	54.2	58.6	4.4	hypochromic microcytic	hypochromic microcytic	2	1	1

113	17	9.6	10.7	1.1	499	478	-21	10.3	12.32	2.05	9.26	11.86	2.6	30	49	19	29.4	30.9	1.5	64.2	69.2	5	hypochromic microcytic	hypochromic microcytic	1	0	1
114	17	8.3	9.7	1.4	563	527	-36	8.56	11.84	3.28	8.42	11.35	2.93	25	49	24	27	30.6	3.6	58.9	65.3	6.4	hypochromic microcytic	hypochromic microcytic	2	1	1
115	18	8.4	9.2	0.8	526	497	-29	8.64	11.27	2.63	8.73	11.91	3.18	25	47	22	27.8	29.6	1.8	57.3	62.1	4.8	hypochromic microcytic	hypochromic microcytic	2	1	1
116	17	10	11.2	1.2	470	448	-22	10.1	14.33	4.19	10.31	15.51	5.2	32	55	23	29.6	31.4	1.8	67.2	72.7	5.5	hypochromic microcytic	hypochromic microcytic	1	0	1
117	19	9.1	10.2	1.1	502	482	-20	9.47	11.97	2.5	10.31	13.27	2.96	28	48	20	29.1	30.8	1.7	62.1	66.6	4.5	hypochromic microcytic	hypochromic microcytic	1	1	0
118	19	7.6	8.8	1.2	575	549	-26	7.25	11.54	4.29	8.14	12.84	4.7	27	48	21	27.2	29.5	2.3	53.3	59.3	6	hypochromic microcytic	hypochromic microcytic	3	2	1
119	19	10.1	11.3	1.2	487	461	-26	11.8	16.92	5.11	10.51	14.95	4.44	35	58	23	29.4	31.7	2.3	68.1	73.2	5.1	hypochromic microcytic	hypochromic microcytic	1	0	1
120	18	8.5	9.4	0.9	511	484	-27	8.12	12.39	4.27	10.3	13.14	2.84	28	47	19	28.2	30.5	2.3	59	65.5	6.5	hypochromic microcytic	hypochromic microcytic	2	1	1
121	19	8.2	9.3	1.1	496	467	-29	7.79	11.97	4.18	9.93	12.87	2.94	26	48	22	28.2	29.7	1.5	56.1	60.2	4.1	hypochromic microcytic	hypochromic microcytic	2	1	1
122	17	8.7	9.9	1.2	534	499	-35	8.52	12.76	4.24	9.71	12.98	3.27	29	49	20	26.9	30	3.1	57.7	62.7	5	hypochromic microcytic	hypochromic microcytic	1	1	0
123	19	8.5	9.6	1.1	560	524	-36	8.73	11.95	3.22	9.11	11.47	2.36	28	49	21	28.1	29.6	1.5	58.2	64	5.8	hypochromic microcytic	hypochromic microcytic	2	1	1
124	17	7.5	8.3	0.8	568	545	-23	7.28	9.97	2.69	9.67	11.79	2.12	20	39	19	26.2	28.1	1.9	51.1	54.5	3.4	hypochromic microcytic	hypochromic microcytic	3	2	1
125	18	8.6	9.7	1.1	551	523	-28	8.91	12.29	3.38	9.34	12.36	3.02	29	49	20	28.9	30.2	1.3	58.4	64.2	5.8	hypochromic microcytic	hypochromic microcytic	2	1	1
126	19	9.4	10.6	1.2	529	496	-33	9.86	12.65	2.79	10.23	13.46	3.23	24	48	24	29.5	31.7	2.2	61.1	65.7	4.6	hypochromic microcytic	hypochromic microcytic	1	0	1
127	18	10.2	11.2	1	489	458	-31	11.7	16.26	4.61	11.92	14.97	3.05	33	53	20	30.6	31.2	0.6	68	72.4	4.4	hypochromic microcytic	hypochromic microcytic	0	0	0
128	19	9.5	10.6	1.1	503	455	-48	10.2	13.57	3.33	10.87	13.85	2.98	30	49	19	29.7	31.9	2.2	65.3	68.1	2.8	hypochromic microcytic	hypochromic microcytic	1	0	1
129	18	7.7	8.9	1.2	576	547	-29	8.14	12.54	4.4	7.23	11.15	3.92	22	46	24	27.2	29.7	2.5	53.7	59.2	5.5	hypochromic microcytic	hypochromic microcytic	3	2	1
130	17	9.4	10.2	0.8	504	477	-27	10.9	13.92	3.03	9.38	14.24	4.86	29	45	16	29	30.1	1.1	64.2	66.4	2.2	hypochromic microcytic	hypochromic microcytic	1	1	0

Sr. No.	Age (year)	HB0	HB1	HB Diff	TIBC0	TIBC1	TIBC Diff	SrFerratin0	SrFerratin1	Sr ferratin diff.	SrTransferin0	SrTransferin1	SrTransferin_diff	SrIron0	SrIron1	SrIron_diff	MCHC0	MCHC1	MCHC_diff	MCV0	MCV1	MCV_diff	Blood_smear0	Blood_smear1	Pallor 0	Pallor 1	Pallor diff
1	17	8.4	9.1	0.7	529	501	-28	7.79	10.11	2.32	8.85	10.15	1.3	25	30	5	28.1	29.2	1.1	60.1	62.5	2.4	hypochromic microcytic	hypochromic microcytic	2	1	1
2	18	8.5	9.1	0.6	537	522	-15	6.98	8.42	1.44	7.9	9.18	1.28	19	28	9	28.2	29.1	0.9	61.2	63.2	2	hypochromic microcytic	hypochromic microcytic	2	2	0
3	19	9.1	9.6	0.5	501	484	-17	8.95	10.31	1.36	9.78	11.51	1.73	28	31	3	29	30.1	1.1	64.8	67.3	2.5	hypochromic microcytic	hypochromic microcytic	2	1	1
4	18	7.9	8.4	0.5	539	517	-22	8.23	10.71	2.48	8.12	11.91	3.79	23	29	6	27.4	28.5	1.1	57.2	58.7	1.5	hypochromic microcytic	hypochromic microcytic	2	1	1
5	18	8.4	8.9	0.5	537	506	-31	8.9	10.2	1.3	8.27	10.74	2.47	27	38	11	28.6	29.6	1	58.7	60.1	1.4	hypochromic microcytic	hypochromic microcytic	2	2	0
6	18	7.6	8.2	0.6	561	549	-12	7.61	10.41	2.8	8.25	10.77	2.52	20	29	9	27.4	28.6	1.2	54.8	57.2	2.4	hypochromic microcytic	hypochromic microcytic	3	3	0
7	17	9.1	9.9	0.8	498	476	-22	9.15	11.47	2.32	9.77	13.51	3.74	23	32	9	29.6	30.4	0.8	62.9	64.2	1.3	hypochromic microcytic	hypochromic microcytic	1	0	1
8	18	10.1	10.9	0.8	481	461	-20	11.26	13.75	2.49	11.5	12.11	0.61	31	39	8	30.4	31.6	1.2	68.2	70.5	2.3	hypochromic microcytic	hypochromic microcytic	2	1	1
9	18	7.5	8.1	0.6	562	546	-16	8.1	10.5	2.4	8.31	10.47	2.16	21	27	6	27.8	28.8	1	55.3	57.6	2.3	hypochromic microcytic	hypochromic microcytic	3	3	0
10	18	8.7	9.2	0.5	528	515	-13	9.44	10.74	1.3	8.43	10.89	2.46	28	34	6	29.1	30.2	1.1	59.9	62.2	2.3	hypochromic microcytic	hypochromic microcytic	2	1	1
11	19	9.2	9.8	0.6	542	537	-5	9.57	12.09	2.52	9.88	12.6	2.72	35	44	9	30.4	31.2	0.8	69.8	71.5	1.7	hypochromic microcytic	hypochromic microcytic	2	1	1
12	19	7.4	8.1	0.7	571	553	-18	7.69	9.48	1.79	7.65	10.48	2.83	15	25	10	26.9	28.2	1.3	55.7	57.7	2	hypochromic microcytic	hypochromic microcytic	3	3	0
13	19	7.6	8.4	0.8	535	519	-16	8.26	9.69	1.43	7.75	10.56	2.81	26	31	5	27	28.4	1.4	56.2	58.3	2.1	hypochromic microcytic	hypochromic microcytic	3	2	1
14	19	9.5	10.2	0.7	498	479	-19	9.61	11.55	1.94	9.15	11.56	2.41	31	43	12	29.4	30.5	1.1	62.2	65.2	3	hypochromic microcytic	hypochromic microcytic	1	1	0
15	18	10.4	11.2	0.8	477	456	-21	12.14	14.95	2.81	11.96	15.49	3.53	37	48	11	30.8	31.7	0.9	69.6	72.1	2.5	hypochromic microcytic	hypochromic microcytic	0	0	0
16	18	9.5	9.9	0.4	484	464	-20	9.42	11.13	1.71	9.51	11.31	1.8	29	39	10	29.5	30.1	0.6	65.1	67.3	2.2	hypochromic microcytic	hypochromic microcytic	1	1	0
17	17	8.6	9.3	0.7	523	501	-22	8.4	10.77	2.37	8.77	11.14	2.37	27	32	5	28.5	29.5	1	58.3	61.3	3	hypochromic microcytic	hypochromic microcytic	2	1	1
18	17	9.2	9.9	0.7	531	511	-20	9.12	11.56	2.44	9.99	12.24	2.25	30	37	7	29.2	30.5	1.3	64.2	67.5	3.3	hypochromic microcytic	hypochromic microcytic	1	1	0

19	18	7.6	8.2	0.6	557	539	-18	6.55	8.49	1.94	7.67	9.15	1.48	18	29	11	27.7	28.5	0.8	54.3	56.8	2.5	hypochromic microcytic	hypochromic microcytic	3	3	0
20	19	10.3	11	0.7	489	476	-13	10.94	13.15	2.21	12.88	15.12	2.24	34	44	10	30.1	31.8	1.7	68.6	71.7	3.1	hypochromic microcytic	hypochromic microcytic	0	0	0
21	19	8.5	9.2	0.7	487	470	-17	8.28	9.78	1.5	8.45	10.21	1.76	21	28	7	28.1	29.7	1.6	59.2	62.1	2.9	hypochromic microcytic	hypochromic microcytic	2	1	1
22	17	9.4	9.8	0.4	512	478	-34	9.17	10.43	1.26	9.15	11.41	2.26	27	39	12	29.1	30	0.9	64.8	65.5	0.7	hypochromic microcytic	hypochromic microcytic	1	1	0
23	16	7.8	8.5	0.7	542	514	-28	7.47	9.98	2.51	8.11	10.67	2.56	22	36	14	27.6	29	1.4	56.2	58	1.8	hypochromic microcytic	hypochromic microcytic	3	2	1
24	16	8.2	9.1	0.9	514	488	-26	8.49	11.79	3.3	8.36	12.14	3.78	23	31	8	28.4	29.9	1.5	57.3	60.2	2.9	hypochromic microcytic	hypochromic microcytic	2	1	1
25	17	9.5	10	0.5	542	511	-31	9.13	11.25	2.12	10.25	13.16	2.91	29	40	11	29.9	30.8	0.9	65.8	67.9	2.1	hypochromic microcytic	hypochromic microcytic	1	1	0
26	19	9.2	9.8	0.6	519	518	-1	9.23	11.13	1.9	9.14	10.79	1.65	29	28	-1	29.7	30.7	1	62.7	64.1	1.4	hypochromic microcytic	hypochromic microcytic	1	1	0
27	18	10.1	10.9	0.8	509	496	-13	11.89	14.12	2.23	10.97	13.58	2.61	35	45	10	30.5	31.2	0.7	68.9	71.2	2.3	hypochromic microcytic	hypochromic microcytic	1	0	1
28	19	8.2	9.1	0.9	547	521	-26	8.16	10.56	2.4	8.76	10.41	1.65	24	35	11	28.5	29.4	0.9	58.3	60.2	1.9	hypochromic microcytic	hypochromic microcytic	2	1	1
29	19	8.6	8.9	0.3	531	508	-23	8.91	9.24	0.33	8.37	9.53	1.16	29	27	-2	28.4	29	0.6	57.2	58.3	1.1	hypochromic microcytic	hypochromic microcytic	2	2	0
30	19	9.1	9.8	0.7	501	489	-12	9.27	11.81	2.54	9.35	11.74	2.39	29	42	13	29.6	30.7	1.1	63.1	65.7	2.6	hypochromic microcytic	hypochromic microcytic	1	1	0
31	19	9.6	10.2	0.6	486	474	-12	10.26	12.96	2.7	11.42	13.98	2.56	27	34	7	27.7	28.6	0.9	54.9	57.5	2.6	hypochromic microcytic	hypochromic microcytic	1	1	0
32	18	8.2	8.4	0.2	532	521	-11	8.43	8.92	0.49	8.27	9.93	1.66	27	36	9	28.8	29.2	0.4	58.1	59	0.9	hypochromic microcytic	hypochromic microcytic	2	2	0
33	18	9.6	10.1	0.5	534	506	-28	9.68	12.69	3.01	9.78	11.55	1.77	31	43	12	29.8	30.2	0.4	66.2	67.4	1.2	hypochromic microcytic	hypochromic microcytic	1	1	0
34	17	7.5	8.4	0.9	547	519	-28	7.24	10.98	3.74	8.21	10.72	2.51	24	37	13	26.7	28.2	1.5	55.9	58.2	2.3	hypochromic microcytic	hypochromic microcytic	3	2	1
35	17	9.2	9.9	0.7	491	476	-15	10.63	12.94	2.31	10.55	12.92	2.37	39	44	5	29.5	30.4	0.9	68.9	71.4	2.5	hypochromic microcytic	hypochromic microcytic	1	1	0
36	18	9.2	9.4	0.2	526	505	-21	9.75	11.02	1.27	10.03	11.77	1.74	28	35	7	29.4	29.8	0.4	64.5	65.5	1	hypochromic microcytic	hypochromic microcytic	1	1	0
37	19	7.2	7.9	0.7	552	524	-28	7.34	8.89	1.55	7.46	9.97	2.51	16	29	13	26.8	28.1	1.3	56.2	58.4	2.2	hypochromic microcytic	hypochromic microcytic	3	3	0
38	19	9.3	9.7	0.4	516	501	-15	9.74	11.61	1.87	10.23	11.95	1.72	31	35	4	29.5	30.5	1	63.1	65.4	2.3	hypochromic microcytic	hypochromic microcytic	1	1	0
39	18	8.1	8.7	0.6	529	507	-22	7.89	9.64	1.75	8.91	10.25	1.34	25	36	11	28.5	29.7	1.2	58.4	60.4	2	hypochromic microcytic	hypochromic microcytic	2	2	0

40	19	9.3	10	0.7	502	489	-13	9.56	10.98	1.42	10.21	11.14	0.93	29	37	8	26.7	28.1	1.4	55.3	58	2.7	hypochromic microcytic	hypochromic microcytic	1	1	0
41	17	8.2	8.8	0.6	516	498	-18	8.62	10.24	1.62	8.34	11.17	2.83	26	41	15	28.6	28.9	0.3	59.1	58.7	-0.4	hypochromic microcytic	hypochromic microcytic	2	2	0
42	19	10.2	10.8	0.6	486	475	-11	10.44	10.87	0.43	11.17	11.55	0.38	34	46	12	30.5	31.5	1	69.3	74.5	5.2	hypochromic microcytic	hypochromic microcytic	1	0	1
43	18	8	8.9	0.9	524	509	-15	8.27	10.56	2.29	8.42	9.87	1.45	19	30	11	27.4	29	1.6	54.9	58.8	3.9	hypochromic microcytic	hypochromic microcytic	2	2	0
44	19	8.1	8.9	0.8	521	499	-22	8.92	11.38	2.46	8.82	10.41	1.59	25	31	6	28.8	29.4	0.6	56.1	59.3	3.2	hypochromic microcytic	hypochromic microcytic	2	2	0
45	18	10.4	10.9	0.5	475	436	-39	11.46	13.92	2.46	12.21	14.76	2.55	28	35	7	31	31.6	0.6	68.2	70.6	2.4	hypochromic microcytic	hypochromic microcytic	0	0	0
46	19	9.1	9.9	0.8	511	478	-33	10.25	13.79	3.54	10.16	12.78	2.62	34	44	10	30.7	31.8	1.1	66.7	69.9	3.2	hypochromic microcytic	hypochromic microcytic	1	1	0
47	17	8.9	9.8	0.9	487	475	-12	8.45	11.26	2.81	8.61	10.68	2.07	23	39	16	28.6	30.1	1.5	62.4	65.6	3.2	hypochromic microcytic	hypochromic microcytic	2	1	1
48	16	7.6	8.4	0.8	551	527	-24	6.91	9.15	2.24	8.29	9.75	1.46	18	29	11	27.4	28.1	0.7	55.2	58.2	3	hypochromic microcytic	hypochromic microcytic	3	2	1
49	16	9.4	10.2	0.8	487	484	-3	11.43	14.97	3.54	10.41	14.96	4.55	37	43	6	29.5	30.4	0.9	68.4	71.2	2.8	hypochromic microcytic	hypochromic microcytic	1	1	0
50	17	8.4	9.3	0.9	504	487	-17	8.29	10.94	2.65	9.13	11.51	2.38	22	35	13	28.9	30.4	1.5	59.8	63.4	3.6	hypochromic microcytic	hypochromic microcytic	2	1	1
51	18	9.2	10.1	0.9	517	485	-32	9.36	11.84	2.48	9.76	12.23	2.47	28	37	9	29.7	30.5	0.8	63.7	67.2	3.5	hypochromic microcytic	hypochromic microcytic	1	1	0
52	19	9.4	10.2	0.8	521	480	-41	9.18	11.76	2.58	10.37	11.85	1.48	30	40	10	29.1	30.8	1.7	64.1	67.3	3.2	hypochromic microcytic	hypochromic microcytic	1	1	0
53	18	7.2	7.8	0.6	571	541	-30	7.95	9.16	1.21	7.95	9.52	1.57	22	37	15	26.6	28.1	1.5	53.4	56.2	2.8	hypochromic microcytic	hypochromic microcytic	3	3	0
54	18	9.6	10.2	0.6	471	463	-8	9.54	11.18	1.64	9.82	12.79	2.97	29	35	6	29.4	30	0.6	67.7	70.9	3.2	hypochromic microcytic	hypochromic microcytic	1	0	1
55	19	8.4	8.7	0.3	541	524	-17	8.87	10.29	1.42	9.27	10.76	1.49	26	30	4	28.5	29.1	0.6	57.3	58.7	1.4	hypochromic microcytic	hypochromic microcytic	2	2	0
56	19	7.1	7.9	0.8	567	551	-16	7.76	9.45	1.69	8.38	9.96	1.58	19	31	12	26.6	27.7	1.1	52.9	55.2	2.3	hypochromic microcytic	hypochromic microcytic	3	3	0
57	18	8.4	9.2	0.8	526	498	-28	8.25	10.74	2.49	9.5	11.16	1.66	26	36	10	28.4	29.3	0.9	58.7	61.8	3.1	hypochromic microcytic	hypochromic microcytic	2	1	1
58	16	9.1	9.9	0.8	515	486	-29	9.35	11.86	2.51	9.67	12.25	2.58	31	41	10	29	30.2	1.2	62.7	65.4	2.7	hypochromic microcytic	hypochromic microcytic	1	1	0
59	17	9.6	10.2	0.6	542	521	-21	9.56	12.23	2.67	9.51	12.47	2.96	31	38	7	29	30.1	1.1	62.2	65.4	3.2	hypochromic microcytic	hypochromic microcytic	1	1	0
60	19	8.1	8.9	0.8	512	493	-19	8.06	10.71	2.65	9.14	10.87	1.73	27	39	12	27.2	28.2	1	56.8	59.4	2.6	hypochromic microcytic	hypochromic microcytic	2	2	0

61	18	7.5	8.4	0.9	534	506	-28	7.55	9.46	1.91	8.27	10.99	2.72	28	37	9	26.9	28.4	1.5	55.6	57.5	1.9	hypochromic microcytic	hypochromic microcytic	3	2	1
62	19	10.1	10.8	0.7	509	483	-26	10.28	12.24	1.96	10.35	12.26	1.91	36	42	6	30.4	31.7	1.3	66.7	69.6	2.9	hypochromic microcytic	hypochromic microcytic	1	0	1
63	18	10.2	10.9	0.7	490	478	-12	10.05	12.97	2.92	11.24	14.97	3.73	34	44	10	29.9	30.7	0.8	66.4	69.5	3.1	hypochromic microcytic	hypochromic microcytic	1	0	1
64	18	8.6	9.4	0.8	521	507	-14	9.12	11.78	2.66	9.87	11.57	1.7	27	39	12	28.7	30.2	1.5	57.3	61.2	3.9	hypochromic microcytic	hypochromic microcytic	2	1	1
65	17	7.3	8	0.7	567	551	-16	7.15	10.99	3.84	7.79	10.41	2.62	16	29	13	26.7	28	1.3	56.8	59.1	2.3	hypochromic microcytic	hypochromic microcytic	3	3	0
66	18	8.2	8.9	0.7	497	476	-21	8.51	11.92	3.41	8.27	11.21	2.94	28	35	7	28.1	29.4	1.3	56.4	58.9	2.5	hypochromic microcytic	hypochromic microcytic	2	2	0
67	19	7.6	8.3	0.7	551	531	-20	7.54	10.17	2.63	8.26	10.17	1.91	27	37	10	27.5	28.7	1.2	56.7	58	1.3	hypochromic microcytic	hypochromic microcytic	3	2	1
68	19	9.4	9.9	0.5	486	467	-19	10.37	14.04	3.67	10.18	13.24	3.06	35	43	8	30.9	31.6	0.7	69.2	71.7	2.5	hypochromic microcytic	hypochromic microcytic	1	1	1
69	19	8.5	9.1	0.6	539	501	-38	8.61	11.16	2.55	9.23	10.78	1.55	28	37	9	28.7	29.5	0.8	58.2	60.7	2.5	hypochromic microcytic	hypochromic microcytic	2	1	1
70	17	9.2	9.9	0.7	518	495	-23	9.02	12.31	3.29	9.73	12.74	3.01	29	38	9	29.9	30.7	0.8	62.3	65.9	3.6	hypochromic microcytic	hypochromic microcytic	1	1	0
71	17	7.5	8.2	0.7	551	540	-11	7.22	10.19	2.97	8.35	10.34	1.99	21	32	11	26.6	28.1	1.5	55.2	57.1	1.9	hypochromic microcytic	hypochromic microcytic	3	2	1
72	16	8.4	9.1	0.7	526	511	-15	8.54	10.26	1.72	8.23	10.11	1.88	25	37	12	28.5	29.9	1.4	59.2	62.4	3.2	hypochromic microcytic	hypochromic microcytic	2	1	1
73	17	9.1	9.8	0.7	527	509	-18	9.47	12.16	2.69	9.62	12.54	2.92	30	35	5	29.7	30.8	1.1	60.1	63.3	3.2	hypochromic microcytic	hypochromic microcytic	1	1	0
74	19	9.2	10.1	0.9	517	509	-8	9.62	13.41	3.79	10.92	13.71	2.79	27	40	13	29.2	30.8	1.6	64.7	67.4	2.7	hypochromic microcytic	hypochromic microcytic	1	1	0
75	18	8.8	9.1	0.3	516	512	-4	8.65	11.51	2.86	9.72	11.56	1.84	28	37	9	28.4	29.2	0.8	57.8	59.7	1.9	hypochromic microcytic	hypochromic microcytic	2	2	0
76	18	10.1	10.4	0.3	467	451	-16	10.25	12.14	1.89	10.21	12.87	2.66	39	43	4	30	30.4	0.4	67.5	69.5	2	hypochromic microcytic	hypochromic microcytic	1	1	0
77	18	8.5	9.4	0.9	544	517	-27	8.91	11.66	2.75	9.76	11.67	1.91	29	38	9	28.6	30.2	1.6	59.6	62.8	3.2	hypochromic microcytic	hypochromic microcytic	2	1	1
78	19	7.2	8.1	0.9	568	550	-18	7.24	10.12	2.88	8.46	10.24	1.78	17	29	12	26.5	28.4	1.9	53.7	56.1	2.4	hypochromic microcytic	hypochromic microcytic	3	3	0
79	17	8.7	9.1	0.4	541	518	-23	8.47	11.45	2.98	9.51	12.45	2.94	26	37	11	28.4	29.4	1	59.8	61	1.2	hypochromic microcytic	hypochromic microcytic	2	1	1
80	17	9.1	9.8	0.7	517	487	-30	9.18	10.95	1.77	9.24	12.21	2.97	27	35	8	29.6	31.7	2.1	62.1	65.2	3.1	hypochromic microcytic	hypochromic microcytic	1	1	0
81	18	10.1	10.9	0.8	492	478	-14	10.15	12.06	1.91	10.17	12.27	2.1	33	44	11	30.2	31.5	1.3	66.4	69.5	3.1	hypochromic microcytic	hypochromic microcytic	1	0	1

82	17	9.5	10.1	0.6	512	507	-5	9.27	11.93	2.66	9.81	11.74	1.93	29	41	12	29.2	30.4	1.2	64.6	66.8	2.2	hypochromic microcytic	hypochromic microcytic	1	1	0
83	18	8.5	9.2	0.7	531	516	-15	8.21	11.18	2.97	8.92	10.81	1.89	28	42	14	27.5	29.2	1.7	57.4	60.2	2.8	hypochromic microcytic	hypochromic microcytic	2	1	1
84	19	7.4	8.1	0.7	559	537	-22	7.23	9.19	1.96	8.52	10.31	1.79	19	29	10	27.4	29.2	1.8	55.8	58	2.2	hypochromic microcytic	hypochromic microcytic	3	2	1
85	17	9.8	10.6	0.8	484	472	-12	10.11	13.52	3.41	10.1	12.97	2.87	30	45	15	29.1	30.3	1.2	64.2	66.6	2.4	hypochromic microcytic	hypochromic microcytic	1	0	1
86	18	9.2	9.9	0.7	517	503	-14	9.15	11.13	1.98	9.41	11.36	1.95	27	43	16	28.9	29.7	0.8	62.1	64.8	2.7	hypochromic microcytic	hypochromic microcytic	1	1	0
87	17	7.6	8.4	0.8	542	528	-14	7.97	10.84	2.87	8.41	11.35	2.94	23	36	13	26.4	28.4	2	55.3	57.1	1.8	hypochromic microcytic	hypochromic microcytic	3	2	1
88	17	8.3	8.7	0.4	529	524	-5	8.34	9.27	0.93	8.12	10.19	2.07	25	30	5	28.8	29	0.2	57.5	58.1	0.6	hypochromic microcytic	hypochromic microcytic	2	2	0
89	18	9.2	10.1	0.9	520	511	-9	9.16	12.23	3.07	8.34	11.27	2.93	25	37	12	29.7	31.6	1.9	62.2	65.1	2.9	hypochromic microcytic	hypochromic microcytic	1	1	0
90	18	9.8	10.7	0.9	512	504	-8	9.21	12.06	2.85	9.78	12.71	2.93	29	38	9	29.5	30.9	1.4	63.3	66.4	3.1	hypochromic microcytic	hypochromic microcytic	1	0	1
91	18	7.2	8.1	0.9	565	541	-24	7.29	9.27	1.98	8.31	10.29	1.98	17	34	17	26.5	28.3	1.8	55.6	56.6	1	hypochromic microcytic	hypochromic microcytic	3	3	0
92	18	8.7	9.1	0.4	536	529	-7	8.72	10.56	1.84	8.64	11.35	2.71	25	31	6	28	29.2	1.2	59.4	61.4	2	hypochromic microcytic	hypochromic microcytic	2	1	1
93	18	7.4	8.2	0.8	557	530	-27	8.52	10.16	1.64	7.41	10.16	2.75	21	33	12	27.1	28.8	1.7	56.7	57.4	0.7	hypochromic microcytic	hypochromic microcytic	3	2	1
94	18	8.5	9.3	0.8	534	511	-23	8.83	11.69	2.86	8.43	11.4	2.97	24	38	14	28.5	29.6	1.1	59.8	61.4	1.6	hypochromic microcytic	hypochromic microcytic	2	1	1
95	18	10.2	11.1	0.9	489	470	-19	10.38	13.18	2.8	10.31	12.09	1.78	28	42	14	28.7	30.4	1.7	62.4	66.3	3.9	hypochromic microcytic	hypochromic microcytic	1	0	1
96	17	7.9	8.4	0.5	541	521	-20	8.24	10.17	1.93	8.72	10.54	1.82	21	30	9	28.1	28.8	0.7	56.2	58.9	2.7	hypochromic microcytic	hypochromic microcytic	2	2	0
97	19	8.7	9.3	0.6	526	511	-15	9.27	12.23	2.96	9.75	11.67	1.92	26	36	10	28.3	30.4	2.1	59.5	61.4	1.9	hypochromic microcytic	hypochromic microcytic	2	1	1
98	17	7.5	8.2	0.7	551	516	-35	8.17	10.19	2.02	8.41	10.45	2.04	25	35	10	27.1	28.6	1.5	56.1	57.6	1.5	hypochromic microcytic	hypochromic microcytic	3	2	1
99	18	7.6	8.1	0.5	562	551	-11	7.64	9.51	1.87	8.79	10.67	1.88	20	28	8	27	28.1	1.1	55.7	57.8	2.1	hypochromic microcytic	hypochromic microcytic	3	3	0
100	17	9.4	10.2	0.8	541	518	-23	9.76	12.41	2.65	9.57	12.46	2.89	30	40	10	29.1	30.8	1.7	61.2	64.7	3.5	hypochromic microcytic	hypochromic microcytic	1	1	0
101	17	8.5	9.2	0.7	509	489	-20	8.64	9.56	0.92	9.47	10.15	0.68	24	36	12	28.4	30.7	2.3	59.2	61.6	2.4	hypochromic microcytic	hypochromic microcytic	2	1	1
102	17	9.1	9.7	0.6	504	479	-25	9.51	11.49	1.98	9.76	11.67	1.91	28	36	8	28.9	30.5	1.6	59.4	62.1	2.7	hypochromic microcytic	hypochromic microcytic	1	1	0

103	18	9.7	10.2	0.5	524	501	-23	9.35	12.94	3.59	10.43	12.35	1.92	31	41	10	29.9	30.5	0.6	63.1	66.9	3.8	hypochromic microcytic	hypochromic microcytic	1	1	0
104	18	7.4	8.3	0.9	574	552	-22	7.45	9.36	1.91	7.62	11.59	3.97	21	29	8	27.6	29.5	1.9	55.9	58.7	2.8	hypochromic microcytic	hypochromic microcytic	3	2	1
105	19	8.6	9.2	0.6	529	498	-31	9.17	10.94	1.77	9.02	11.27	2.25	27	35	8	28.8	30.5	1.7	59.6	61.7	2.1	hypochromic microcytic	hypochromic microcytic	2	1	1
106	19	7.7	8.4	0.7	555	549	-6	8.21	9.75	1.54	8.76	10.58	1.82	24	31	7	26.9	28.7	1.8	56.4	57.9	1.5	hypochromic microcytic	hypochromic microcytic	3	2	1
107	18	9.3	10.1	0.8	501	489	-12	9.54	11.47	1.93	9.61	12.54	2.93	29	38	9	28.5	30.7	2.2	62.2	63.3	1.1	hypochromic microcytic	hypochromic microcytic	1	1	0
108	18	10.1	10.7	0.6	492	472	-20	10.42	11.97	1.55	10.59	12.61	2.02	33	39	6	30.7	31.6	0.9	67.7	69.9	2.2	hypochromic microcytic	hypochromic microcytic	1	0	1
109	18	7.9	8.3	0.4	537	516	-21	8.76	10.15	1.39	7.84	10.42	2.58	19	27	8	27.8	29.5	1.7	55.9	57.1	1.2	hypochromic microcytic	hypochromic microcytic	2	2	0
110	17	8.7	9.1	0.4	518	495	-23	8.82	10.76	1.94	9.51	12.48	2.97	21	30	9	28.6	29.8	1.2	59.5	62.8	3.3	hypochromic microcytic	hypochromic microcytic	2	1	1
111	19	7.4	8.1	0.7	566	544	-22	7.51	10.43	2.92	7.65	9.39	1.74	23	33	10	27.8	29.7	1.9	52.2	53	0.8	hypochromic microcytic	hypochromic microcytic	3	3	0
112	17	9.9	10.7	0.8	451	433	-18	10.05	11.59	1.54	10.61	12.56	1.95	30	40	10	27.7	29.3	1.6	56.9	61.3	4.4	hypochromic microcytic	hypochromic microcytic	1	0	1
113	18	9.1	9.8	0.7	501	489	-12	9.13	11.17	2.04	9.38	12.46	3.08	26	34	8	29	30	1	60.6	63.2	2.6	hypochromic microcytic	hypochromic microcytic	1	1	0
114	19	8.9	9.5	0.6	553	541	-12	9.14	11.81	2.67	9.19	12.58	3.39	24	37	13	29.1	30.7	1.6	59.5	62.3	2.8	hypochromic microcytic	hypochromic microcytic	2	1	1
115	17	10.6	11.3	0.7	494	474	-20	11.58	14.22	2.64	11.57	14.38	2.81	32	43	11	28.7	30.7	2	63.6	65.4	1.8	hypochromic microcytic	hypochromic microcytic	0	0	0
116	17	8.7	9.1	0.4	520	507	-13	8.69	10.54	1.85	9.28	10.21	0.93	29	36	7	28.6	30.1	1.5	59.7	61.6	1.9	hypochromic microcytic	hypochromic microcytic	2	1	1
117	18	9.2	9.6	0.4	524	501	-23	9.46	11.32	1.86	9.65	11.57	1.92	28	34	6	29.1	29.5	0.4	60.7	62.3	1.6	hypochromic microcytic	hypochromic microcytic	1	1	0
118	18	7.8	8.2	0.4	565	547	-18	8.55	10.46	1.91	8.44	10.38	1.94	20	29	9	27.4	28.2	0.8	57.1	59.2	2.1	hypochromic microcytic	hypochromic microcytic	3	2	1
119	17	9.7	10.5	0.8	498	484	-14	10.71	13.59	2.88	9.94	13.81	3.87	29	38	9	28.9	30.2	1.3	65.1	67.1	2	hypochromic microcytic	hypochromic microcytic	1	0	1
120	17	8.4	9.1	0.7	564	552	-12	8.72	11.66	2.94	8.64	11.58	2.94	26	33	7	28.6	29.2	0.6	59.2	61.4	2.2	hypochromic microcytic	hypochromic microcytic	2	1	1
121	18	9.6	10.1	0.5	522	512	-10	10.77	13.63	2.86	9.58	12.53	2.95	30	35	5	29.1	30.2	1.1	63.3	66.4	3.1	hypochromic microcytic	hypochromic microcytic	1	1	0
122	17	8.6	9.2	0.6	527	514	-13	8.93	11.84	2.91	8.97	10.76	1.79	28	34	6	28.5	29.8	1.3	59.5	61.7	2.2	hypochromic microcytic	hypochromic microcytic	2	1	1
123	18	9.4	10.2	0.8	501	482	-19	9.67	13.96	4.29	9.65	13.54	3.89	31	41	10	29.2	30.6	1.4	63.1	64	0.9	hypochromic microcytic	hypochromic microcytic	1	1	0

124	17	8.1	8.6	0.5	529	517	-12	8.57	10.46	1.89	8.68	10.59	1.91	29	34	5	28.3	29.7	1.4	53.4	54.7	1.3	hypochromic microcytic	hypochromic microcytic	2	2	0
125	19	8.9	9.3	0.4	501	498	-3	9.65	11.79	2.14	8.69	11.46	2.77	27	36	9	28.8	29.9	1.1	59.4	62.5	3.1	hypochromic microcytic	hypochromic microcytic	2	1	1
126	19	7.7	8.2	0.5	541	519	-22	8.13	9.94	1.81	8.42	9.09	0.67	24	31	7	27.1	28.7	1.6	51.5	52.4	0.9	hypochromic microcytic	hypochromic microcytic	3	3	0
127	18	9.4	10.1	0.7	498	487	-11	9.78	12.65	2.87	9.92	11.87	1.95	30	37	7	29.5	30.2	0.7	63.7	66.1	2.4	hypochromic microcytic	hypochromic microcytic	1	1	0
128	18	9.8	10.2	0.4	501	487	-14	10.76	13.71	2.95	10.71	13.64	2.93	25	30	5	29.3	30.5	1.2	63.1	64.4	1.3	hypochromic microcytic	hypochromic microcytic	1	1	0
129	18	9.3	10.2	0.9	517	499	-18	9.25	12.29	3.04	9.83	11.72	1.89	28	38	10	29.4	30.6	1.2	62.7	64.3	1.6	hypochromic microcytic	hypochromic microcytic	1	1	0
130	18	10.1	10.6	0.5	487	461	-26	10.66	13.45	2.79	11.27	13.98	2.71	34	39	5	30.8	31.2	0.4	65.3	67.9	2.6	hypochromic microcytic	hypochromic microcytic	1	0	1

Faculty of Ayurved, Tilak Maharashtra Vidyapeeth, Pune &

Department of Dravyaguna, Government Ayurved College, Nanded

PHYSICOCHEMICAL & PHYTOCHEMICAL EVALUATION OF ARDRA & SHUSHKA DOSAGE FORMS OF BHRUNGARAJ PANCHANGA & ITS CLINICAL TRIAL IN MANAGEMENT OF IRON DEFICIENCY ANAEMIA

CASE REPORT FORM I - SCREENING

BEFORE TREATMENT

Prepared by Dr. Sanjivani Shekokar

VISIT ZERO baseline examination

1. Code No. (Of clinical trial)
2. Centre _____
3. OPD / IPD No:
4. Name of the Patient _____
5. Gender: Female
6. Date of Birth: Age (in yrs.) -
7. Educational status: Illiterate / Read and Write / Primary School / Middle School / High School / College / Other (specify)
8. Occupation: Student / Others
   Indicate school hours: . . . . . .
9. Religion: Hindu / Muslim / Christian / Jain / Others
10. Address Permanent postal address with phone number and email if any

CRITERIA FOR DIAGNOSIS

(Textual references & WHO guidelines for Iron Deficiency Anemia)

Sr no	Clinical presentation	Duration
1	Pallor	
2	Palpitations	
3	Vertigo	
4	Tinnitus	
5	Weakness	
6	Loss of Appetite	
7	Dyspnoea on exertion	
8	Oedema	
9	Giddiness	
10	Koilonychias	
11	Brittleness of nails	

Classification of Iron Deficiency Anemia on the basis of gradation of Hb %

Severe – Hb % < 7 gm %

Moderate – Hb % 7-10 gm %

Mild – Hb % 10-12 gm %

(Threshold value for Hb % in children is 11-13 gm %)

Normal values of other factors in children are as follows;

Serum Iron – 50 – 120 micro gm/dl

MCHC – 32 -36 gm/dl

TOTAL IRON BINDING CAPACITY –

Male – 250mcg/dl **Female –** 400 mcg/dl

Serum Transferin – Male – 10-50

Female – 15-50

Preliminary investigations

Hb % -

Serum ferretin -

Serum Iron -

Total Iron Binding Capacity -

MCHC -

Blood film -

CRITERIA FOR INCLUSION

	YES (1)	NO (2)
1. Age between 11 years to 19 years	☐	☐
2. Adolescent females	☐	☐
3. Hb % 7-11 gm %	☐	☐

CRITERIA FOR EXCLUSION

1. Severe anaemia i.e. Hb - < 7 gm %.	☐	☐
2. Age below 11 years and more than 19 years	☐	☐
3. Acute infections	☐	☐
4. Anaemia other than IDA	☐	☐
5. Lack of assent	☐	☐
6. Patients receiving Iron supplements	☐	☐
7. Carcinoma of any type	☐	☐
8. Known hyper sensitivity to Iron or the protocol drug	☐	☐
9. Intolerance to Oral iron	☐	☐
10. Excessive bleeding condition	☐	☐
11. Any other systemic disorder	☐	☐
12. HIV positive cases	☐	☐

- -

This case is accepted / not accepted for the study

Allocated study group as per randomization: Group I / Group II / Group III

Date: _____ Signature of the Investigator_____

Faculty of Ayurved, Tilak Maharashtra Vidyapeeth, Pune &
Department of Dravyaguna, Government Ayurved College, Nanded

ASSENT FORM
CERTIFICATE BY INVESTIGATOR
(Investigator's copy)

I certify that I have disclosed all details about the study in the terms easily understood by the patient.

Date:_____ Signature of guardian/parents_____

Name of parent & patient _____

CONSENT BY SUBJECT

I have been informed to my satisfaction, by the attending physician, the purpose of the clinical trial and the nature of drug treatment and follow-up, including the laboratory investigations to be performed to monitor and safeguard my body functions.

I am also aware of my right to opt out of the trial at any time during the course of the trial without having to give the reasons for doing so.

I, exercising my free power of choice, hereby give my consent to include my child as a subject in the clinical trial on "PHYSICOCHEMICAL & PHYTOCHEMICAL EVALUATION OF ARDRA & SHUSHKA DOSAGE FORMS OF BHRUNGARAJ PANCHANGA & ITS CLINICAL TRIAL IN MANAGEMENT OF IRON DEFICIENCY ANAEMIA."

Date:_____ Name of the Subject:_____

Signature or Thumb impression:_____

Date:_____ Name of gardian/parent:_____

Signature or Thumb impression: _____

Relationship: _____

Aayauvao-d Ô^klTI iTLk maharaYT,/ iva_yaapIz puNao va
Saasaikya Aayauvao-d mahaiva_yaalaya va $gNaalaya naaMdoD

palakaMcaI p`t

$gNaacao AanaumatIp~a

malaa yaa sampUNa- icaik%saoSaI saMbaMQaIt sava- maaihtI doNyaat AalaolaI Asauna %yaacao p`yaaojana va sva$p hyaa saMbaMQaI sava- maaihtI malaa imaLalaolaI Aaho va maI maaJyaa palyaalaa %yaa saazI sahBaagaI k$ [cCIt Asauna %yaa saazI maI sahkaya- krNyaasa tyaar Aaho %yaaca p`maaNao jyaa kahI r@ta [%yaadI tpaasaNyaa krayavyaa Aahto %yaa maaJyaa palyaasaMd-Baat krNyaasa tyaar Aaho.

maI yaa Pa`yaaogaatuna kQaIhI baahor pDNyaacao malaa svaatM~ya icaik%sakaMnaI idlaolao Aaho
%yaa nausaar maI yaa p`yaaogaat maaJao palyaalaa sahBaagaI krNyaacaI AnaumatI dot Aaho

palakacaI sahI

idnaaMk

$gNaasah naato

Faculty of Ayurved, Tilak Maharashtra Vidyapeeth, Pune &
Department of Dravyaguna, Government Ayurved College, Nanded

ASSENT FORM

CERTIFICATE BY INVESTIGATOR

(Subjects/guardian's copy)

I certify that I have disclosed all details about the study in the terms easily understood by the patient.

Date:_____ Signature of guardian/parents_____

Name of parent & patient _____

ASSENT BY SUBJECT

I have been informed to my satisfaction, by the attending physician, the purpose of the clinical trial and the nature of drug treatment and follow-up, including the laboratory investigations to be performed to monitor and safeguard my child's body function.

I am also aware of my right to opt out of the trial at any time during the course of the trial without having to give the reasons for doing so.

I, exercising my free power of choice, hereby give my consent to include my child as a subject in the clinical trial on "PHYSICOCHEMICAL & PHYTOCHEMICAL EVALUATION OF ARDRA & SHUSHKA DOSAGE FORMS OF BHRUNGARAJ PANCHANGA & ITS CLINICAL TRIAL IN MANAGEMENT OF IRON DEFICIENCY ANAEMIA."

Date:_____ Name of the Subject:_____

Signature or Thumb impression:_____

Date:_____ Name of guardian/parents:_____

Signature or Thumb impression: _____

Relationship: _____

Investigator / patient interaction tentative calander

Visit	Day	Date
Visit 0 (subject recruitment)	Baseline	
Visit 1 (pre treatment period)	1st	
Visit 2 (treatment period)	8th	
Visit 3 (treatment period)	16th	
Visit 4 (treatment period)	24th	
Visit 5 (treatment period)	31th	
Visit 6 (treatment period)	45th	
Visit 7 (treatment period)	52 nd day	

VISIT ONE

CASE REPORT FORM II – HISTORY

1. Code No. (Of clinical trial):
2. Sr. No. of Subject:
3. Name of the Subject

4. Chief complaint with duration -

Symptoms:-	**Duration**
1. Pallor of conjunctiva -	
2. Pallor of nails -	
3. Pallor of palms -	
4. Pallor of tongue -	
5. Weakness -	
6. Dyspnoea on exertion -	
7. Gidiness -	
8. Vertigo -	
9. Koilynochia -	
10. Brittleness of nails -	
11. Loss of appetites -	
12. Tinnitus -	
13. Oedema -	

Other complaints -

History of Present illness:-

Weakness - Duration: for the last days/ weeks/ months/ years

Giddiness -

Pallor -

Dyspnoea on exertion -

Treatment History:

Healthe care- Catagory Details / Response / State discontinuing Duration

Ayurveda -

Hospitalization -

Traditional -

Homeopathy -

Others -

Family History

Relative - Dead/Alive Health status Treatment history

Father -

Mother -

Other relatives -

Personal History:

Aspect Details:-

School hours -

Exercise - minimum / moderate / heavy

Hours of rest in a day -

Appetite- good / poor / moderate, state during the attack

Diet - Veg / Non-veg / mixed

Breakfast - Mid morning

Lunch -

Dinner -

Evening snacks -

Fruits - regular/ occasional -

Cold water - regular/ occasional -

Brevarages - regular/occasional -

Buttermilk/curds - regular/ occasional -

Spicy food - regular/ occasional -

Fried items - regular/ occasional -

Ice cream - regular/occasional -

Cucumber - regular/ occasional -

Sleep - Sound / disturbed / good-At night / Difficulty in falling asleep / staying asleep

Reasons if disturbed:

Daytime naps:

Koshtha:- Mrudu / Madhyam / Krura.

Emotional influences – Anxiety / Tension / Depression / Irritation / Anger / Fear.

Habits:

Coffee- Occasional / Regular / Continued / Reduced / Stopped

occasional / Regular / Continued / Reduced / Stopped

Others- Occasional / Regular / Continued / Reduced / Stopped

Gyneacological history:

Menstrual cycle …… regular,irregular.

Menarche age…….. bleeding days…….

Menorrhagia, metrorrhagia, dysmenorrhoea, leucorrhoea Menopause.

Aatura Bala Pramana Pariksha –

1) Prakrutitah – Sharir - Vata / Pitta / Kapha

Manasik – Satvaja / Rajas / Tamas.

2) Saratah - Pravara / Madhyam / Avar

3) Samhananatah - Pravara / Madhyam / Avar

4) Pramanatah - Pravara / Madhyam / Avar

5) Satmyatah - Pravara / Madhyam / Avar

6) Satvatah - Pravara / Madhyam / Avar

7) Aahara shaktitah –

Abhyavaran Shakti - Jaran Shakti –

Agni - Sama / Vishama / Manda / Tikshna

8) Vyayama Shaktitah - Pravara / Madhyam / Avar]

Srotas Pariksha (Strotodushti, etc)

1) Pranavaha –

Nasa - Shwasana -

2) Annavaha - Anannabhilasha / Arochak / Chardi / Annadvesha /Pipasa

3) Udakavaha – Jivha / Talu / Oshta / Kantha / Pipasa

4) Rasvaha – Hrillas / Gaurava / Jvara / Agnimandya.

5) Raktavaha – Yakrut / Pleeha / Akshi Shwetata / Hasta tala krushnata

6) Mansavaha – Shotha /Mansashosha / Akshikuta shotha / Twak krushnata

7) Medovaha – Sweda / Snigdhata / Sthulata / Shithilangata / Madhurasyata

8) Asthivaha – Asthishula/ nakha krushnata / nakha bhangurata

9) Majjavaha – Bhrama / tandra

10) Purishavaha - Mala – consistency - Ghana / drava / grathita / alpa

Malpravrutti - sakashta / sashabda /sarakta / sashula/ saphena / Picchila / sadaha

Mala - Sama / niram

Bowel habit – Regular / Irregular No. of vegas - per day

Time taken to deficate –

Color – Pita / Krushna / other

11) Mutravaha – Micturation - No. of vegas in day / night

12) Swedavaha- perspiration – alpa / madhyam / bahu

Dnyanendriya and Karmendriya parikshana –

Chakshu Twak Shrotra Ghrana

Jivha Hasta Pada Vaka

Payu Upastha Mana

Ashtavidha parikshana –

Nadi - , Shabda - , Sparsha - , Druga - , Akruti - ,

Mal - , Jivha - , Mutra -

Systemic examination – RS, CVS, GIT, CNS

General Examination:

Aspects Details of -

1. Built - Slender, lanky, muscular, stocky, obese
2. Nourishment - Good, fair, poor
3. Height in cm -
4. Weight in kg
5. B.M.I Weight in KG /(Height in meters)
6. Pallor of - conjunctiva, nails, palm, tongue
7. Kolynochia -
8. Nails - pink, pallor, bluish
9. Conjunctiva - pink, pallor, bluish
10. Oedema - Foot, ankle, leg, sacral hands, face,
11. Pitting/ non-pitting
12. Teeth Caries
13. Gums - Spongy, bleeding, unhealthy
14. Pulse /Min - regular, irregular, full, week
15. B. P mm of Hg -
16. Temperature -

Investigations

Sample	Investigation	Result	Normal range
Urine	Physical characters		
	Color	Pale yellow	
	Appearance	Clear	
	Reaction	Acidic	
	Microscopic examination		
	Pus cells /hpf		
	RBCs /hpf		
eBlood			
	Hb (g/dl)		
	Serum ferretin		
	Serum transferin		
	Serum iron		
	Total iron binding capacity		
	MCHC	Blood film	

Outcome measures and before treatment scores

Sr. No.	Criteria	Score / Value	Comments
1	Pallor of conjunctiva		
2	Pallor of Nail bed		
3	Pallor of palms		
4	Pallor of tongue		

* (Accuracy & Reliability of Pallor for Detecting Anaemia: A hospital– based diagnostic accuracy study, Ashwini Kalantri, et al. Plos one, dept. of Medicine, MGIMS, Wardha, Maharashtra)

ADVERSE EVENTS DURING THE TRIAL

Does the patient have any symptoms with medication in trial group? Yes / No
Severity, Mild-1, Moderate-2, Severe-3
Mild- No interference with usual activity, ***Moderate**- Significant interference with usual activities,
***Severe**- Prevents usual activities
Serious*

Unrelated: A reaction that does not follow a reasonable temporal sequence from the administration of the drug; or a known adverse reaction pattern of the suspected drugs could have been produced by the patients clinical stage, intermittent illness, trauma, accidents etc:

Date: _____ Signature of Investigator

_____

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A Phytopharmacological Review of Prospective of *Bhrungaraj* (*Eclipta alba Hassk.*)

Review Article

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Abstract

*Eclipta alba Hassk.(*compositeae*)* is an important small branched annual herbaceous plant in Ayurveda described first by *Bhavprakasha* and is widely used for treating various ailments in the Indian system of medicine. Aim: Aim of this review is to provide comprehensive information on the pharmacological activities of various part of *Eclipta alba Hassk*. Setting and design: This is a contribution which provides a comprehensive review on ethnomedicinal uses, chemical composition, and the pharmacological profile of *Eclipta alba Hassk*. as an important medicinal plant. Material and methods: All the relevant universally accepted electronic databases were searched with respect to the terms "Bhrungaraj", "False Daisy", "*Eclipta erecta,*", "*Eclipta prostate,*", "*Verbesina alba,* "& *Verbesina prostrate*" including Indian classical texts, pharmacopoeias, Ayurvedic books, journals, etc., for information without specific timeline. Complete information of the plant has been collected manually. Result and conclusion: The collected data reflects that many ethno-medicinal claims have been confirmed through the modern in-vitro and in vivo pharmacological studies using different extracts and their isolates of *Eclipta alba Hassk*. The isolation of active constituents, their biological actions, clinical safety and validation of traditional uses of *Eclipta alba* could provide leads for further scientific research. The information collected here will be useful to set up research protocols for modern drugs and Ayurvedic formulation development.

Keywords: *Bhrungaraj*, *Eclipta alba*, *Eclipta erecta*, *Eclipta prostate, Hepatoprotective*

Introduction

Bhrungaraj is well known drug for hair disorders from the very ancient time. It is described by *Bhavaprakash*, *Raj Nighantu*, *Bheshajjya ratnavali* and many ayurvedic texts. It is known by its synonyms like *Kesharaj*, *Kesharanjana*(1), *Markava* , etc.

The genus name comes from the Greek word meaning "Deficient," with reference to the absence of the bristles and awns on the fruits. The specific *Eclipta alba* means white which refers to the color of the flowers(2).

The herb is being used for its curative properties as antimytotoxic, analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, and for immunomodulatory properties and it is considered as a good rejuvenator. It is an active ingredient of many herbal formulations used for liver disorders and enhances liver cell generation .It is used for its tonic and diuretic action in hepatic and spleen (3) enlargement.

It is also useful in *Krumi* (worm infestation), *Shotha*(oedema), *Pandu*(anemia)(4), etc. also useful for wound healing and skin diseases. Several formulations are prepared from this drug like *Bhrungaraj Tail*, *Bhrungaraj Swaras*, etc which are popularly used for hair treatment in hair disorders also (5). Still there is a large market trade of oils & medicaments prepared from *Bhrungaraj*. Various chemical constituents (6) are separated from *Bhrungaraj* & are being clinically tested for various hepatic disorders, etc.

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Botanical description (7)

Eclipta alba (L.) Hassk. (Syn. *Eclipta prostrata* L.) is commonly known as False Daisy, yerba de tago, and bhringraj, a plant belonging to the family Asteraceae. Root is well developed, cylindrical, greyish in color. It is also named 'kehraj' in Assamese and karisalankanni in Tamil. Floral heads are 6-8 mm in diameter, solitary, white, achene compressed and narrowly winged.

Eclipta alba is a herbaceous tufted plant that may be prostrate or grow up to 50cm in erect form. The stems and leaves are covered with white hairs. Sometimes the stems may be reddish. The leaves are simple, opposite and attached to the stem without petiole. The inflorescences are white on a hemispherical heads of 1cm in diameter. The figures of Eclipta entire plant and its different parts are shown in Figure 1-4.

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Fig. 1 Entire Plant

Fig. 2 Entire Plant

Fig. 3 Inflorescence

Fig.4 Roots of Eclipta alba

Botanical classification (8):-

- Kingdom – Plantae
- Unranked – Angiosperms
- Unranked – Eudicots
- Unranked – Asterids
- Order – Asterales
- Family – Asteraceae
- Genus – Eclipta
- Species – Eclipta alba
- Botanical name - *Eclipta alba L. Hassk*
- Synonyms – *Eclipta erecta, Eclipta prostate, Verbesina alba, Verbesina prostrate*

Vernacular names or Synonyms:-

- Sanskrit – Bhrungaraj, Kesharaj, Markava, Kesharanjana, Kesharaj, etc.
- Hindi – Bhangara, Bhangarayya
- Punjabi – Bhangara, dodhak, Babri.
- Marathi – Maka
- Gujarat –Bhangaro.
- Bengali – Kesuriya, Kesuti.
- Tamil – Kaikeshi.
- Telgu – Galagara, Gunta, Galijaeru.
- Arabic – Kadim-ul-bint, Radim-el-bint.
- Malyalam – Cajenneam,Kanni.
- Konkani - Mako, Kajalamavu.

Gunadharma –

The properties of *Bhrungaraj* are well illustrated in *Bhava Prakash nighantu*, *Guduchyadivarga* /240-241.

Guna Ruksha laghu, *Rasa Katu Tikta*, *Vipak Katu & Virya Ushna*

Doshakarma – Kapha Vata shamak.

Species

It is of three varieties – Yellow (flowered), white (flowered) & black (fruiting) (9)

The Yellow is *Wedelia calendulaceae*, this herb has yellow flowers. The black variety is a variety of the white one, called *Kala Bhrungaraj*, Black & white-*Eclipta alba*.

Parts used

Herb roots & leaves, *Panchanga*, *beeja* all parts are used.

Cultivation & propagation

Vegetative propagation by using buds of length of 5cm.It can be planted in well prepared beds as above seed beds or nursery bags. The plants will be ready for transplanting within 30 to 45 days.

Harvesting

The whole plant is plucked after 9 to 10 month. The fresh plants are chopped & dried in shade. The seeds are collected when it turns black in color. The average matured plants give 2500 Kg. per acre of dry materials.

Distribution of Eclipta sp. Worldwide (10)

It is usually found on poorly drained, wet areas; along streams and ditches in marshes and on the dikes of rice paddies. However, it is also common in lawns and in upland conditions where rainfall is about 1200mm or more.

It can grow under wet, saline conditions but is often a weed of drier sites in plantation crops. *E. alba* is a native of Asia, but has a general distribution over the world. It is on all continents in tropical, subtropical and warm temperate regions.

Habitat

Sub herbaceous annual or perennial tufted plant described as a prostrate or grows up to 50cm in erect form. In reality, it develops at the base of the main stem, several long stems, prostrate, rooting at the nodes. It grows commonly in moist places as a weed all over the world. It is widely distributed throughout India, China, Thailand, and Brazil.

Phytoconstituents

Eclipta alba (L) has wide variety of active constituents as described in **Table no 1**,

The constituents are coumestan derivatives like wedololactone[1.6%], and alkaloidal principles like ecliptine, glyoide like demethylwedelolactone, desmethyl-wedelolactone-7 glucoside present in leaves and other constituents are ecliptal(11), ß-amyrin, luteolin-7-O-glucoside in aerial parts, hentriacontanol, heptacosano in roots, stigmasterol. All the parts of *Eclipta alba* and chemical constituents are used as anticancer, antileprotic, analgesic, antioxidant, antimyotoxic, antihaemorrhagic, antihepatotoxic, antiviral, antibacterial, spasmogenic, hypotensive, ovicidal, promoter for blackening and growth of hair (12).

Table 1: Chemical constituents and biological activities of parts of *Eclipta alba*

Sr no.	Part	Formulation	Constituents	Biological Activity
1.	Leaves	Juice	Stigmasterol,a-terthienymethanol, Wedelolactone[1.6%], Desmethylwedelolactone, Desmethyl-wedelolactone-7-glucoside	Skin diseases, allergic Urticaria, Asthma, Inflatulence, Colic and liver affections, Bronchitis, Enlarged glands, Dizziness, Vertigo, Blurred vision
2.	Roots	Powder/ juice	Hentriacontanol, Heptacosanol& Stigmasterol4, Ecliptal12-1	Liver tonic, Emetic, Purgative, Antiseptic to ulcers, Wounds in cattle
3.	Aerial parts	Juice	ß-amyrin & Luteolin-7-0-glucoside, Apigenin, Cinnaroside, Sulphur compounds	
4.	Stems	Paste	Wedelolactone	
5.	Seeds		Sterols	Sexual debility, Tonic, Aphrodisiac
6.	Twigs of the plant	Paste	Unnamed alkaloid	
7.	Whole plant	Paste	Large amounts of resin, Ecliptine, Reducing sugar, Nicotine, Stigmastero, Triterpene saponin, Eclalbatin togetherwith a -amyrin, Ursolic acid, Oleanolic acid.	Rejuvenating, Age-sustaining tonic, Detoxifying, Deobstruent, Antiseptic herb in vitiated blood, Anaemia, Splenic and liver enlargements, Catarrhal jaundice, Hyperacidity, Gastritis, Dysentery, Anticatarrhal, Spasmogenic, Hypotensive properties

In *Auyrveda* medicine, the leaf extract is considered as a powerful liver tonic, rejuvenator, and hair growth promoter and used for dyeing hair and tattooing. *Eclipta alba* also has traditional external uses in athlete foot, eczema and dermatitis, on the scalp to address hair loss and the leaves have been used in the treatment of scorpion stings. It is widely used as anti-venom agent against snakebite in China and Brazil (13) (Mors, 1991).

Wedelolactone is a large amount of resin & an alkaloidal principle ecliptine(14) is obtained. Wedelolactone is found in yellow & white variety.

Uses

Shotha, *Vrana*, *Savarnikarana*, *Kesha Vyadhis*, *Shleepada*, *granthi*, *Shirashula*, Greying of hair, etc.

It is also used as application in Hepatic & Splenic enlargements & in various chronic skin diseases. It is also useful internally in *Yakrut vyadhis*, *Yakrut vruddhi*, Spleenic enlargements, jaundice, *Udarashula*, etc. In *Krumi* with Castor oil, *Shwasa*, *Kasa* & in *Mutradaha*.

Useful in Serpent bite, scorpion bite, chronic glandular swellings & other skin diseases & Alopecia, etc. Leaf juice is used as hepatic tonic.

Pharmacological activities of *Bhrungaraj*

The crude extract shows wound healing property and it counteracts CCl4-induced inhibition of the hepatic microsomal drug metabolizing enzymes. The restoration of loss of hepatic lysosomal acid phosphatase and alkaline phosphatase by CCl4 was significantly seen by using *Eclipta alba* showing the hepatoprotective activity of *Eclipta alba* which is by regulating the levels of hepatic microsomal drug metabolizing enzymes (16). The fresh plant is used as a helpful medication by AIDS patients in part showed potential as a therapeutic agent against Giardia intestinalis infections also (17, 18). It shows antimicrobial and antioxidant properties (19) and is being used in pilex formulation along with other ingredients reported to decrease the bleeding time of the patient (20). Leaf extract is being used in oedema. It is used in the treatment of paronychia (21).

Hepatoprotective activity

The study of hepatoprotective effect of the ethanol/water (1:1) extract of whole plant of *Eclipta alba* has been conducted at subcellular levels in rats against CCl4-induced hepatotoxicity which showed that *Eclipta alba* significantly counteracted CCl4-induced inhibition of the hepatic microsomal drug metabolizing enzymes. The restoration of loss of hepatic lysosomal acid phosphatase and alkaline phosphatase by CCl4 was observed. This study elaborated shows that hepatoprotective activity of *Eclipta alba* is mainly based on regulation of the levels of hepatic microsomal drug metabolizing enzymes [(16)]. Bi-herbal ethanolic extract (BHEE) of combination of leaves of *Eclipta alba* and seeds of *Piper longum* was administered orally at a dose level of 50 mg/kg body weight once for 14 days which was found to restore the elevated serum marker enzymes such as SGOT, SGPT, ALP, LDH, ACP, GGT and 5' Nucleotidase, due to CCl4 treatment. It was observed that the biochemical parameters like total protein, total bilirubin, total cholesterol, triglycerides, and urea were also restored towards normal levels (22).

The study of hepatoprotective activity of methanolic extract of leaves and the chloroform extract of roots of *Eclipta alba* was carried out using carbon tetrachloride for inducing liver damage and assessment of Lysosomal enzymes level in wistar albino rats. The methanolic extract of leaves and the chloroform extract of roots of *Eclipta alba* showed very significant activation and respectively causing 72.8% & 47.96% reduction in the lysosomal enzyme. It was observed that the triterpenoid eclabasaponin fraction from methanolic extract of leaves produced significant increase (78.78%) and the alkaloidal fraction (60.65%) reduction of carbon tetra chloride induced increase in lysosomal enzyme in the blood. The chloroform extract of roots having the coumestan fraction and triterpenoidal saponin fraction from produced very significant (75.6%) and (52.41%) respectively reduction of carbon tetra chloride induced increase in lysosomal enzyme levels in blood (23).

Antihyperlipidemic activity

The aqueous leaf extract of the *Eclipta. prostrata* was given orally to the rats and it has been observed that in the atherogenic diet induced hyperlipedemic model, significant reduction in total cholesterol, triglycerides, total protein was seen along with a significant elevation in the high density lipoprotein cholesterol levels. The dose of about 200mg/kg of the extract showed better results as compared to 100mg/kg (24). In another study the animal model containing Charles River Sprague-Dawley CD rats (specific pathogen-free/viral antibody-free Crj/Bgi male, 180 ± 10 g) were fed the experimental diets which were supplemented with 0 mg (control), 25 mg (E25), 50 mg (E50), or 100 mg (E100) of a freeze-dried butanol extracted fraction of E. prostrata per kilogram of diet for 6 weeks and control group which was untreated. The results were reported as significant reduction of serum triacylglycerol and total cholesterol, low-density lipoprotein-cholesterol levels and elevation in the high-density lipoprotein in the E50 and E100 groups respectively as compared with the untreated control group (25).

The butanol extract fraction of *Eclipta prostrata* (Linn) was found to reduce serum lipid levels effectively and improve antioxidant activities in CD rats.

Antioxidant action

In this study Charles River Sprague-Dawley CD rats were orally fed with50mg/kg and 100mg/kg dose alcoholic extract of *Eclipta prostrata* and the study revealed that the extract reduced serum hydroxyl radical (nmol/mg protein per minute) and serum lipid peroxide (nmol/mg protein) levels to some level as compared to untreated group. The 100mg/kg dose of the extract of Eclipta significantly reduced Carbonyl content of oxidatively modified proteins (26).

Antioxidant activity of *Eclipta prostrata* was determined by parameters such as- FRAP radical scavenging activity, reducing activity, and DPPH assay. It was observed that the antioxidant capacity was increased as the concentration of the extracts was increased from 25 to 100mg/ml against α-tocopherol as reference drug . The antioxidant activity of the hexane, ethyl acetate, ethanol and water extracts of *E. prostrata* were also determined by ferric thiocynate (FTC) method used to determine the amount of peroxide formed and that react with ferrous chloride (FeCl2) to form a reddish ferric chloride (FeCl3) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases so Hexane, ethyl acetate, ethanol and water extract of Eclipta showed antioxidant activities at various concentration (50, 100, 250 and 500 in µg/mL) in the increasing order of the concentration. Ethanolic extract at the concentration of 500 µg/mL showed maximum i.e. 77.62% which is close to the reference compound used α-tocopherol (80.06%) (19).

Action on immune system

Immunomodulatory action of *Eclipta alba* was observed by the protection of neuronal tissues. So, *Eclipta alba* proves to be a potential memory modulator (27).

Methanol extracts of whole plant of *E. alba* (1.6% wedelolactone) was used to assess then immunomodulatory activity at five dose levels (dose-response relationship) ranging from 100 to 500 mg/kg by

using carbon clearance, antibody titer and cyclophosphamide immunosuppression parameters the study showed significant increase in phagocytic index and antibody titre and the F ratios of the phagocytic index and WBC count were observed to be also significant (28).

In another experiment the aqueous leaf extract *Eclipta alba* was fed to a fish (tilapia, Oreochromis mossambicus) at 0, 0.01, 0.1 or 1% levels as a diet for about 3 weeks and after each week, non-specific humoral (lysozyme, antiprotease and complement) and cellular (myeloperoxidase content, production of reactive oxygen and nitrogen species) responses and disease resistance were noted against Aeromonas hydrophila which showed increased activity of non-specific immune parameters. Thus the study results indicated that dietary intake of E. alba aqueous leaf extract can enhance the non-specific immune responses and disease resistance of O. mossambicus against A. Hydrophila(29).

Anti-inflammatory and analgesic effect

Anti-inflammatory activity was studied in Albino wistar carrageenin and egg white induced hind paw edema rats by using methanolic extract of *Eclipta* after oral administration. The dose of100 and 200 mg/kg showed significant activity in carrageenin and egg white induced hind paw edema in rats which was compared with indomethacin (10 mg/kg) and cyproheptadine (8 mg/kg) which were used as control (30). The study of analgesic effect on albino mice was conducted using ethanolic and alkaloidal extract of *Eclipta alba.* The Standard experimental models such as the tail clip method, the tail flick method and the acetic acid induced writhing response all were used which showed that both the ethanol extract as well as the total alkaloids produced good analgesic activity in the different models of rats. The alkaloidal extract fraction was found to be the most efficacious in all models used for testing (31, 32).

Antidiabetic effect

The antidiabetic action was studied in alloxan induced diabetic rats using the leaf suspension of *Eclipta alba* (2 & 4g/kg) orally. It was observed that there was significant reduction in blood glucose level and glycosylated hemoglobin A well as decreased activity of glucose-6 phosphatase and fructose1, 6-bisphosphatase and increase in the activity of liver hexokinase. The study revealed potent antihypergylcemic activity of oral administration of *Eclipta alba* suspension possess in the alloxan induced diabetic rats (33). *Eclipta alba* has been used as an ingredient in polyherbal formulation like Pan-five which were scientifically and clinically proved to possess Antidiabetic and diuretic activity by acting upon pancreas through the mode of restoration and regeneration of pancreatic β-cell activity (34).

Effect on hair growth

Eclipta alba is known for it hair growth promoting action hence used in hair oil preparations and also to maintains the black hair (35). Alopecia is a dermatological disorder with its psychosocial implications on patients having hair loss The study was conducted on shaved denuded skin of albino rats using Petroleum ether & ethanolic extracts in 10%w/v quantity which was incorporated into oleaginous cream (water in oil cream base) which was applied topically in one group and Minoxidil 2% solution was applied topically and served as positive control for comparison (36) The duration required for hair growth initiation as well as completion of hair growth cycle was recorded.. The result of treatment with petroleum ether extracts were better than the positive control minoxidil 2% treatment(36).

Anticancer Activity

The study of anticancer activity was carried out in Swiss albino mice using methanol extract of *Eclipta alba* against Ehrlich Ascites Carcinoma (EAC). . The methanolic extract of *Eclipta alba* was administered orally in the wistar albino mice at a dose of 250 and 500 mg/kg body weight for 9 consecutive days. The parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span in experimental animal models were applied for assessment of cancer. The study proved that the methanolic extract of *Eclipta* showed significant results in the parameter like increased the life span of EAC treated mice and also maintained the restoration of the hematological parameters as compared to the EAC bearing mice. This proved the anticancer activity of the methanolic extract of *Eclipta alba* in the tested animal models Coumestans which are active constituents of Eclipta are commonly known for its action as phytoestrogens which act as chemopreventive agent in breast and prostate cancer (37) The another compound in *Eclipta protrata* is Dasyscyphin-C (saponins) which is a newer isolated compound also reported to have anticancer-cytotoxic activity in an invitro study on study (38) in HeLa (Human cervical carcinoma) & vero cell lines , it showed a good anticancer-cytotoxic activity on HeLa cells. The hepatic stellate cell line (HSCs) of rat was used as in-vitro assay system in this study where the methanolic extract of aerial parts of Eclipta prostrata was used. The study revealed significant inhibitory activity on HSCs proliferation (39).

Antibacterial activity

The antibacterial activity study was conducted using the aerial parts of *Eclipta alba* which were extracted in various solvents like acetone, ethanol, methanol, water and hexane against selected strains of gram positive and gram negative bacterial species by agar well diffusion methods. The MIC and MBC methods were also applied (40) for the study.

The *Eclipta alba* extract in the solvent Hexane showed high antibacterial activity against the following bacteria - *S.aureus, B.cereus, E.coli, S.typhi, K.pneumoniae, S.pyogenes* and *P.aeruginosa as compared to the inhibitory activity of* standard antibiotics (Ciprofloxacin 25 µg/ml).

The other extract of *Eclipta alba* like acetone, ethanol, methanol and aqueous extracts showed intermediate activity against *S.aureus, B.cereus, E.coli,*

S.typhi, K.pneumoniae, P.aeruginosa, P.mirabilis and S.pyogenes in comparison with standard antibiotics (Ciprofloxacin 25 µg/ml) *The study* showed maximum MIC (Mean inhibitory concentration) of 90.0µg/ml shown by *E.coli* and *S.aureus* (below 100µg/ml),and the MIC of 125.0µg/ml shown by *E.coli*, *K.pneumoni, P.mirabilis* and *S.typhi proved* better (100-500µg/ml) as such by the action of acetone, ethanol, methanol and hexane extracts on test bacterial species respectively.

MIC between (500-1000µg/ml) was considered to be good.

MBC results were having similar results to MIC and confirmation was made by observing the absence of growth of bacteria in the culture plates after 24 hrs of incubation at 37ºC.

Thus the Eclipta proved to be a potent antibacterial plant drug.

This antibacterial and hepatoprotective action of *Eclipta alba* extract can be utilized to formulate a potent dry to combat the bacterial and hepatotoxic infections.

Memory enhancing activity (41)

The extract of 100 and 200 mg/kg extract of *Eclipta alba* was administered in rat to evaluate transfer latency (TL) on an elevated plus maize which was considered as a measure of acquisition and learning to assess spatial habitual learning from 20 min. to 144 hour. The results revealed significant improvement in retrieval of memory.

Other pharmacological actions of *Eclipta alba*

The methanolic extract *Eclipta prostrata* contains free carboxylic acid at C-28 position in echinocystic acid derivatives which showed antifibrotic activity in a study conducted to study the antiproliferative activity of triterpenoid from hepatic stellate cells in rats (42).

In an another study Ethanolic and ethyl acetate fractions of Eclipta prostrata were tested for antibacterial activities against *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysentrae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* and were found to be potent antibacterial (43)

Ethanopharmacological review showed that *Eclipta prostrata* is being used combined with a non-plant material which is used to bath children suffering from malnutrition for 9 days and used as self medication by AIDS patients in various parts of southern Thailand (17,44).

It has been reported that the formulations like 16 parts of *Eclipta prostrata* (*bhringaraj*), 1 part of *Triphala* formula {Emblica officinalis (amalaki), 1 part of *Caltropis gigantean* (arka) and 1 part of *Smilax officinalis* (sariva) mixed with 80 parts of sesame oil and boiled to make a medicated oil which is reported to be used in skin diseases (45)

Conclusion

Eclipta alba is an important medicinal plant having remarkable activities for curing several diseases. Its chemical constituents have wide activities on living cells. Thus the review of literature shows the significant pharmacological activities of *Bhrungaraj* i.e. *Eclipta alba* like hepatotoxicity, antiproliferative, antidiabetic, hypolipedemic and its potential to inhibit the growth of the bacteria and fungus also.

Further scope of Study

The investigation of the plant by the isolation of the newer molecules which will be helpful for the study of the pharmacological activities thus contributing to the human trials.

References

1. Chunekar KC, Pandey GS , *Guduchyadi varga*, *Bhavprakash Nighantu*, Chaukhamba Vishvabharati Publication,Varanasi, , Shlokaa no. 240-241, 2002, 429
2. Pandeu M, Singh GN, Sharma R, Lata S, Antibacterial activity of Eclipta alba Hassk., Journal of applied pharmaceutical science, (01) 07, 2011:104 -107
3. Mithun NM, Shashidhara S, Vivekumar R , *Eclipta alba* (L.) A Review on its Phytochemical and Pharmacological Profile, Pharmacologyonline, 2011, 1: 345-357
4. Chunekar KC, Pandey G, *Guduchyadi varga*, *Bhavprakash Nighantu*, Chaukhamba Vishvabharati Publication,Varanasi, , Shlokaa no. 242,2002 429
5. Oza Z, Mishra K, *Dhanvantari nighantu,* 4th edn, Chaukhamba orientalia publication,Varanasi, 2004
6. Wagner A, Geyer H, Kiso B, Hikino Y, & Rao GS, Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulacea*, Planta Med. , Vol-52, 1986, 370-374
7. Khare CP, Encyclopedia of Indian Medicinal plants, Springer-Verlag Berlin Heidelberg, New York, 2004, 198
8. Chopra RN, Nayar, SL, Chopra, IC,1955, *Glossary of Indian Medicinal plants.* C.S.I.R., New Delhi.
9. Chokotia L, Vashistha P, Sironiya R , Matoli H, Pharmacological Activities Of *Eclipta alba* (L.), International Journal Of Research And Development In Pharmacy And Life Sciences, Vol. 2, 2013/4, 499-502
10. Holm LG, Plucknett DL, Pancho JV, Herberger JP, The world's worst weeds, Distribution and Biology, East-West Center by the University Press. Hawaii. - Galinato M., Moody K., Piggin C. M. 1999. Upland rice weeds of South and Southeast Asia. IRRI. Philippines.
11. Wagner H, & Fessler B, In vitro 5- Lipoxygenase inhibition by *Eclipta alba* extracts and the coumestan derivative wedelolactone, Planta Med., Vol-52, 1986, 374-377
12. Daniel M, Medicinal plants chemistry and properties, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 2006, 149
13. Mors 1991 snake bite
14. Upadhyay E, Pandey RK, Jha MB, Pandey VB, Eclalbatin,a triterpene saponin from *Eclipta alba*, J. Asian. Nat. Prod. Res., Vol- 3, 2001, 213-217
15. Lucas DS, *Dravyaguna vijnyan*, vol 2, 2008, Chaukhamba vishvabharati publication, Varanasi, 1st edition
16. Saxena AK, Singh B, Anand KK, Hepatoprotective effects of Eclipta alba on subcellular levels in rats. Journal of ethnopharmacology. 1993; 40(3): 155-61

17. Sawangjaroen N, Subhadhirasakul S, Phongpaichit S, Siripanth C, Jamjaroen K, Sawangjaroen K. The in vitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand. Parasitol Res. 2005; 95(1): 17-21.
18. Supinya Tewtrakul, Sanan Subhadhirasakul, Sopa Kummee. Anti-HIV-1 integrase activity of medicinal plants used as self medication by AIDS patients. Songklanakarin J. Sci. Technol. 2006; 28(4): 785-90
19. Karthikumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves of Eclipta prostrata (L). Sci. Res. Essays. 2007; 2(4): 101-04
20. Mukherjee DR, Poddar H. Pilex therapy in Piles - a Preliminary Report. The antiseptic. 1976; 10: 541
21. Abdul viqar khan, athar ali khan, Ethnomedicinal uses of Eclipta prostrate. Indian journal of traditional knowledge, 2008; 7(2): 316-20
22. Samudram P, Rajeshwari Hari, Vasuki R, Geetha, Sathiya moorthi P. Hepatoprotective activity of Bi-herbal ethanolic extract on CCl4 induced hepatic damage in rats. AJBR. 2008; 2(2): 61-65
23. Lal V.K, Amit Kumar, Prashant Kumar, Kuldeep Singh Yadav, Screening of Leaves and Roots of *Eclipta alba* for Hepatoprotective Activity, Arch. Appl. Sci. Res, 2010; 2(1): 86-94.)
24. Dhandapani R. Hypolipidemic activity of Eclipta prostrata (L.) L. leaf extract in atherogenic diet induced hyperlipidemic rats, 2007; 45: 617-19.
25. Dae-Ik Kima, Sung-Hyen Lee, Jin-Ho Choia, Hyun Soon Lillehoj, Mi-Hee Yu, Gun-Soon Lee, The butanol fraction of Eclipta prostrata (Linn) effectively reduces serum lipid levels and improves antioxidant activities in CD rats. Nutrition Research. 2008; 28: 550-54.)
26. Bhaskar rao D, Ch ravi kiran, Madhavi Y, Koteshwara rao P, Raghava rao T. Evaluation of antioxidant potential of clitoria ternate L. and Eclipta prostrata L. Indian journal of biochemistry and biophysics. 2009; 46: 247-52.
27. Otilia Banji, David Banji, Annamalai Ar, Manavalan R, Investigation on the effect of *Eclipta alba* on animal models of learning and memory, Indian J physiol pharmacol, 2007; 51(3):274-78
28. Jayathirthaa MG, Mishraa SH. Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and Centella asiatica. Phytomedicine, 2004; 11(4): 361-65
29. Christybapita D, Divyagnaneswari M, Dinakaran Michael R, Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of Oreochromis mossambicus, Fish and shellfish immunology, 2007; 23(4): 840-52
30. Arunachalam G, Subramanian N, Pazhani GP, Ravichandran V, Anti-inflammatory activity of methanolic extract of Eclipta prostrata L. (Astearaceae). African Journal of Pharmacy and Pharmacology, 2009; 3(3): 97-100
31. Amritpal singh, Samir malhothra, Ravi subban. Anti-inflammatory and analgesic agents from Indian medicinal plants. International journal of integrative biology, 2008; 3(1):58-72
32. Mahesh Sawant, Jolly C. Isaac, Shridhar Narayanan. Analgesic studies on total alkaloids and alcohol extracts of *Eclipta alba* (Linn.) Hassk. Phytotherapy research, 2004; 18(2): 111-13
33. Ananthi J, Prakasam A, Pugalendi KV, Antihyperglycemic Activity of *Eclipta alba* Leaf on Alloxan-induced Diabetic Rats, Yale Journal of Biology and Medicine,2003; 76:97-102
34. Hemalatha S, Ayyappan T, Shanmugan S, Nagavalli D, Shrivijaya kirubha T, Evaluation of antidiabetic and diuretic activity of polyherbal formulation, Indian Journal of Traditional Knowledge, 2006; 5(4): 468-70
35. Rupali thorat, Varsha jadhav, Vilasrao kadam, Ninad sathe, Ashwini save, Vikas ghorpade, Evaluation of a herbal hair oil in reducing hair fall in human volunteers, 2009; 6: 974-79
36. Roy RK, Mayank Thakur, Dixit VK, Hair growth promoting activity of *Eclipta alba* in male albino rats, Arch Dermatol Res., 2008; 300: 357-64.)
37. Neerja Kaushik-Basu, Alain Bopda waffo, Tanaji Talele T, Amartya Basu, Paulo Costa R R, Alcides da Silva J. M, Stefan Sarafianos G, Francois Noel, Identification and characterization of coumestans as novel HCV NS5B polymerase inhibitors, 2008; 36 (5): 1482-96
38. Khanna, kannabiran, Anticancer-cytotoxic activity of saponins isolated from the leaves of Gymnema sylvestre and *Eclipta alba* on HeLa cells, International journal of green pharmacy, 2008; 1: 227-29
39. Mi Kyeong Lee, Na Ry Ha, Hyekyung Yang, Sang Hyun Sung,Gun Hee Kim, Young Choong Kim, Antiproliferative activity of triterpenoids from Eclipta prostrata on hepatic stellate cells, 2008; 15 (9): 775-80
40. Manoj Kumar Pandey, G.N.Singh, Rajeev Kr Sharma and Sneh Lata, Antibacterial activity of *Eclipta alba* (L.) Hassk, Journal of Applied Pharmaceutical Science, 01 (07); 2011: 104-107
41. Banji OD, Annamalai AR, Manavalan R, Investigation on the effect of Ecipta alba on animal models of learning and memory, Indian J. Phusiol. Pharmacol: 2003, Vol. 51, (3), 274-278.
42. Sagar B.P, Sangwan A, Panwar A, Sangwan A. In-vitro Production of Anti-hepatotoxic Compounds in Cultures of *Eclipta alba* Linn. and *Silybum marianum* gaertn. 2006; 6: 88.
43. Karthikumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves of Eclipta prostrata (L). Sci. Res. Essays. 2007; 2(4): 101-04.
44. Cheryl Lans, Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine, Journal of Ethnobiology and Ethnomedicine, 2007; 3(3): 1-12
45. Bensky Dan, Andrew Gamble, Chinese Herbal Medicine: Materia Medica, 1986, Seattle: Eastland Press.

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