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# COMPARATIVE ANALYSIS OF HAEMATINIC EFFECT OF DHATRYARISTA WITH STANDARD FERROUS SULPHATE IN TANNIC ACID INDUCED IRON DEFICIENCY ANAEMIA IN ALBINO WISTAR RATS

**Srivastava V.[1], Sarkar S.[1]*, Pattanayak C.[2], Mishra M.[3] and Jena J.[4]**

Department of Pharmacology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha.

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***Corresponding Author**
**Sarkar S.**
Department of Pharmacology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha.

## ABSTRACT

**Background:** Iron deficiency anemia affects more than 1.2 billions individuals worldwide, and iron deficiency in the absence of anemia is even more frequent. Iron deficiency is caused by physiologically increased iron requirements in children, adolescents, young and pregnant women, by reduced iron intake, or by pathological defective absorption or chronic blood loss. Treatment modalities include both oral and parenteral therapies. **Objectives:** a) To study the improvement in haemoglobin after giving dhatryarista in IDA. b) To compare the effect of Dhatryarista (in different concentrations) with standard oral formulation Ferrous sulphate in terms of haemoglobin. **Materials and methods:** The study was conducted for the period of two months in department of Pharmacology KIMS, bhubaneswar. Total 24 Albino wistar rats were taken and divided into 4 groups containing 6 rats each. Anaemia was induced by adding tannic acid in diet of 24 rats by 21days. After induction of anaemia, Group II, Group III and Group IV rats were given standard drug (Ferrous sulphate), Dhatryarista sample 1 and Dhatryarista sample 2 respectively, alongwith normal diet for next 30 days. Blood samples were taken in day 1, day 21 and day 53. **Results:** All the values are expressed as a Mean ± SEM (standard error of mean).The data were analyzed by one way ANOVA and post hoc methods. using socscistatistics.com. Significant decrease in haemoglobin were observed, when compared between day 1 and day 21, while significant increase in haemoglobin was observed after 30 days of administration of test and standard drug, when compared between day 21 and day 53. **Conclusion:** The administration of tannic acid to rats caused anaemia characterized by reducing hematological parameters. The oral administration of Dhatryarista in the dose of

4.32ml/kg/day P.O and 8.64ml/kg/day P.O significantly increased haemoglobin level. The results demonstrated that higher doses of the plant extract didnot show any signs of acute toxicity in animals.

**KEYWORDS:** Iron deficiency anaemia, Dhatryarista, Ferrous sulphate, Amalaki, Haemoglobin.

## 1. INTRODUCTION

Iron deficiency anaemia or *Panduroga* is defined as *Pitta dominant Tridoshaja* disease where *Vivarnata of Twaka* (discolouration of skin) is mainly *Pandu* (pallor/yellowish-whitish) due to *Alpa Rakta* (reduced blood) or *Vidushya Rakta* (vitiated blood). Anaemia is defined as qualitative and quantitative reduction of circulating RBC and/or the percentage of haemoglobin concentration in relation to standard age and sex.[1] Iron deficiency anaemia is a common type of anaemia in which blood lacks adequate healthy red blood cells. Red blood cells carry oxygen to the body's tissues. As the name implies, iron deficiency anaemia is due to insufficient iron. In women of childbearing age, the most common cause of iron deficiency anaemia is a loss of iron in the blood due to heavy menstruation or pregnancy. A poor diet or certain intestinal diseases that affect how the body absorbs iron can also cause iron deficiency anaemia.

The state of IDA is being managed with the supplementation of external iron containing drugs for which several types of modern medicines are available in the market. These modern iron formulations contain one or the other types of iron salts such as, ferrous fumarate, ferrous sulfate, ferrous glycine sulfate, ferric ammonium citrate, ferric hydroxide polymaltose complex, iron choline citrate, iron dextran, ferrous calcium citrate, iron sorbitol citrate, colloidal iron hydroxide, ferrous gluconate, ferric hydroxide, and ferrous succinate. It has been further reported that the long-term treatment of IDA with these drugs is associated with constipation, heart burn, nausea, gastric discomfort and diarrhea.

*Dhatryarishta*, a non iron formulation, is indicated in *Pandu roga* by *Acharya Charaka*. As most of the Ayurvedic formulations are found effective against IDA, their usage should be fostered at all level in addition to modern allopathic medicines. This study focuses about the haematinic effect of Dhatryarista as compared to allopathic medicines available. It contains four major ingredients- amalaki, pippali, honey and sugar. Amalaki (Emblica officinalis Gaertn) has been described in Phalasava and Sarkara as a separate Asava

yoni by Acharya Charaka.[2] Asava yoni itself denotes the fermenting base for Asava-Arishta. Dhatryarishta is indicated in the context of Panduroga in Charaka Samhita Chikitsasthan.[3] The same reference is available in Chakradatta,[4] Bhaishajya Ratnavali,[5] and Sahastrayogam,[6] in Arishtaprakaran.

## 3. OBJECTIVES

- To study the improvement in haemoglobin after giving dhatryarista in IDA.
- To compare the effect of Dhatryarista (in different concentrations) with standard oral formulation Ferrous sulphate in terms of haemoglobin.

## 4. MATERIALS AND METHODS

The study was conducted in the animal house, located in department of pharmacology after getting approval from Institutional Animal ethics committee of KIMS, Bhubaneswar. The study period was two months (60days).

Dhatri Swarasa (Amalaki Juice), Madhu (Honey)- one-eighth of Swarasa, Krishna(Pippali) half Kudava (96 g), and Sarkara (Sugar) are the four ingredients of Dhatryarishta as per classic method.

***Amalaki*** ***Pippali***

**Preparation of Dhatryarista sample**

The normal human dose trial compounds i.e Dhatryarista is 48ml/day for an adult (Jyothi et al, RGUHS).[7] The dose of drug is fixed by extrapolating the human dose to rats on body surface area ratio as per the table of Pagets and Barnes (1969) which will be 4.32 ml/kg body weight.

Rat dose per kg body wt= 0.018× Human dose× 5= 0.018 × 48ml × 5= 4.32 ml/kg.

**A. Animals required**

a. Species/common name: Wistar albino rats
b. Age/ weight: adult/ 100-200gm
c. Gender- Male
d. Number to be used- 24
e. Source of animal- Institutional Animal house, Kalinga Institute of Medical Sciences, KIIT university, Bhubaneswar, Odisha

Twenty four adult albino rats of either sex weighing 100–200 g were used for this study. The animals were allowed to acclimatize in the research laboratory for 1 week before the commencement of the study. The animals had been maintained under standard conditions (room temperature 25°C±3, humidity 35–60%, and light and dark period 12/12 h). All animals were fed with food and water ad libitum. All the animal testing were done under the approval of Institutional Animal Ethical Committee (IAEC) of KIMS, Bhubanesawar.

**B. Induction of anaemia**

Induction of anaemia was done was done by adding tannic acid to the basal diet of the rats. The rats were fed on the basal diet containing tannic acid for 3 weeks. 20g tannic acid/kg was added to the basal diet at the expense of the whole diet,[8] which was given to all 24 rats belonging to Group I to Group IV. After 21 days, iron deficiency anaemia was induced in those rats. From day 22, Group II rats were started on standard treatment available which is ferrous sulphate, Group III were given Dhatryarista sample 1 (4.32ml/kg P.O) and Group IV were given Dhatryarista sample 2 (8.64ml/kg P.O), alongwith normal diet, for next 30 days. The blood samples were collected on day 1, day 21 and day 53.

**C. Grouping of animals**

**Table 1: Shows grouping of the animals with respective treatment group. Ferrous sulphate and two concentrations of Dhatryarista were started from day 22 after induction of anaemia in groups II, III and IV respectively.**

| Grouping | No of animals | Drug |
|---|---|---|
| **Group I** | 6 | Control - Basal diet + Tannic acid (normal diet started after day 22) |
| **Group II** | 6 | Ferrous sulphate (Normal diet + $FeSo_4$ 40mg/kg P.O) |
| **Group III** | 6 | Test group 1 (Normal diet + Sample 1-4.32ml/kg P.O) |
| **Group IV** | 6 | Test group 2 (Normal diet + sample 2- 8.64 ml/kg P.O) |

**D. Toxicity Studies**

There was no mortality observed with oral administration of Dhatryarista even at the higher dose 8.64ml/kg P.O. Doubling the dose had no toxic effect on the normal behaviour of the rats. Hence, 1ml and 2ml dose per day were selected and administered.

**E. Statistical analysis**

All the values are expressed as a Mean ± SEM (standard error of mean). The data were analyzed by one way ANOVA and post hoc methods. using socscistatistics.com. A level of $P < 0.05$ was considered as statistically significant. A level of significance was noted and interpreted accordingly.

**4. OBSERVATION AND RESULTS**

The whole study was conducted in 24 healthy albino rats. These rats, divided from Group I to Group IV with six rats in each group, were given basal diet including tannic acid (20g/kg) for next 21 days for the induction of anaemia. From day 22, Group II rats were started on standard treatment available which is ferrous sulphate, Group III were given Dhatryarista sample 1 (4.32ml/kg P.O) and Group IV were given Dhatryarista sample 2 (8.64ml/kg P.O), along with normal diet, for next 30 days.

Blood samples were taken on day 1 and then on day 21 (after induction) and then on day 53 for checking the efficacy of the drugs provided to rats. Blood was withdrawn from retro-orbital vein of rats. Samples were added to a tube containing ethylenediaminetetraacetic acid after 4 weeks of treatment. The haemoglobin was determined at day 53rd, using an automatic blood cell counter.


**Table 2: Hb level of rats before and after induction and after 30 days of treatment. Values are expressed as mean±SD, one way ANOVA and post hoc was done, * and # shows P<0.05- statistically significant, ** and ## shows P<0.005 - statistically very significant, * indicates increase while # indicates decrease.^ significant difference between group IV and II.**

| Grouping | Day 1 Hb% | Day 21 Hb% | Day 53 Hb% |
|---|---|---|---|
| Group I | 13.64 ± 0.31 | 8.78 ± 0.55## | 9.22 ± 0.45 |
| Group II | 13.23 ± 0.71 | 9.28 ± 0.43## | 12.74 ± 0.28** |
| Group III | 13.44 ± 0.68 | 8.54 ± 0.52## | 13.24 ± 0.43** |
| Group IV | 13.25 ± 0.46 | 9.13 ± 0.34## | 14.92 ± 0.54**^ |

After adding tannic acid to the diet, there was a significant decrease in haemoglobin on day 21. On applying statistics, it was observed that a significant decrease in haemoglobin were observed, when compared between day 1 and day 21 (#P<0.05- statistically significant, ##P<0.005 - statistically very significant), while significant increase in haemoglobin was observed after 30 days of administration of test and standard drug, when compared between day 21 and day 53(*P<0.05- statistically significant, **P<0.005 – statistically very significant). The results showed that the rats of the Group II–IV have almost completely recovered at the end of two months. (Table 2). We applied Tukey HSD post hoc analysis and observed that there was significant difference between haemoglobin levels in groups II, III, IV as compared to group I on day 53, while no significant difference was observed within comparison of groups II & III as well as group III and IV. But a significant difference was noted on comparison of Group II and IV, considering that the haemoglobin levels of rats getting higher dose of Dhatryarista were more as compared to the standard drug ferrous sulphate. This implies that doubling the dose of dhatryarista produces beneficial effect instead of toxic effect.

## 5. DISCUSSION

Iron deficiency is the most common form of nutritional deficiency. The size and number of red blood cells are reduced. There is a spectrum of iron deficiency ranging from iron depletion, which causes no physiological impairments, to iron-deficiency anemia, which affects the functioning of several organ systems. The terms anemia, iron deficiency, and iron-deficiency anemia are often used interchangeably, but are not equivalent. Anemia can only be diagnosed as irondeficiency anemia when there is additional evidence of iron deficiency. A diagnosis of iron-deficiency anemia can be made if haemoglobin concentration or hematocrit value increases after a course of therapeutic iron supplementation (Centers for Disease Control and Prevention, 1998).

Tannins are a group of compounds belonging to the phenolic class of secondary metabolites in plants. These compounds, in particular tannic acid, exhibit a wide variety of activity and physiological functions.[9] A number of adverse nutritional effects have been attributed to tannins. It has been demonstrated that feeding growing animals diets containing these compounds brings about several physiological and biochemical effects. These effects are reflected by growth inhibition, negative nitrogen balances, reduced intestinal absorption of sugars and amino acids, reduced immune response and increased liver and protein catabolism.[10] Also, foods with high tannin content inhibit iron absorption from meals.[11]

*Dhatryarista* is a derived from amalaki which had been in use as an herbal prepartion for treatment of IDA. *Amalaki* was taken in 12 kg amount, *Sarkara* was added at 10% of the weight of Amalaki, that is, 1.2 kg, and honey was added one-eighth of the *Swarasa* obtained from *Amalaki*, and 48 g of *Pippali Churna* as *Prakshepa* was added (one-twenty-fifth of *Sarkara*).

Amalaki is an Amla rasa pradhana and can increase Raktha and hence in Rakthalpatha, Amla preeti is seen. It also being a rich source of Vit.C helps in absorption of iron. Hence it is used in anaemia.[12] Amalaki is worshipped as an auspicious fruit since ancient time and respected as a symbol of good health. The festival Amala Navami is celebrated at the begining of winter season of Hindu calender, where Amalaki tree is socially and religiously propagated among people and cultivated for promotion of good health. When the fruit is dried, the main ingredient, water, is mostly eliminated, and the remaining constituents are present in considerably larger proportions. The pulpy portion of fruit, dried and freed from the nut contains gallic acid, sugar, albumin, moisture, gum, crude cellulose. The edible fruit tissue

contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. A research showed that 8.75 mg of natural vitamin C complex from Amalaki is equivalent to 100mg of the most commonly used synthetic vitamin C. Amalaki possesses the highest level of heat and storage stable vitamin C known to man. Phyllembin from fruit pulp is identified as ethyl gallate.[13] It has mild depressant action of CNS and spasmolytic action. The fruit also contains considerably higher concentration of most minerals and amino acids than apples.

*Pipali* fruits contain 1% volatile oil, resin, a waxy alkaloid, a terpenoid substance and alkaloids piperine and piperlongumine. Two new piperidine alkaloids namely pipermonaline and piperundecalidine were isolated from fruit. Dried spikes are acrid, stomachic, carminative, tonic, laxative, digestive, emollient, mild thermogenic and antiseptic. They are useful in anorexia, dyspepsia, vomiting, flatulence, colic, diarrhea, gastric disorders and insomnia.[14]

*Madhu* is used as an important dravya in many Ayurvedic formulations, wherein it has its own manifold pharmacological activities. It is also the most commonly used adjuvant as it acts as a suitable vehicle due to its '*Yogavahi'* (catalytic) property. Honey contains dextrose and fructose which are known as invert sugars (50-90%) and water. It also contains 0.1 - 100% of sucrose and small quantities of other carbohydrates, volatile oil, pigments and plant parts especially pollen grains. It also contains vitamin B & C. Acid constituents are Formic, Acetic, Malic, Citric, and Succinic acids. It also contains an enzyme named Invertase. It is laxative, demulcent and emollient. It possesses nutritive properties. The fatty acids present in honey stimulate peristalsis and digestion. It has beneficial effect on the digestion and appetite of those weak stomach and loose bowels. It decreases flatulence and increases general metabolism.

In present study, different doses of dhatryarista were administered in rats along with standard drug ferrous sulphate. Human equivalent dose is 48ml/day. In rats, required dose is 4.32ml/day but in this study, we are giving the higher dose so as to observe any kind of toxicity. We included 30 rats in this experiment which were divided as 6 rats in 5 groups. Blood samples were collected at Day 1 for measuring the baseline parameters. Group I rats were kept on normal basal diet while Groups II, III, IV, and V were put on basal diet including tannic acid for next 21 days. On Day 21, again blood samples were collected. From day 22, ferrous sulphate and Dhatryarista sample 1 & 2 were started in groups III, IV and V

respectively for nest 30 days. Blood samples were again collected on day 53. It was seen that the recovery was progressive such that after 4 weeks of continuous treatment, the haemoglobin concentration was higher in the treated groups than in the control groups. It was also observed that the recovery of the treated groups was dose related with the highest dose of 8.64 ml/kg affecting the highest change.

Animals are similar to humans in terms of reduction of haemoglobin, which is indicative of anaemia.[15] In IDA, all the parameters are markedly reduced especially haemoglobin. Dhatryarista has shown to increase the red cell indices in drug sample groups without causing any signs of toxixity.

## 5. CONCLUSION

The administration of tannic acid to rats caused anaemia characterized by reducing hematological parameters. The oral administration of Dhatryarista in the dose of 4.32ml/kg/day P.O and 8.64ml/kg/day P.O significantly increased haemoglobin level. And also, the results demonstrated that higher doses of the plant extract didnot show any signs of acute toxicity in animals. This result supports the traditional use of dhatryarista in the treatment of anemia.

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## CONFLICT OF INTEREST

Nil.

## REFERENCES

1. Sen SK. Essentials of Clinical Diagnosis. 7th ed. Calcutta: The standard Book House, 1994; 14.
2. Acharya Vaidya Jadavaji Trikamji., editor. Chakrapanidatta's Commentary. Varanasi: Chaukhambha Sanskrit Sansthan; Reprint; Charaka's Charaka Samhita, 2004; 134: 25/49.
3. Acharya Vaidya Jadavaji Trikamji., editor. Chakrapanidatta's Commentary. Varanasi: Chaukhambha Sanskrit Sansthan; Reprint; Charaka's Charaka Samhita, 2004; 531. Chi.16/111-3.

4. Chaukhambha Sanskrit Sansthan. 49-51. Vol. 8. Varanasi: Panduroga Chikitsa Prakarana; Reprint; Chakrapanidatta's Chakradatta by Indradev Tripathi, 2005; 83.
5. Bhaishajya Ratnavali Sri Govindas's. In: Chaukhambha Sanskrit Sansthan. sixth edition. 120-1. Shastri Kaviraj Sri Ambikadatta., editor. Varanasi: Panduroga Chikitsa Prakarana, 2002; 12: 275.
6. Chaukhambha Sanskrit Prathisthan. Delhi: Arishta Prakaran; Reprint; Sahastrayogam by Dr. Ramnivas Sharma and Dr. Surendra Sharma, 2007; 167.
7. Jyothi KB A Clinical Study On The Efficacy Of Dhatryarishta In Pandu W.S.R To Iron Deficiency Anemi; Rajiv Gandhi University of health sciences, 2010.
8. Kaosar A, Kazuki S, Satoshi I, Hiroshi H. Effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. Biosci. Biotechnol, Biochem., 2004; 3(68): 584-592.
9. Carbonaro M, Grant G, Pustai A. Evalution of polyphenol bioavailability in rat small intestine. Eur. J. Nutr., 2001; 40(2): 84-90.
10. Marzo F, Urdaneta E, Santidrian S. Liver proteolytic activity in tannic acid-fed birds. Poultry Sci., 2002; 81: 92-94.
11. South PK, Miller DD. Iron binding by tannic acid: Effects of selected ligands. Food Chem., 1998; 63(2): 167-172.
12. Ram Karan Sharma, Vaidya Bhagavan Dash. Charaka Samhita translation. Varanasi; Chaukamba Sanskrit Series, 2002.
13. Ayurveda for Promotion of Health." Journal of Ayurveda, Jan-March 1985; 3.
14. The useful plants of India; with notices of their chief medicinal value in commerce, medicine and the arts.
15. Ashaolu JO, Ukwenya VO, Okonoboh AB, Ghazal OK, Jimoh AA. Effect of monosodium glutamate on hematological parameters in wistar rats. Int J Med Med Sci 2011; 3: 219-22.