ORIGINAL ARTICLE

Phyto-Based Iron Supplementation: Development and Evaluation of a Hematinic Herbal Tea

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Abstract

Aim: Anemia is a condition where the amount of hemoglobin (Hb) in the blood is below the normal level, or there are fewer red blood cells (RBCs) than normal. Hematinics are substances that are essential to the proper formation of the components of blood. **Materials and Methods:** The leaves of *Asparagus racemosus*, *Boerhavia diffusa, and Phyllanthus emblica* were used for preparing herbal tea in the present study. The herbs were evaluated for physicochemical parameters, such as extractive values and ash content and phytochemical screening was also carried out. The formulated herbal tea bags were evaluated for physicochemical parameters, spectroscopic analysis, total phenolic, and total flavonoid content. Acute oral toxicity studies and pharmacological evaluation of herbal tea for hematinic activity (phenylhydrazine-induced anemia) was carried out in rats. **Results and Discussion:** The results revealed significant change in RBC, packed cell volume, and Hb in anemic rats. **Conclusion:** From this study, it is concluded that the herbal tea bag composed of *A. racemosus*, *P. emblica,* and *B. diffusa* possesses significant hematinic activity.

Key words: Anemia, hemoglobin, *Asparagus racemosus*, *Boerhavia diffusa* and *Phyllanthus emblica*, phenylhydrazine-induced anemia

INTRODUCTION

Anemia is a condition where the amount of hemoglobin (Hb) in the blood is below the normal level, or there are fewer red blood cells (RBC) than normal. Anemia possess severe health burden in India and is one of the world's major public health problems.[1] It is characterized by the Hb count lower than 13 g/dL in male and 12 g/dL in female according to the World Health Organization and iron deficiency is the most common and widespread nutritional disorder in the world. It is affecting a large number of children and women in developing countries and it is the only nutrient deficiency that is also significantly prevalent in industrialized countries. Iron deficiency anemia is affecting more than 30% of the world's population.[2,3]

Hematinics are substances that are essential to the proper formation of the components of blood. Hematinic substances are essential to the proper formation of the components of blood. Iron being a hematinic substance is a very essential component of RBCs and the muscles that assist in the transportation of oxygen throughout the body.[4] When the blood is deficient in RBCs, in Hb, or in total blood volume, it can cause a variety of complications, including fatigue and stress on bodily organs.[5]

Developing new herb tea products from indigenous plants will provide novel uses for underutilized plants. It will further provide consumers with new alternatives to traditional teas. Moreover, the research will bring to light the potential of underutilized plants for food product development. The research will broaden understanding of the sensory characteristics and

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preferences of herb teas in particular and beverages in general. It will further advance research in herb tea product development. Many plant drugs have been traditionally used as hematinics. In the present research, herbs, such as *Asparagus racemosus, Boerhavia diffusa,* and *Phyllanthus emblica* were screened for iron content following traditional claims of the plant.

MATERIALS AND METHODS

The leaves of *A. racemosus*, *B. diffusa, and P. emblica* were collected from Tirupati, India in the month of January 2024 and it was identified and authenticated. Phenylhydrazine from Fisons Ltd., Guildford, UK was used to induce anemia.

Animals

Wister albino rats (150–200) were housed in the animal house of Sree Vidyanikethan College of Pharmacy and they were maintained under standard laboratory conditions. They were fed with standard rat feed and water ad libitum. All the experiments involving animals have been performed after getting approval from the Institute's Animal Ethical Committee of Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh.

Preparation of plant extracts

The leaves of *A. racemosus*, *B. diffusa,* and *P. emblica* were collected, washed and shade dried. After complete drying, they were powdered and stored in airtight container. Dried powdered drugs were used to prepare the extract. About 50 g of shade-dried powders of each plant material were extracted with water by maceration. The solvent was evaporated and the accurate weight of the extracts was noted. Extractive values (%) were calculated with reference to air-dried drug.

Physicochemical evaluation

Physicochemical constants, such as ash values, extractive values, and moisture content were determined as per the standard procedures given by Khandelwal.[6]

Phytochemical screening

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents as per the standard procedures given by Khandelwal.[6]

Formulation of herbal tea bags

Sample preparation

All plant materials were carefully inspected and all foreign materials were removed. The samples were then gently rinsed in tap water and spread thinly on paper and shade dried. After drying the plant materials were collected and crushed unevenly. They were stored in glass bottles with tight lids and labeled.

Preparation of tea bags

Tea bags were prepared by using white muslin cloth. The white muslin cloth was purchased from the local market and washed to remove any debris. The cloth was then dried and tea bags were stitched in any required form. The tea bags were filled with the required amount of herbs and then packed.[7] They were stored in airtight glass containers. Each tea bag contains the quantities of herbs as mentioned in Table 1.

Evaluation of herbal tea

Physicochemical evaluation

The physical characteristics, such as color, taste, and odor were evaluated. pH and solubility were also evaluated.[8]

Spectroscopic evaluation

Ultraviolet (UV)-visible spectrophotometer

1 g of tea powder was weighed and added to 100 mL of hot water (80°C) and soaked for 1 min. The above extract was filtered. Then 1 mL of extract was diluted to 10 mL and absorbance was noted at 280 nm. The above experiment was repeated for 2, 3, 4, and 5 min and readings were noted. The same procedure was followed for the cold water (8°C) and readings were noted at 280 nm.[8]

Atomic absorption spectroscopy (AAS)

The iron content present in the hematinic herbal tea was determined by using an Analyst 700 atomic absorption spectrometer.[9]

Total phenolic content

0.5 g of sample was weighed and dissolved in 100 mL of distilled water. From this solution, 0.1 mL was taken and 1.25 mL of Folin Ciocalteu reagent was added and left for 5 min to react. To that solution, 2.5 mL of sodium bicarbonate was added and made up to 10 mL. It was kept for 30 min to complete the reaction. The absorbance was measured to that

Table 1: Formulation for herbal tea

S. No.	Drug	Quantity (g)
1	*Asparagus racemosus*	1
2	*Boerhavia diffusa*	1
3	*Phyllanthus emblica*	1
4	Salt	q.s

The above formula was calculated for 200 mL of hot water (i.e., 3.25 g)

solution at 765 nm. The total phenolic content was estimated from the calibration curve.[10]

Total flavonoid content

The total flavonoid content was estimated by aluminum trichloride ($AlCl_3$). To 0.5 mg/mL of formulation, 5 mL of 2% $AlCl_3$ was added and mixed with the same amount of methanol. Then absorbance was noted at 415 nm using a UV-VIS spectrophotometer along with a blank sample, which contains 5 mL of formulation and 5 mL of methanol devoid of $AlCl_3$. The total flavonoid content was determined using a standard curve of quercetin.[11]

Acute oral toxicity test

Healthy Wister albino rats of 150–200 g weight, maintained under standard laboratory conditions were used for acute oral toxicity test according to the Organization for Economic Co-operation and Development (OECD) guideline 423. In each group, three rats were taken for the experiment. The first group received a single oral dose of 2000 mg/kg body weight of hematinic herbal tea. The rats were kept overnight for fasting before the administration of hematinic herbal tea by oral gavage. Food was withheld for 3–4 h. The rats were observed individually at least once during the first 30 min after dosing, then periodically during the first 24 h (with special attention during the first 4 h), and thereafter for a period of 14 days. Daily observations were noted, such as changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems (tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma) and behavior pattern. Animals were observed continuously for 2 h for behavioral profile, neurological profile, and autonomical profile. The mortality and morbidity was observed after 24 h. The results were recorded and noted.[12]

Pharmacological evaluation of herbal tea for hematinic activity: Phenylhydrazine induced anemia in rats

Anemia was induced in rats by intraperitoneal injection of phenylhydrazine at 40 mg/kg for 2 days. Rats that developed anemia with Hb concentration below 11 g/dL were selected for the study. Those animals were divided into 5 groups, each containing 6 animals. Group I served as Normal control (only vehicle), Group II was anemic control (phenylhydrazine for 2 days), Group III was standard (hematinic syrup), Group IV received herbal tea (200 mg/kg) and Group V received herbal tea (400 mg/kg). The blood was collected by retro-orbital puncture of experimental animals after an overnight fast. The blood was collected before and after induction of anemia with phenylhydrazine and after 1, 2, 3, and 4 weeks of treatment with herbal tea. Blood was collected in ethylenediaminetetraacetic acid bulb for hematological determination. The RBC count, packed cell volume (PCV), and Hb count were determined at 1, 2, 3 and 4 weeks. The mean cell volume, mean cell Hb (MCH), and MCH concentration were determined.[13,14]

RESULTS AND DISCUSSION

Physicochemical evaluation

The physicochemical constant is an important parameter in detecting adulteration. In the evaluation of crude drug, ash values, extractive values, and moisture contents are important parameters. The results are noted in Tables 2 and 3.

The results obtained from the present study may play a major role in setting particular standards for the plants *A. racemosus* L., *B. diffusa* L., *P. emblica* L., which broadens its botanical, pharmacognostic, pharmacological, and economical importance. These parameters prove beneficial in the identification of the plants.

Phytochemical screening

All the plant extracts were subjected to preliminary phytochemical screening and the results were recorded in Table 4. According to this report, the plant extract contains carbohydrates, alkaloids, glycosides, tannins, flavonoids, phenols, saponins, and proteins.

Physicochemical evaluation, spectroscopic analysis, total phenol content, and total flavonoid content of formulation

The result of the physicochemical analysis of the formulation is given in Tables 5 and 6. By UV analysis, it showed that herbal tea can be extracted at any temperature; however, in hot water, it showed better results [Table 7]. The result of

Table 2: Physicochemical parameters

S. No.	Plant names	Total ash % W/W	Acid insoluble ash % W/W	Water soluble ash % W/W	Moisture content % W/W
1	*Asparagus racemosus*	9	0.5	0.07	3
2	*Boerhavia diffusa*	8	1.6	0.6	4
3	*Phyllanthus emblica*	10.2	2.7	3.7	2

Table 3: Extractive values of herb powders

S. No.	Plant names	Alcohol soluble extractive value % W/W	Water soluble extractive value % W/W
1	*Asparagus racemosus*	5.6	10.2
2	*Boerhavia diffusa*	12.6	15.4
3	*Phyllanthus emblica*	6.8	9.6

Table 4: Phytochemical investigation of the plant extracts

S. No.	Phytochemical screening	*Asparagus racemosus*	*Boerhavia diffusa*	*Phyllanthus emblica*	combined
1	Carbohydrates	+	+	+	+
2	Alkaloids	–	–	–	+
3	Glycosides	–	+	–	+
4	Tannins	+	–	+	+
5	Steroids	–	–	–	–
6	Triterpenoids	–	–	–	–
7	Flavonoids	+	+	+	+
8	Phenols	–	–	–	+
9	Saponins	–	+	+	+
10	Proteins	+	–	+	+

"+" indicates presence; "–" indicates absence

Table 5: Physical characters and pH of the formulation

S. No.	Parameters	Formulation
1	Color	Red
2	Odor	Characteristic
3	Taste	Hot and spicy
4	pH	6.2

Table 6: Solubility of the formulation

S. No	Solvent	Solubility
1	Water	soluble
2	Methanol	soluble
3	Ethanol	soluble
4	Chloroform	Insoluble
5	Benzene	Insoluble
6	Petroleum Ether	Insoluble

AAS of the formulation is given in Table 8. The total phenolic content was found to be 7.8 µg/mL, which is equivalent to gallic acid and the total flavonoid content was found to be 6.7 µg/mL which is equivalent to quercetin.

Acute oral toxicity study

The acute toxicity of Hematinic Herbal Tea was carried out as per OECD-423 guidelines for safe dose administration to animals. The hematinic herbal tea was found to be non-toxic up to the dose of 2000 mg/kg body weight and did not cause any death of the tested animals. However, a dose of 200 and 400 mg/kg was chosen for the animal studies.

Pharmacological evaluation of herbal tea for hematinic activity: Phenylhydrazine induced anemia in rats

The changes in the hematological parameters of the rats during the study are expressed in the form of charts [Figures 1-7]. The Hb, PCV, and RBC in phenylhydrazine-induced rats decreased significantly ($P < 0.05$) while MC and MCH increased giving rise to macrocytic anemia. After 1 week of treatment of anemic rats with hematinic herbal tea for groups 4 and 5 and standard hematinic syrup for group 3 has reversed the effect of phenylhydrazine resulting significant ($P < 0.05$) increase in Hb, PCV, and RBC. During the experimental period, the Hb, PCV, and RBC of the anemic negative control (untreated anemic rats, group 2) also increased but at a very slow rate.

The Hb and RBC reached to normal range in the 2nd week of the experiment and PCV reached to normal range in the 3rd week of the experiment. The Hb, RBC, and PVC of groups 4 and 5 reached to normal level after 2nd week of treatment and reached the maximum in the 3rd week. At this point the Hb and RBC were significantly ($P < 0.05$) higher in group 5 rats than in normal control rats and groups 3 and 4 rats. By this, we can conclude that the response to treatment was dose-related. The results showed a significant increase in the Hb, PCV, and RBC in the anemic rats in the 1st week

Table 7: Absorbance of formulation at different temperatures

S. No.	Water	Absorbance at 280 nm				
		1 min	2 min	3 min	4 min	5 min
1	Hot (80°C)	0.752	0.784	0.810	0.823	0.998
2	Cold (8°C)	0.406	0.429	0.502	0.528	0.531
3	Room temperature	0.328	0.598	0.649	0.769	0.841

Table 8: Atomic absorption spectroscopy of formulation

Test	Specification	Result
Iron	Not more than 100 ppm	78.50

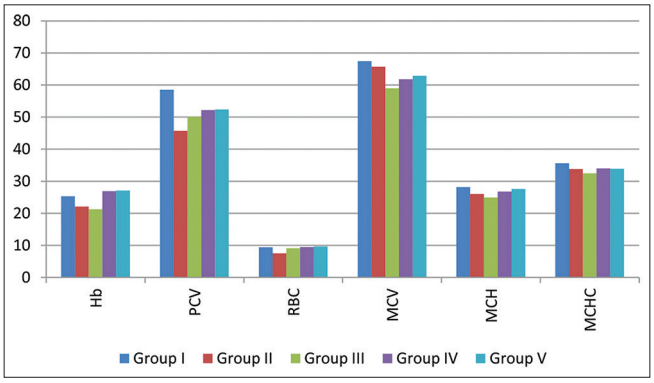

Figure 1: Hematological parameters of rats after 4 weeks of treatment with formulation

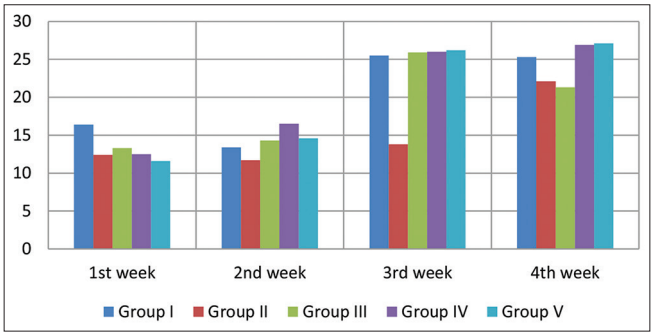

Figure 2: Changes in hemoglobin concentration per group during experimental period

of the studies, though there showed a higher increase in the groups treated with hematinic herbal tea than the standard and control groups.

By the results obtained the Hb contents and RBC were significantly increased which may be due to the presence of different phytochemicals, especially poly phenols (flavonoids) and iron. In phenylhydrazine-induced anemia, it has been reported that the RBC is damaged by oxidative stress by increasing the formation of reactive oxygen species. However, by the phytochemical screening of the extracts, it revealed the presence of flavonoids, saponins, and alkaloids,

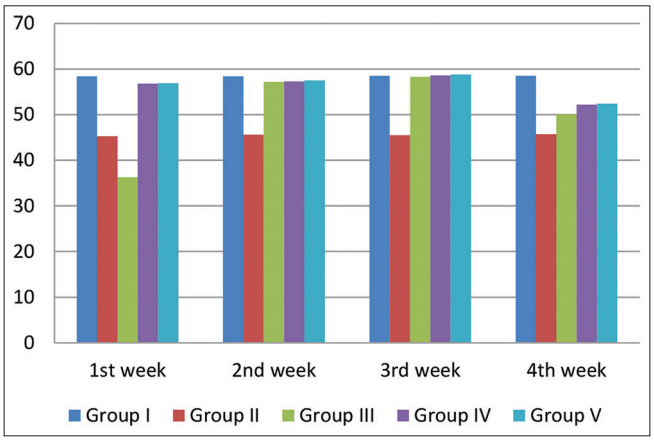

Figure 3: Changes in packed cell volume concentration per group during experimental period

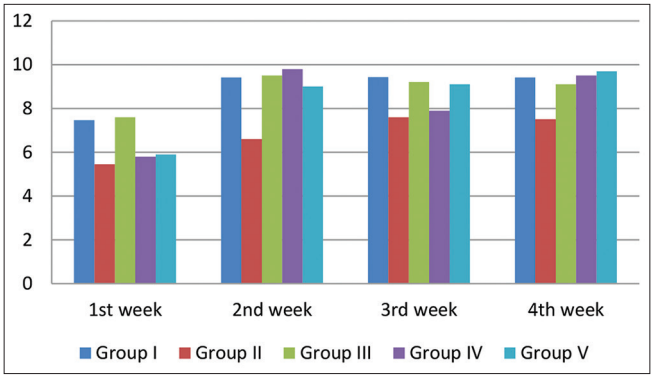

Figure 4: Changes in red blood corpuscles (red blood cells) concentration per group during experimental period

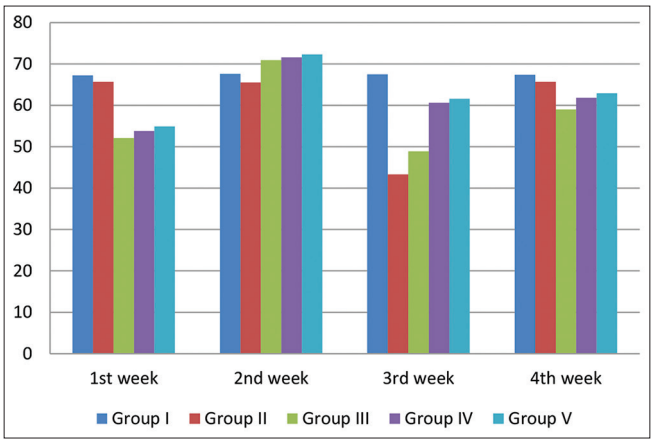

Figure 5: Changes in mean corpuscular volume concentration per group during experimental period

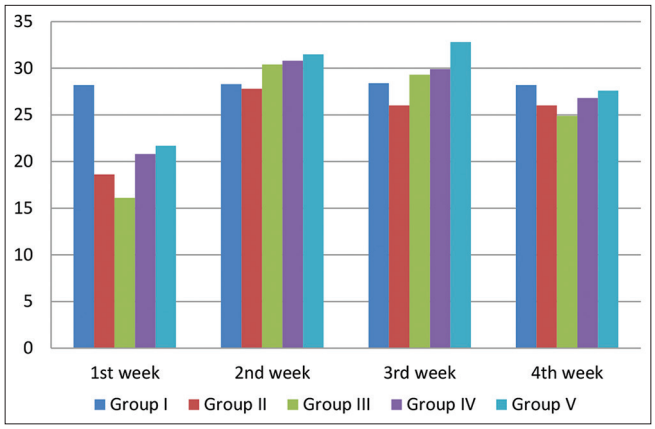

Figure 6: Changes in mean corpuscular hemoglobin concentration per group during experimental period

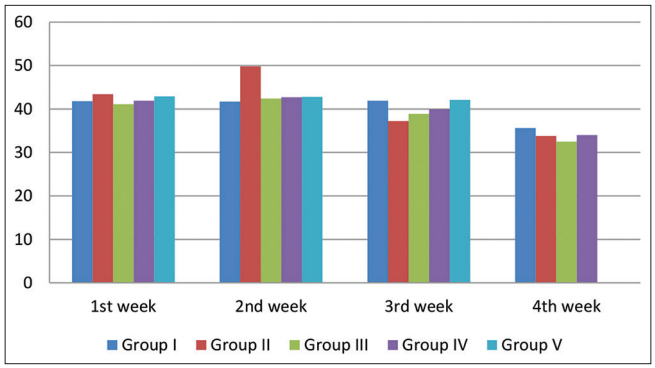

Figure 7: Changes in mean corpuscular hemoglobin concentration per group during experimental period

which act as antioxidants and it may reverse the damage of the phenylhydrazine-induced anemia by preventing or repairing the damage done to the RBC.

CONCLUSION

The present study entitled "Formulation and Evaluation of Herbal Tea from Some Edible Herbs for Hematinic Activity" includes the study of physicochemical parameters, analytical evaluation, and pharmacological evaluation of herbal tea. The formulation was subjected to hematinic activity. The effect of this formulation was determined by phenylhydrazine-induced anemia in rats and it was assessed by the biochemical parameters. The results showed that the formulation possess hematinic activity. Blood samples were collected every week by retro-orbital puncture for up to 4 weeks. The dose of 200 mg/kg and 400 mg/kg of the formulation was given to the animals for the treatment of anemia. The results revealed significant change in RBC, PCV, and Hb in anemic rats. From this study, it is concluded that *A. racemosus*, *P. emblica*, and *B. diffusa* possess hematinic activity and can be used in the form of herbal tea. In the meantime, an increase in the consumption of tea should be encouraged. The present study clearly infers that the combination of plants can be used for the treatment of anemia. However, more studies have to be done by exploring the individual plants and its constituents. It also reveals that consuming these herbs in the regular diet will enhance blood Hb levels.

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