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COMBINED EFFECT OF DRIED FIGS WITH JAGGERY IN PHENYLHYDRAZINE INDUCED ANEMIA IN RATS

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ABSTRACT

Anemia is the utmost communal nutritional insufficiency disorder in the world. Anemia can affect many systems in the body and produce a variety of symptoms and signs. The present treatments available for anemia are not safe and effective. Figs and jiggery are conventionally utilized in the treatment of anemia. Hence, the present study was aimed to investigate additive effect of figs and jaggery in phenylhydrazine induced anemia in rats. Phenylhydrazine induced anemia is an effective model to study and evaluate various new therapeutic modalities. In the current study, two days administration of phenylhydrazine has significantly decreased RBC count, hemoglobin, hematocrit levels and increased MCV, MCH, MCHC when compared to normal control group indicating induction of anemia. 28 days treatment with diet supplemented with figs, jiggery and combination of figs and jaggery has significantly increased RBC, hemoglobin, hematocrit levels and decreased MCV, MCH, MCHC levels when compared to anemic control group. Outcomes of the present research work concluded that consuming diet supplemented with figs, jiggery and combination of figs and jaggery could reduce the risk of anemia.

KEYWORDS: Anemia, phenylhydrazine, figs, jaggery, hemoglobin, serum iron.

INTRODUCTION

Anemia is the most common nutritional deficiency disorder in the world. World Health Organization (WHO) defines anemia as a condition in which the hemoglobin (Hb) content of blood is lower than normal (Men 13.5-17.5 g/dl and Women 12-15.5g/dl) caused by the paucity of one or more vital nutrients, irrespective of the basis of such insufficiencies.[1] Anemia can influence numerous systems in the body and produce an assortment of side effects and signs that influence wellbeing including weakness, fatigue and drowsiness.[2]

According to WHO, the prevalence of anemia in pregnant women is 14% in developed and 51% in developing countries and 65-75% in India alone.[3] As for its distribution by age group, it is estimated that 47.4% anemia reached in 2-3 years old children, 25.4% in school age children, 41.8% in pregnant women, 30.2% in non-pregnant women and 23.9% in elderly 23.9.[4] The present treatments available for anemia are not safe and effective. Early diagnosis, iron replacement therapies and executing the underlying cause persist as the core challenges in the management of anemia. The treatment of anemia is mainly focused on nutritional supplementation.[5,6]

Figs (*Ficus carica*) are traditionally used for the treatment of anemia due to their rich source of iron. In traditional medicine, the fruit, root and leaves of *Ficus carica* are utilized in the treatment different diseases, for example, gastrointestinal (colic, heartburn, loss of craving, loose bowels), respiratory (sorethroats, hacks and bronchial issues) and cardiovascular issues and as mitigating and antispasmodic cure.[7] Similarly, jaggery is known for its beneficial effects in anemic conditions as it is rich in ascorbic acid and iron that produce heat and give instant energy to human body. In Ayurveda, it is used as medicine, blood purifier and base material for syrups.[8,9]

Hence, the current study was aimed to investigate additive effect of figs and jaggery in phenylhydrazine induced anemia in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Phenylhydrazine (PHZ) was acquired from Sigma Aldrich, Hyderabad. Drabkin solution for estimation of hemoglobin was obtained from Arkray Inc., Growers solution and EDTA were obtained from S D Fine-Chem Limited. Total Iron Binding Capacity (TIBC) kit was

procured from Coral Clinical System, Hyderabad, Telangana, India.

Experimental Animals

Female wistar rats (200-250g) were obtained from Sai Nath Agencies, Telangana, India. They were maintained with 12hr light/dark cycle, in temperature and humidity controlled room. Animals were allowed to free access to food and water *ad libitum*. After one week of acclimatization, animals were randomly selected for study. All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines (320/CPCSCEA dated 03-01-2001). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (GPRCP/IAEC/23/19/02/PCL/AE-1-Rats-M/F-30), G. Pulla Reddy College of Pharmacy, Hyderabad, Telangana, India.

Experimental Design

Animals with normal ranges of hemoglobin 11.5-16.1 g/dl and RBC count 6.76-9.75 10^6/mm^3 were selected for study. They were divided into 5 groups (n=6). Except in normal group, anemia was induced in all groups using phenylhydrazine (40 mg/kg i.p) for 2 days.[10,11]

Table I: Animal grouping.

Group	Treatment
Normal	Normal Diet
Anemic	Phenylhydrazine (40 mg/kg i.p.) for 2 Days + Normal diet
Treatment - I	Phenylhydrazine (40 mg/kg i.p.) + Diet supplemented with Figs for 28 days
Treatment - II	Phenylhydrazine (40 mg/kg i.p.) + Diet Supplemented with Jaggery for 28 days.
Treatment - III	Phenylhydrazine (40 mg/kg i.p.) + Diet supplemented with combination of Figs and Jaggery for 28 days.

Evaluation of Hematological and Biochemical Parameters

On 14th and 29th day, blood was collected from retro orbital plexus. Hematological parameters such as Red Blood Cell (RBC) count, Hemoglobin (Hb), Packed Cell Volume or Hematocrit (PCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and biochemical parameter i.e., serum iron concentration were evaluated. Hemoglobin concentration was estimated using cyanmethemoglobin method, RBC count was estimated manually using visual haemocytometer method, hematocrit was determined using Macro method and serum iron content was estimated by Ferrozine method using iron and TIBC kit.[12-14]

Statistical Analysis

Data expressed as mean ± SEM were analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Graph Pad Prism Software (8.0 version). $^{\alpha}P < 0.0001$ compared with normal control, $^{a}P < 0.0001$, $^{b}P < 0.001$, $^{c}P < 0.01$ compared with anemic control were taken as significant.

RESULTS

Hematological Parameters

According to the Table II, administration of phenylhydrazine (40 mg/kg) for 2 days has significantly induced anemia. Decreased hemoglobin, RBC count, hematocrit levels and increased MCH, MCV and MCHC values was observed on both 14th and 29th day in anemic control group when compared to normal control group indicating the induction of anemia by PHZ. However, treatment with figs, jaggery and combination of figs and jaggery showed significant increase in the concentration of hemoglobin, RBC count, hematocrit and decreased MCH, MCV and MCHC values on 14th and more significantly on 29th day, when compared to anemic control group.

Table II: Effect of figs, jaggery and combination of figs and jaggery on hematological parameters.

S. No.	Groups (n=6)	Red Blood Cells (Million/mm^3)		Hemoglobin (g/dl)		Hematocrit (%)		Mean Corpuscular Hemoglobin (pg)		Mean Corpuscular Volume (%)		Mean Cell Hemoglobin Concentration (%)	
		14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day
1.	Normal	10.93 ± 0.024	10.93 ± 0.024	17.01 ± 0.67	17.01 ± 0.67	53.45 ± 0.90	54.45 ± 0.90	16.36 ± 0.18	15.57 ± 0.17	61.97 ± 0.70	59.02 ± 1.15	25.14 ± 0.46	23.08 ± 0.45
2.	Anemic	8.152 ± 0.17$^{\alpha}$	8.853 ± 0.063$^{\alpha}$	11.52 ± 0.04$^{\alpha}$	12.86 ± 0.26$^{\alpha}$	42.50 ± 0.71$^{\alpha}$	49.08 ± 0.37$^{\alpha}$	18.47 ± 0.28$^{\alpha}$	17.62 ± 0.29$^{\alpha}$	70.97 ± 0.34$^{\alpha}$	68.57 ± 0.44$^{\alpha}$	31.20 ± 0.73$^{\alpha}$	29.13 ± 0.74$^{\alpha}$
3.	Treatment - I	9.320 ± 0.045^{a}	10.09 ± 0.077^{a}	15.91 ± 0.38^{a}	17.22 ± 0.19^{a}	45.92 ± 0.58^{c}	52.83 ± 0.50^{b}	16.24 ± 0.38^{a}	14.92 ± 0.28^{a}	62.78 ± 0.88^{b}	58.93 ± 0.98^{a}	25.17 ± 0.74^{a}	22.17 ± 0.87^{a}
4.	Treatment - II	9.427 ± 0.040^{a}	10.05 ± 0.08^{a}	17.22 ± 0.19^{a}	17.95 ± 0.50^{a}	45.67 ± 0.42^{c}	53.67 ± 0.33^{a}	16.72 ± 0.16^{b}	15.68 ± 0.15^{a}	63.00 ± 1.23^{b}	59.33 ± 1.30^{a}	25.50 ± 0.88^{a}	23.40 ± 1.01^{a}
5.	Treatment - III	9.60 ± 0.16^{a}	10.35 ± 0.13^{a}	16.25 ± 0.83^{a}	18.18 ± 0.60^{a}	49.22 ± 0.61^{a}	55 ± 0.36^{a}	16.88 ± 0.07^{c}	15.80 ± 0.27^{b}	62.83 ± 1.68^{b}	59.67 ± 1.64^{a}	25.47 ± 0.61^{a}	23.10 ± 0.81^{a}

Values expressed as mean ± SEM were analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{\alpha}P < 0.0001$ compared with normal control, $^{a}P < 0.0001$, $^{b}P < 0.001$, $^{c}P < 0.01$ compared with anemic control.

Biochemical Parameters

Decreased serum iron content was observed on both 14th and 29th day in anemic control group when compared to normal control group indicating the induction of anemia by PHZ. However, treatment with figs, jaggery and combination of figs and jaggery showed significant increase in the concentration of serum iron content on 14th day and more significantly on 29th day, when compared to anemic control group (Table III).

Table III: Effect of figs, jaggery and combination of figs and jaggery on serum iron concentration.

S. No.	Groups (n=6)	Iron (µg/dl)	
		14th day	28th day
1.	Normal	73.33 ± 0.91	73.33 ± 0.91
2.	Anemic	39.82 ± 2.08$^{\alpha}$	39.82 ± 2.08$^{\alpha}$
3.	Treatment - I	82.27 ± 2.01^{a}	91.93 ± 1.74^{a}
4.	Treatment - II	71.22 ± 2.31^{a}	83.63 ±2.04^{a}
5.	Treatment - III	86.70 ± 3.08^{a}	99.33 ± 2.04^{a}

Values expressed as mean ± SEM were analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{\alpha}P < 0.0001$ compared with normal control, $^{a}P < 0.0001$, compared with anemic control.

DISCUSSION

Anemia is a condition that arises if the red blood cells are not able to transport adequate amount of oxygen to the tissues of the body.[3] It can be treated with oral and injectable iron, erythropoietin, blood, transfusion and hyperbaric oxygen. Increasing side effects and non-compliance of conventional treatments is demanding the need for investigation of new safer approaches for the management of anemia.[15] Nutritional substances rich in iron are proved to be beneficial in controlling anemia but, the study shows that dietary iron has poor bioavailability. It has been also proved that ascorbic acid enhances the bioavailability of dietary iron in the circulation.[16] Dried figs are proved to be useful for increasing hemoglobin level in blood and thus prevents from anemia.[17] Jaggery has rich content of ascorbic acid and iron for which, it was predicted that the combination will have additive effect.

Many experimental models are used for investigation of anti-anemic potential activity. Phenylhydrazine induced toxic anemia in female rats offers a model for research into the pathogenesis of hemolytic anemia and the influence of anemia on other physiological processes or the course of associated diseases.[18] Therefore, the current study used phenylhydrazine induced anemic model for the evaluation of anti-anemic potentials of combination of figs and jaggery.

According to the results of the present study, administration of phenylhydrazine (40 mg/kg) for 2 days has significantly induced anemia. PHZ causes oxidative stress and generate reactive oxygen species (ROS) in RBCs that reacts with haemoglobin which consequences in oxidative degradation and peroxidation of lipid in RBC.[19] Decreased haemoglobin, RBC count, haematocrit levels, serum iron content and increased MCH, MCV and MCHC values was observed on both 14th and 29th day in anaemic control group when compared to normal control group indicating the induction of anemia by PHZ. However, treatment with figs, jaggery and combination of figs and jaggery showed significant increase in the concentration of haemoglobin, RBC count, haematocrit and serum iron content and decreased MCH, MCV and MCHC values on 14th and more significantly on 29th day, when compared to anaemic control group. This increased in the haematological and biochemical parameters may be due to nutrient (iron) rich dried figs and ascorbic acid from jaggery presented protective effect on RBC from ROS generated by phenylhydrazine. Hence, it can be stated that the consumption of combination of jaggery with figs could be more effective in treatment of anemia than the figs and jaggery alone.

CONCLUSION

From the outcomes of the present research work, it can be concluded that the consumption of figs and jaggery is effective in treating anemia and can enhance the progression of haematological parameters and serum iron concentrations. It also suggests that the consumption of combination of jaggery with figs could be more effective than the figs and jaggery alone.

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