



Original Article

Exploring the Anti-Anemic Potential of *Bauhinia variegata* Linn Leaves Powder Biscuits in Animal Modeling

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ABSTRACT

Globally, Anemia is one of the most prevalent disease. In developing countries women and children suffered more from anemia. **Objective:** To develop and explore the anti-anemic potential of iron enriched biscuits by using *Bauhinia variegata* leaves powder. **Methods:** Longitudinal study design was followed to check the therapeutic effect of *Bauhinia variegata* leaves powder biscuits in anemia-induced rats. For this purpose, dried powder of *Bauhinia variegata* leaves powder was prepared and functional biscuits were developed by utilizing *Bauhinia variegata* leaves powder and lemon juice. Thirty male anemia-induced albino rats were randomly divided into three groups G1 (standard diet), G2 (biscuits with 10 g *Bauhinia variegata* leaves powder) and G3 (biscuits with 10 g *Bauhinia variegata* leaves powder and 10 mL lemon juice). Feed intake, water intake, total body weight gain and hemoglobin levels of rats were determined after an interval of 15 day for study period of two months. The results obtained was statistically analyzed. **Results:** The results showed that functional biscuits prepared by using *Bauhinia variegata* leaves powder (high in iron) and lemon juice (rich in Vitamin C) significantly improved the feed intake, water intake, total body weight gain and hemoglobin levels of rats during 60 days study period. **Conclusions:** Conclusively, *Bauhinia variegata* leaves powder and lemon juice in combination are effective against anemia.

INTRODUCTION

Anemia is one of the prevalent diseases among all the developing countries worldwide. It is defined as low hemoglobin level due to inability of the red blood cells to carry the oxygen to the other tissues. In anemia the hemoglobin concentration diminished below 13 g/dL and 12 g/dL in men and women, respectively [1, 2]. Iron deficiency is one of the main cause for anemia, which results in about 50 % of anemic cases and 90,000 deaths. However, other reasons for anemia are malaria, HIV and folate deficiency. Anemia affects more women and children as compared to men [3]. Synthetic medicines can be used for anemia treatment, but due to high cost, increased awareness

about the adverse effects of medicines, low absorption of supplements than that of food might delay the treatment process. Therefore, Natural sources could be used as natural remedy for the treatment of anemia [4]. Kachnar (*Bauhinia variegata* Linn) is medium sized plant and belongs to family Leguminosae, includes more than 200 species. The different parts of *Bauhinia variegata* in particular the leaves contained variety of macronutrients like fat, protein & fiber, micronutrients (iron, calcium, phosphorous & vitamin C) and phytochemicals (quercitroside, rutoside, isoquercitroside, 5,7 dimethoxy flavanone-4-o-L rhamnopyrosyl-β-D-glycopyranoside, etc.) [5]. Kancher

leaves have various types of health effects such as anticarcinogenic activity, antimicrobial activity, anti-diabetic activity, antiulcer activity, haematinic activity, anti-inflammatory activity, haemagglutinating activity, immunomodulatory activity, hepatoprotective activity, antitumor activity, antibacterial activity, antioxidant activity, hypolipidemic activity and wound healing activity [6]. The production of bakery products increases 10 % per year. In snacks, biscuits have different appealing features including high consumer demand and good shelf-life and good food quality. Biscuits consist of three major ingredients that are white flour, powdered sugar and ghee [7]. The calculated number of micronutrients is added to improve the nutritional value of the food it is basically known as fortification. Keeping in view the status of particular nutrient in the population programs are designed to deal with the major deficiencies and common foods are fortified with the required nutrients like iron, iodine, B complexes, and other vitamins in the selected population [8]. The trend of Fortification in bakery industry is increasing day by day to combat the malnutrition. Iron fortification in foodstuffs is associated with increased hemoglobin and serum ferritin levels and decreased the prevalence of anemia in children, pregnant women, adolescents, and adults [9].

Keeping in view about present status of anemia worldwide, the current experimental trail was designed to develop functional *Bauhinia variegata* (kachnar) leaves powder biscuits by the addition of kachnar leaves powder and lemon juice. Furthermore, Animal study was conducted to explore the role of *Bauhinia variegata* leaves powder and lemon juice biscuits as natural iron and Vitamin C sources for the cure of anemia.

METHODS

The current research was carried out in Institute of Home sciences and National Institute of Food Science & Technology, University of Agriculture, Faisalabad. *Bauhinia variegata* Linn. (kachnar) leaves were collected from the Ayub Agriculture Research Institute, Faisalabad and sun-dried kachnar leaves powder was prepared and stored in air tight jars [10]. Iron enriched biscuits were prepared by incorporation of white flour, *Bauhinia variegata* (kachnar) leaves powder and lemon juice at the ratio of T0 (100:0:0), T1 (90:10:0) and T2 (80:10:10) with certain modifications by using AACC Method No. 44-15 A, 44-40 [11]. Longitudinal study design was carried out to determine the effect of *Bauhinia variegata* leaves powder biscuits against anemia in male adult albino rats. From the animal house situated in National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, thirty adult male albino rats (average weight 125.53 g) were selected

carefully after getting permission from Institution of Animal Ethics Committee (IAEC) of University of Agriculture, Faisalabad (IRB No. 001474). Cages made up of stainless steel were used to keep those rats. Those cages were placed under standard conditions required for the efficacy ($23\pm 2^{\circ}\text{C}$) temperature, humidity ($55\pm 5\%$) with twelve hours dark-light cycle in department of Pharmacology, University of Agriculture Faisalabad. The male albino rats were selected by random sampling and administrated with standard basal diet for 14 days to meet their requirement for growing ad libitum [1]. Anemia was induced by dissolving Phenyl hydrazine in 0.9 % NCL and then injected intraperitoneally at level of 40 mg/kg for two consecutive days. The anemia was confirmed when the red blood cells and the hemoglobin reduced by almost 30 % [1]. Thirty male albino rats were divided into three groups of ten animals each after acclimatization of one week. 1st groups included anemic rats that received only distilled water and standard cookies throughout the whole trial. 2nd and 3rd group consumed functional biscuits with formulations of (90 % wheat flour and 10 % kachnar leaves powder) and (80 % wheat flour, 10 % kachnar leaves powder and 10 mL lemon juice) respectively. Total feed, water intake and gain in body weight were calculated on weekly basis. After completion of the study period (60 days), animals were overnight fasted and blood was collected from each rat under ether anesthesia via retro-orbital puncture method to obtain serum for the hemoglobin testing analysis. Then the Hb levels were measured by using method as mentioned by [12] at 0 day and then repeated after 15 days throughout the study period (two months). The significance level all parameters were statistically analyzed by applying Two-way ANOVA using SPSS version 23.0 software according to method given by [13].

RESULTS

The findings of current research showed that the feed intake of the rats significantly increased from G0 to G2. The results presented in Table 1 further exhibited that feed intake of G0, G1 and G2 increased from 16.22 ± 1.05 , 16.27 ± 1.77 and 16.32 ± 2.05 g/rat/day at the 1st week to 18.54 ± 1.89 , 19.09 ± 2.76 and 19.32 ± 2.82 g/rat/day respectively at 8th week.

Table 1: Feed intake (g/rat/day) of anemic rats

Experimental Groups	Study period							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
G ₀	16.22 $\pm 1.05c$	16.44 $\pm 1.12c$	16.62± 1.23c	16.94 $\pm 1.45c$	17.52 $\pm 1.67c$	17.76 $\pm 1.87c$	18.30± 1.34c	18.54 $\pm 1.89c$
G ₁	16.27 $\pm 1.77b$	16.57 $\pm 1.67b$	16.82± 2.12b	17.65 $\pm 2.21b$	18.04 $\pm 2.33b$	18.45 $\pm 2.45b$	18.77± 2.54b	19.09 $\pm 2.76b$
G ₂	16.32 $\pm 2.05a$	16.72 $\pm 2.14a$	17.43± 2.26a	17.78 $\pm 2.30a$	18.34 $\pm 2.4a$	18.67± 2.65a	19.04± 2.69a	19.32 $\pm 2.82a$

Alphabets determine (p-value less than 0.05) difference. Table 2 showed that the water intake of the rats significantly increased from the control group (G0) to treatment groups (G1 and G2). According to results, the water intake of G0, G1 and G2 increased from 27.22 ± 0.72 , 27.36 ± 0.89 and 27.88 ± 0.98 g/rat/day at the start of research to 28.65 ± 1.17 , 29.77 ± 1.72 and 30.05 ± 1.82 g/rat/day respectively at the end of study period (8 weeks).

Table 2: Water intake (g/rat/day) of anemic rats

Experimental Groups	Study period							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
G ₀	27.22 $\pm 0.72c$	27.32 $\pm 0.79c$	27.57 $\pm 0.82c$	27.74 $\pm 0.88c$	27.93 $\pm 0.95c$	28.28 $\pm 0.99c$	28.49 $\pm 1.05c$	28.65 $\pm 1.17c$
G ₁	27.36 $\pm 0.89b$	27.47 $\pm 0.92b$	27.62 $\pm 1.03b$	27.83 $\pm 1.08b$	28.25 $\pm 1.14b$	28.67 $\pm 1.32b$	29.32 $\pm 1.5b$	29.77 $\pm 1.72b$
G ₂	27.88 $\pm 0.98a$	27.92 $\pm 1.04a$	28.22 $\pm 1.12a$	28.44 $\pm 1.23a$	28.78 $\pm 1.34a$	29.43 $\pm 1.4a$	29.76 $\pm 1.52a$	30.05 $\pm 1.82a$

Alphabets determine (p-value less than 0.05) difference. Significant increase in the weight gain was observed in the rats but the percentage was less in the rats treated with controlled diet as compared to the rats belonged to G1 and G2. The weight gain was directly proportional to the feed and water intake and the time period also matter a lot. The highest percentage of gaining weight was shown in G2 and the minimum weight gain was observed in G0 with values (129.15 ± 4.11 to 182 ± 4.02 g/rat) and (125.22 ± 4.02 to 158 ± 4.02 g/rat) respectively from start of the research till end of study period (60 days) (Figure 1).

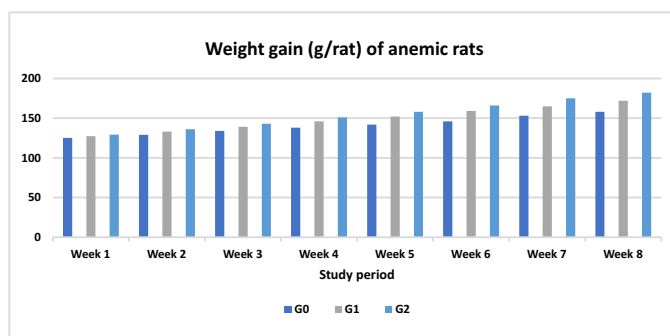


Figure 1: Graphical representation of Body weight gain (g/rat) of anemic rats

After inducing anemia in albino rats, they were weighed again and the hemoglobin test was performed. The results showed that anemia was successfully induced in the rats. After that rats stopped having standard rat diet and fed by the experimental food. Rats were divided into three groups of 5 rats in each group. Significant increase in the hemoglobin level was observed by the reading or values of the hemoglobin tests of all rats after 0 to 60 days but the increase of hemoglobin level was minimum in G0 (6.63 ± 0.38 – 9.20 ± 0.66 g/dL) and maximum in G2 (6.82 ± 0.77 – 13.22 ± 0.92 g/dL) (Figure 2).

P calculated by one-way analysis of variance (ANOVA). The majority of the females (50.5%) had normal weight, whereas 23.7% were overweight and 22% were obese. Deficiency of vitamin D increased more than 3-fold ($P = 0.002$) in the overweight group, whereas obesity increased the risk of deficiency by more than 4-fold ($P = 0.0009$) (Table 4). No association was noted between vitamin D status and dressing type or diet.

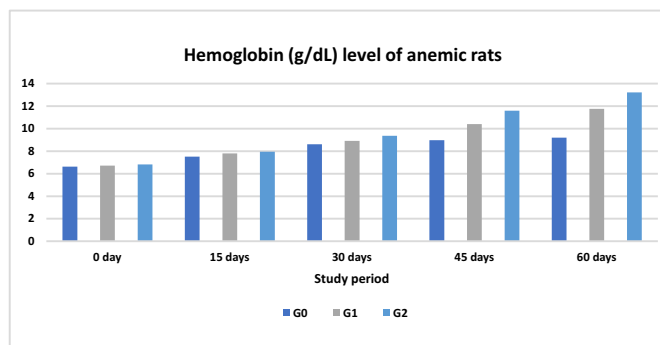


Figure 2: Graphical representation of Hemoglobin (g/dL) Level of Anemic rats

DISCUSSION

The consequences of current research showed that the kanchar leaves powder increased the nutritional profile of functional biscuits by increasing their iron and vitamin C levels [14]. Vitamin C play a significant role in the appropriate utilization of iron and also increase the intestinal absorption of non-haem iron by decreasing ferric ion to a ferrous form [15, 16]. Animal study to evaluate the capacity of kachnar leaves powder and lemon juice biscuits to overcome anemia depicted that feed intake, water intake and body weight gain of anemic male albino rats significantly increased from (G0 to G2) during the study period of 60 days. The findings of present research are in association with the study performed by Kulkarni and Garud, who reported that alcoholic extract of *B. variegata* increased the feed intake, water intake and weight of the diabetic- rats [17]. According to another previous study findings, significant increase in the hemoglobin level was observed in the treated rats after 0 to 60 days due to high amount of iron and Vitamin C in kanchnar leaves powder and lemon juice respectively. The results obtained were in agreement with the study conducted by Kansal et al., who concluded that kanchnar leaves have significant effect on hemoglobin level of anemic patients [19]. Furthermore, Anti-anemic potential of beetroot, pineapple and papaya juice in phenylhydrazine treated wistar rats was assessed and concluded that the functional juice increased the hb level with improved iron status in animal modeling [20].

CONCLUSIONS

The consumption of iron enriched biscuits of kachnar leaves was helpful in curing the iron deficiency anemia and the lemon juice was seemed to effective in improving the absorption of the iron present in the kachnar leaves powder biscuits.

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Authors Contribution

Conceptualization: SA¹

Methodology: UA, SA¹

Formal analysis: SH

Writing-review and editing: SA², AM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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