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Original Article

ANTI-ANEMIC ACTIVITY OF SPROUTS OF *VIGNA RADIATA* L. IN MALE ALBINO RATS

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ABSTRACT

Objective: To evaluate the anti-anemic activity of sprouts of *Vigna radiata* L. against phenyl hydrazine induced anemic rats.

Methods: Rats were divided into 4 groups of 6 each. Group 1 was given normal saline and served as control and all other groups were given 40 mg/kg b. w of phenyl hydrazine for 2 d to induce anemia. Group 3 was treated with Bioferon (230 mg/kg) and served as the standard. Group 4 was treated with sprouted *Vigna radiata* L. (600 mg/kg bw). All the treatments were given orally. On completion of the experimental period, all the test substance/vehicle-treated rats were sacrificed and the plasma separated was used for estimating various biochemical as well as hematological parameters as per standard procedures.

Results: The experimental rats treated with sprouted *Vigna radiata* L. at the dose level 600 mg/kg bw for 13 d revealed significant changes in biochemical and hematological parameters compared to phenyl hydrazine induced anemic rats.

Conclusion: The present study concluded that the sprouted *Vigna radiata* L. inhibits anemia induced by phenyl hydrazine in male albino rats.

Keywords: Anemia, Phenyl hydrazine, *Vigna radiata* L., Bioferon.

INTRODUCTION

Anemia is described as a reduction in Red Blood Cell (RBC) mass or blood hemoglobin (Hb) concentration resulting in a decrease in the oxygen-carrying capacity of the blood. The prevalence of anemia in the developing countries tends to be 3 to 4 times higher than in the developed countries. Anemia affects the physical and mental development of an individual leading to decreased working capacity, which in turn affects the development of the country. Since the technological advancement and economic development of a nation depend heavily on its trained human resources, the behavioral effects of anemia are highly relevant. Consequently, if anemia is highly prevalent in a country, it can substantially affect its intellectual and economic potential.

The World Health Organization (WHO) estimates the number of anemic people worldwide to be a staggering 2 billion (about 30 % of the world's population) and that approximately 50 % of all anemias can be attributed to iron deficiency and in resource-poor areas, this is frequently exacerbated by infectious diseases, malaria, worm infestation and HIV/AIDS [1]. Even though anemia is associated with nutritional deficiencies, acute or chronic disease, drug use or physiological states such as pregnancy, blood loss, impaired erythropoiesis and abnormal erythrocyte destruction are implicated [2, 3].

Plant and plant products are being utilized as a source of medicine since long. Plant extracts are used as phytotherapeutics and are still a large source of natural antioxidants. Natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restored the optimal balance by neutralizing the reactive species [4]. Particularly, flavonoids and phenolics are considered as potential therapeutic agents. A wide range of ailments and is widely distributed in the plant kingdom and, therefore, an integral part of the diet, with the significant amount reported in vegetables, fruits and beverages [5].

Vigna radiata L. has been consumed as a common food in China for more than 2,000 y. It is well known for its detoxification activities and is used to refresh mentality, alleviate heat stroke, and reduce swelling in the summer. In the book Ben Cao Qiu Zhen, the *Vigna radiata* L. was recorded to be beneficial in the regulation of gastrointestinal upset and to moisturize the skin [6]. The seeds and sprouts of *Vigna radiata* L. are also widely used as a fresh salad vegetable or common food in India, Bangladesh, South East Asia, and western countries [7]. Sprouted *Vigna radiata* L. contains: Water, 88.8; protein, 3.8; fat, 0.2; crude fiber, 0.7; total carbohydrates, 6.6; and ash, 0.6 g/100g; mineral constituents: Ca, 19; P, 64; Fe, 1.3; Na, 5; and K, 223 mg/kg. Yang and Tsou reported that the available iron in sprouts tested with *in vitro* dialysis is increased due to the increased ascorbic acid and reduced phytic acid content in *Vigna radiata* L. during sprouting [8-10]. High levels of proteins, amino acids, oligosaccharides, and polyphenols in *Vigna radiata* L. are thought to be the main contributors to the antioxidant activity [11-14], antimicrobial activity [15-19], anti-inflammatory activity [20-23], antidiabetic effects [24-25], antihypertensive effects [26], antitumor effects [27-29] and antisepsis effects [30-31].

Vigna radiata L. is said to be a traditional source cures paralysis, rheumatism, coughs, fever, liver ailments and for weight reduction. It is employed as a light diet during fever and is considered as a cooling and astringent effect. The pulse is prescribed for vertigo. A decoction of seeds is used an effective treatment for beriberi [32].

In recent years, studies have shown that the sprouts of *Vigna radiata L.* after germination have more obvious biological activities and more plentiful secondary metabolites since relevant bio-synthetic enzymes are activated during the initial stages of germination. Thus, germination is thought to improve the nutritional and medicinal qualities of *Vigna radiata L* [33]. Highly efficient use of *Vigna radiata* L. according to evidence demonstrated from scientific experiments will be beneficial to the application of *Vigna radiata* L. as a health food, medicine, and cosmetic [34].

In the tropical area between 10 to 20% of the population presents less than 10g/dl of hemoglobin. Due to the high prevalence and the possibility of an even further increase, there is the need to prevent it or seek for more cost effective and better treatment strategies. By keeping the above points, the present study has been designed to evaluate the anti-anemic activity of sprouted *Vigna radiata* L. on phenyl hydrazine induced hemolytic anemia in male albino rats.

MATERIALS AND METHODS

Animals

Wistar strain male albino rats, weighing 100-120g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (22±3 °C, humidity 30-70%, 12 h light/dark cycle). The animals were allowed to have standard feed and water *adlibtum*. They were acclimated to the environment for one week prior to experimental use. The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee (IAEC).

Chemicals

All chemicals and reagents were used of analytical grade, purchased from SRL Chemie Pvt. Ltd. and from Hi Media Laboratories Pvt. Limited.

Plant material and preparation of drug

The seeds of *Vigna radiata* L. were purchased from a departmental store during the month of January 2014. The seeds were germinated by soaking them in water for four hours. Collected sprouts were open-air-dried under the shade, pulverized into a moderately coarse powder. The powder was mixed with distilled water just before oral administration.

Experimental design

Anemia was induced by intra peritoneal injection of phenyl hydrazine at 40 mg/kg for 2 d, as described by Diallo *et al.* [35]. Following the injections, rats were divided into four groups of six rats each.

Group I-Control rats received saline

Group II-Phenyl hydrazine treated rats (40 mg/kg per day for 2 d)

Group III-Phenyl hydrazine treated rats with 40 mg/kg per day for 2 d and sprouted *Vigna radiata* L. single dose (600 mg/kg) per day for 13 d.

Group IV-Phenyl hydrazine treated rats with standard Bioferon treated rats-a single dose (230 mg/kg) per day for 13 d.

On completion of the experimental period, animals were anesthetized with thiopentone sodium (50 mg/kg, ip). The blood was collected with and without EDTA as an anticoagulant. Plasma was separated by centrifugation. Plasma was used for the estimation of various biochemical parameters. Hemoglobin was estimated by cyanomethaemoglobin method [36]. WBC, RBC and platelet were counted by Ochei method, 2000 [37].

Hematocrit or Packed Cell Volume (PCV)

$$\text{PCV \%} = \frac{\text{Packed RBC Column height}}{\text{Total blood Column height}}$$

Mean Corpuscular Hemoglobin (MCH)

This indicates the weight of hemoglobin in a single red blood cell and is expressed in picograms (pg) (1pg=10·[12]g).

$$\text{MCH} = \frac{\text{Hemoglobin (g/dL)}}{\text{RBC count Million per cu. mm}}$$

Mean corpuscular Hemoglobin concentration (MCHC)

This denotes the hemoglobin concentration per 100 ml of packed red blood cells and is related to the color of the red cells. This is expressed as the percentage of packed cells.

$$\text{MCHC} = \frac{\text{Hemoglobin (g/dL)}}{\text{PCV \%}} \text{X}100$$

This is expressed as the volume in cubic microns or femtoliters of an average red blood cell.

$$\text{MCV} = \frac{\text{PCV \%}}{\text{RBC count Million per cu. mm}} \text{X}10$$

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust [38]; reduced glutathione was estimated by the method of Moron *et al.*, [39] and serum GOT was estimated by the method of Reitman and Frankel [40].

Statistical analysis

Values were expressed as mean±SD and statistically significant differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons [41]. Statistical analysis carried out by MS-Windows based graph pad Instat software (Graph Pad software, San Diego, CA, USA) 3 version was used. A value of $P<0.05$ was considered statistically significant.

RESULTS

The present study was carried out to assess the anti-anemic activity of sprouted *Vigna radiata* L. The observations made on different groups of experimental and control animals were compared as follows.

Table I represent the effect of sprouted *Vigna radiata* L. on Hemoglobin, WBC, RBC, and platelet content of male albino rats. Group II phenyl hydrazine intoxicated rats showed a significant (8.22±0.87) decrease in the content of Hb when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with sprouted *Vigna radiata* L. significantly (14.47±0.93) increase in the level of Hb when compared to group II. Group II phenylhydrazine induced rats showed a significant (2350±236, 2.30±0.18 and 18833.3±2016) decrease in WBC, RBC and platelet levels respectively when compared to Group I rats. Group III phenyl hydrazine induced rats treated with sprouted *Vigna radiata* L. significant (3450±278, 3.89±0.04 and 219000±1870) increase in WBC, RBC and platelet levels respectively when compared to group II.

Table 1: Effect of sprouted *Vigna radiata L.* on Hemoglobin, WBC, RBC and Platelet count

Parameters	Group I	Group II	Group III	Group IV
Hemoglobin(g/dl)	11.96±1.04	8.22±0.87	14.47±0.93*	12.0±0.02
WBC (Cells x 10^9/l)	4075±245	2350±236	3450±278*	3949±146
RBC (Cells x 10[12]/l)	4.9±0.39	2.30±0.18	3.89±0.04*	4.2±0.17
Platelet(Cells x 10^9/l)	223583±1744	18833.3±2016	219000±1870*	203410±1080

Values were expressed as mean±SD for six rats in each group, *Significantly different from Group II $P<0.05$.

Table 2 represents the effect of sprouted *Vigna radiata* L. on PCV, MCH, MCHC and MCV count in male albino rats. Group II phenylhydrazine intoxicated rats showed a significant (16±0.01) decrease in PCV when compared to Group I rats. Group III rats treated with germinated *Vigna radiata* L. significantly (29±0.11) increase in PCV when compared to group II. Group II phenyl hydrazine induced rats showed a significant increase in MCH, MCHC, MCV when compared to Group I rats. Group III rats treated with germinated *Vigna radiata* L. significantly (33±0.35, 38±0.24 and 48±0.97) decreases in MCH, MCHC, and MCV levels respectively when compared to group II.

Table 3 represents the levels of MDA, GSH, and SGOT in male albino rats. Group II phenyl hydrazine intoxicated rats showed a substantial (7.4±1.24) increase in the level of MDA when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with germinated *Vigna radiata* L. significantly (4.2±1.02) a decrease in the level of MDA when compared to group II. Group II phenyl

hydrazine intoxicated rats showed a significant (2.1±0.54) decrease in the level of GSH when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with sprouted *Vigna radiata* L. significantly (4.0±0.67) increase in the level of GSH as compared to group II. Group II phenyl hydrazine intoxicated rats showed a significant increase (205.3±72.5) in the activity of SGOT when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with germinated *Vigna radiata* L. significantly (79.2±22.8) a decrease in the activity of SGOT when compared to group II.

Table 2: Effect of sprouted *Vigna radiata* L. on PCV, MCH, MCHC and MCV

Parameters	Group I	Group II	Group III	Group IV
PCV (%)	23±0.13	16±0.01	29±0.11*	25±0.02
MCH (pg/cell)	24±0.32	50±0.41	33±0.35*	30±0.58
MCHC (%)	32±0.25	53±0.42	38±0.24*	34±0.03
MCV (cubic micron)	46±0.85	86±1.58	48±0.97*	48±0.02

Values were expressed as mean±SD for six rats in each group, *Significantly different from Group II P<0.05.

Table 3: Effect of sprouted *Vigna radiata* L. on MDA level, GSH and SGOT activity

Parameters	Group I	Group II	Group III	Group IV
MDA(nmole/l)	3.78±0.16	7.4±1.24	4.2±1.02*	3.8±0.6
GSH (mg/dl)	4.63±0.05	2.1±0.54	4.0±0.67*	4.61±0.4
SGOT (IU/l)	49.1±16.6	205.3±72.5	79.2±22.8*	60.3±16.2

Values were expressed as mean±SD for six rats in each group, *Significantly different from group II P<0.05.

DISCUSSION

Anemia is a disease characterized by a reduction in the concentration of hemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient. The present study aimed to evaluate the anti-anemic activity of sprouts of *Vigna radiata* L. against phenyl hydrazine induced anemic rats. Phenyl hydrazine is recognized for its capacity to cause hemolysis both *in-vitro* and *in-vivo* by the formation of aryl and hydroxyl radicals, which have been demonstrated to be associated with its interaction with erythrocytes [42].

Oxidative stress in erythrocytes is considered as an important mechanism of hemolysis. Disruption of membrane integrity arises from fragility, dehydration as well as increased production of reactive oxygen species. Chronic hemolysis leads to loss of hemoglobin. These metabolic changes lead to the depletion of essential nutrients and micronutrients which are required for proper cell function [43]. The accumulation of hydrogen peroxide in addition to the detoxifying capacity of the red cell may lead to the oxidation of essential cellular constituents including membrane phospholipids. Such alterations presumably contribute to the eventual hemolysis of affected cells.

The intoxication of rats with phenyl hydrazine (4 mg/kg for 2 d) resulted in a marked hemolytic anemia characterized by decreased RBC, hemoglobin and PCV [44]. Similar results were obtained in our study when experimental rats were administered phenyl hydrazine in order to induce anemia. Phenyl hydrazine altered the function of RBC by hemolysis characterized by decreased levels of RBC, hemoglobin and PCV. In addition, Ferrali *et al.* [45], observed an increased reticulocytosis, methaemoglobinemia and hemocatheresis in phenyl hydrazine intoxicated rats.

This study is intended to evaluate the effect of sprouted *Vigna radiata* L. on the hemolytic anemia induced by phenyl hydrazine. It has been demonstrated previously that intraperitoneal administration of phenyl hydrazine decreases the hemoglobin concentration, red blood cell number, and hematocrit in rats [46]. This anemia which resulted from the early lysis of the red blood cells was naturally reversed 12 d later by the regeneration of those blood cells due to the increase of the reticulocytes.

The germinated *Vigna radiata* L. could stimulate erythropoiesis process. The increase in the number of young red blood cells (reticulocytes) explains the strong osmotic resistance of the red blood cells in rats treated with the extract. The number of circulating reticulocytes coincided with the increase in MCV, thus suggesting that erythrocyte precursors become enucleated at a more differentiated stage of erythropoiesis. On the other hand, the increase in MCH observed during the experimental period could be indicative of a certain degree of intravascular hemolysis [47].

Indicators of anemia are reduced hemoglobin concentration (Hb), red blood cell count (RBC), WBC and PCV. Animals are similar to humans in that reduction in Hb, RBC and PCV are indicative of anemia. Mean Corpuscular Hemoglobin Concentration (MCHC-the amount of hemoglobin per unit erythrocyte volume) often reduced in hemolytic anemia or increased in the case of massive intravascular hemolysis. Mean Corpuscular Volume (MCV-average volume of the erythrocyte) is often increased in hemolytic anemia as the result of reticulocytosis. Mean corpuscular hemoglobin (MCH-the average amount of hemoglobin per cell) often increased in hemolytic anemia [48]. In the present study, phenyl hydrazine intoxicated rats decrease hemoglobin levels, RBC, WBC, platelet count and PCV whereas; it induces an increase in MCV, MCH, and MCHC. Our results with earlier reports [49], supplementation of germinated *Vigna radiata* L. to phenyl hydrazine intoxicated rats restored the altered hematological parameters.

Lipid peroxidation and the resultant perturbation of the structural integrity of the plasma membrane have long been considered to be capable of initiating the hemolytic response [50], though how the generalized destruction of membrane lipids could stimulate a selective macrophage response was not clear. The most recent reports that lipid peroxidation in nucleated cells correlates with the accumulation of Phosphatidylserine (PS) on the outer leaflet of the lipid bilayer. ROS production was associated with extensive binding of oxidized and denatured hemoglobin to the membrane cytoskeleton. Thus, phenyl hydrazine induced hemolytic injury seems to be derived from oxidative alterations to red blood cell membrane lipids [51]. In the present study, increased lipid peroxidation products, as MDA were observed on phenyl hydrazine, intoxicated rats. Supplementations of germinated *Vigna radiata* L. restored the MDA content suggested that reduced the oxidative damage.

In the present study, a marked decrease in the concentration of GSH was observed in phenyl hydrazine intoxicated rats when compared to control rats. Administration of sprouted *Vigna radiata* L. significantly increases the levels of GSH in phenyl hydrazine intoxicated rats. Enzymes catalyze specific biochemical reactions in the body. Changes in their levels and of cellular damage, the intracellular concentration of the enzymes and the mass properties

alter the functional ability of an organism. The diagnosis of organ disease/damage is aided by measurement of a number of non-functional plasma enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of the affected tissue. The concentration of the enzymes released reflects the severity of the damage. SGOT and SGPT are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die transaminases is released into the bloodstream, where they can be measured they are therefore the index of liver injury [52]. The hepatocellular damage indicated by increased activity of SGOT in serum was observed in this study. Supplementation of sprouted *Vigna radiata* L. to phenyl hydrazine intoxicated rats restored the SGOT activity.

Herbal medicine is increasingly gaining greater recognition from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life [53]. Several plant products are known to exhibit creditable medicinal properties for the treatment of various ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. Keeping in view the present study has evaluated the anti-anemic activity of sprouted *Vigna radiata* L. Phenyl hydrazine, an alkyl hydrazine was chosen to induce hemolytic anemia. Phenyl hydrazine induces the destruction of red blood cells by oxidation stress and many changes in cellular levels resulting in hemolytic anemia. Supplementation of germinated *Vigna radiata* L. to phenyl hydrazine intoxicated rats shows the following results.

- Improved the Hb content.
- Restored the WBC, RBC and platelet count.
- Secondary parameters of erythrocytes such as PCV, MCH, MCHC, and MCV were restored.
- Reduced oxidative damage confirmed by the decreased MDA content.
- Improved the detoxification mechanism by increased GSH content.
- Normalize liver functions as evidenced by SGOT activity.

In developing countries, anemia is one of the major health problems and in India, Lauha bhasma, an iron-based herbo-metallic preparation, is prescribed for treating anemia [54, 55].

CONCLUSION

The results of the present study accomplished that sprouted *Vigna radiata* L. inhibits anemia induced by phenyl hydrazine model similar to those induced by parasites such as *Plasmodium falciparum*. This result supports at least partially the traditional use of sprouted *Vigna radiata* L. in the treatment of anemia. Further investigations are needed to understand the mechanism involved in the anti-anemic action of sprouted *Vigna radiata* L.

CONFLICT OF INTERESTS

Declared None

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