National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Hematinic and antioxidant potential of aqueous extract of *Sesamum indicum* seeds against phenylhydrazine-induced hemolytic anemia in albino rats

Sridhar Prasad Y P[1], Padmaja Hari[2], Shajina M[3], Mirshad P V[4], Fasalu Rahiman O M[4]

[1]Department of Pharmacology, Sapthagiri Institute of Medical Sciences & Research Centre, Bengaluru, Karnataka, India, [2]Department of Physiology, MES Medical College, Perinthalmanna, Kerala, India, [3]Department of Biochemistry, Dr. M.V. Shetty College of Medical Lab Technology, Mangaluru, Karnataka, India, [4]Department of Pharmacology, MES Medical College, Perinthalmanna, Kerala, India

Correspondence to: Padmaja Hari, E-mail: mediresearchdirect@gmail.com

Received: March 09, 2018; **Accepted:** March 31, 2018

ABSTRACT

Background: More than 2 billion people around the world are suffering from anemia. Majority of populations in developing countries are depending on dietary supplementation and herbal medicine for the management of the anemic condition. The seed of *Sesamum indicum* L is used for its nutritional, medicinal, and industrial purposes in many parts of the world, which is also used as hematinics agent in the traditional system. **Aim and Objective:** This study investigated the hematinic and antioxidant potential of aqueous extract of *S. indicum* L. supplementation therapy in a rodent model of hemolytic anemia induced by phenylhydrazine (PHZ). **Materials and Methods:** About 36 adult albino rats were selected, of which six were considered to be normal controls. Anemia was induced by intraperitoneal administration of PHZ in remaining 30 rats. On the basis of hemoglobin (Hb) estimation after 2 days of PHZ administration, 24 rats were selected as anemic and randomly classified them into four groups. Groups II and III served as negative and positive control, while Groups IV and V were administered with the aqueous extract of S*esamum* for 14 days. Rats were sacrificed at the end of 14th day, and their blood samples were collected. **Result:** After the injection of PHZ rats developed hemolytic anemia reflected by a significant decrease in red blood cell (RBC) count, Hb concentration, and hematocrit percentage. Interestingly, therapy with S. *indicum* had significantly reversed these deteriorating effects on PHZ on RBCs, HGB, and HCT. In addition, sesame therapy significantly reversed the decreases in serum levels of total glutathione and activities of superoxide dismutase. **Conclusion:** The result of this study thus indicated that S. *indicum* is effective remedy to manage anemia in humans.

KEY WORDS: Hematinic activity; *Sesamum indicum*; Anemia; Phenylhydrazine; Antioxidant.

INTRODUCTION

Anemia is defined as a condition in which the number of red blood cells (RBCs) or their oxygen-carrying capacity is insufficient to meet the physiological needs of the body. There are over 400 types of anemia, many of which are rare, but in all cases, there is lower than a normal number of circulating RBCs. Many types of anemia exist such as iron deficiency anemia, pernicious anemia, hemolytic anemia, aplastic anemia, megaloblastic anemia, sickle cell anemia, thalassemia, and sideroblastic anemia. Iron deficiency is thought to be the most common cause of anemia globally.[1]

The causes of anemia can be acquired or inherited. Other factors such as lack of access to balance diet, folate, Vitamin B12, Vitamin A deficiencies, chronic inflammation, parasitic

Access this article online

Website: www.njppp.com

Quick Response code

DOI: 10.5455/njppp.2018.8.0310831032018

infection, and inherited disorders can cause anemia.[2] Anemia occurs, and the body makes few RBCs, destroys many RBCs, or loses many RBCs. Iron deficiency anemia has serious consequences for the health and well-beings as well as the social and economic impact of India.[3] If anemia develops during pregnancy, blood does not have enough healthy RBCs to carry oxygen to tissues and to the fetus. Folate deficiency can directly contribute to the certain type of birth defects, such as neural tube abnormalities, (spina bifida) and low birth weight. Iron, folic acid, and vitamin deficiency anemia can be treated with changes in diet plan with the supplementation iron, folic acid, and vitamin.

Man since time immemorial has been using herbs or plant products as medicine for combating aliments, namely, cold, pain, and fever. A vast majority of the rural population depends largely on herbal remedies, and there are so many beneficial advantages related to the usage of these medicines. Herbal treatment is non-invasive and non-toxic, so it can be used safely as an alternative therapy or alongside conventional therapies. A good number of medicinal plants are traditionally employed to treatment for anemia, and some of these plants include dates, grapes, beetroot, broccoli, honey, and pomegranate.[4] It is noted that substances such as flavonoids and phenols have the hematinic activity.[5] *Sesamum indicum* Linn. belonging to the family Pedaliaceae is an important oilseed crop, having seed and oil that are highly valued as a traditional health food, and has a large number of medicinal use. Conventionally, the plant sesame used as wound healing medicines.[6] The studies on *S. indicum* have been carried out by various researchers, and a wide spectrum of its pharmacological actions has been explored such as anticancer, antidiabetic, and antihyperlipidemic activity.[6] Conventionally, *S. indicum* seed is used for the treatment of anemia in several parts of India.[7] Therefore, this research aims to reveal the hematinic and antioxidant activity of black *S. indicum* seed to verify the claim of its medicinal potential.

MATERIALS AND METHODS

The study was conducted on healthy rats, which were kept in the animal house of research laboratory of MES Medical College, Perinthalmanna, for 3 months.

Plant Material

The sesame seeds were brought from the local market of Calicut in the sealed, airtight bags for the analysis. The sample was washed thoroughly with water to remove dirt, rinsed with distilled water, and was allowed to drain and dried under the sun for a day and dried under shade for 2 days. Seeds are ground into fine powder by an electrical blender and kept in the clean plastic can until use.

Preparation of Aqueous Extract

The aqueous extract was prepared by maceration; 1000 g of powder was soaked in about 1 L of distilled water for 7 days. The extract was decanted and the remaining material was re soaked in distilled water. Both the extracts were dried completely by using Rotary vacuum evaporator.

Experimental Animals

Thirty 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*. The study protocols were duly approved by the Institutional Animal Ethics Committee of the institute, MES Medical College, Perinthalmanna. The study was performed in accordance with the CPCSEA guidelines.

Acute Toxicity Studies

There was no mortality observed with oral administration of *S. indicum* even at the highest dose 2000 mg/kg. Both the doses of sesame had no toxic effect on the normal behavior of the rats. Hence, 200 and 400 mg dose per day were selected.[8]

Experimental Design

Albino rats of either sex weighing between 100 and 200 g were divided into five group of six rats each. At first, anemia was induced in rats (except normal control or Group I) by intraperitoneal administration of 40 mg/kg of phenylhydrazine (PHZ) for 2 days (D_0 and D_1). On the 2nd day, hemoglobin (Hb) level of treated rats is estimated, and the treated rat with PHZ whose Hb concentration <13 g/dL was considered as anemic and included in this study. Anemic rats were divided into Groups II–V and treated with the following chart for 14 days.

- Group I: Normal control received 10 mL/kg of corn suspension from day 2 to 14.
- Group II: Anemic control received 10 mL/kg of corn suspension from day 2 to 14.
- Group III: positive control treated with Vitamin B 12 syrup 1 mL/day from day 2 to 14.
- Group IV: Test group treated with aqueous extract of *S. indicum* (AESI) 200 mg/kg from day 2 to 14.
- Group V: Test group treated with AESI 400 mg/kg from day 2 to day 14.

Analysis of Hematological Parameters

After 14 days of treatment, a whole blood sample was collected from retro-orbital plexus under anesthesia. The number, shape, volume, and the color of the RBCs indicate the quality of blood. Samples were added to a tube containing

ethylenediaminetetraacetic acid after 2 weeks of treatment. The RBC number, Hb, hematocrit (PCV), MVC, mean corpuscular Hb (MCH), and MCH concentration (MCHC) were determined at day 14 using an automatic blood cell counter. Antioxidant potential such as glutathione (GSH) and superoxide dismutase (SOD) was also analyzed using the standard procedure.[9,10]

Statistical Analysis

Values were expressed as mean ± SD and statistically significant differences between mean values were determined by ANOVA and *t*-test using SigmaStat; $P < 0.05$ were considered to be statistically significant.

RESULTS

The whole study was conducted on 36 healthy adult albino rats. A total of six rats were considered as normal control, anemia induced by intraperitoneal administration of phenylhydrazine (PHZ) in remaining 30 rats. On the basis of Hb estimation after 2 days of PHZ administration, 24 rats were selected as anemic. The hematological and antioxidant potential of tests were compared to control groups.

Administration of PHZ for 2 days caused a significant decrease ($P < 0.05$) Hb rate in rats of anemic control, standard, test Group I, and test Group II [Table 1]. After the 14 days of treatment with appropriate agents, the result shows an improvement of Hb levels in Vitamin B_{12} syrup-treated group and those which received an AESI [Table 1]. The AESI at dose 400 mg per day allows a faster recovery. In the negative control rats, the Hb, for instance, increased naturally and progressively.

After injection of PHZ to rats, there was a decrease in RBCs on day 2. Significant increases in RBCs were observed after treatment for 14 days. The result showed that the rats of the Group III–V have almost completely recovered at the 2nd week [Table 2].

The administration of PHZ also decreased hematocrit, MCV, MCH, and MCHC at day 2. 1 week of treatment of anemic rats Group III–V with extract reversed the effect of PHZ resulting in a significant increase in PCV, MCV, MCH, and MCHC [Table 3].

The administration of PHZ also decreased the level of antioxidant parameters such as GSH and SOD at day 2. 2 weeks of treatment on anemic rats reversed the effect of PHZ, resulting in a significant increase in GSH and SOD [Table 4].

DISCUSSION

The present study was aimed to evaluate the effect of *S. indicum* seed extracts on the hemolytic anemia induced by PHZ in albino rats. Iron deficiency anemia brings serious economic consequences and obstacles to national development by affecting more than 30% of the world population.[11,12] Hence, the study was relevant to find an effective agent to manage the anemic condition in a cost-effective manner. The study shows that *S. indicum* effectively improves the Hb, RBC, PCV, MCV, MCH, and MCHC levels compared to the negative control group. PHZ 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. It has been demonstrated that intraperitoneal administration of PHZ decreased the Hb concentration, RBC, and PCV. Peter *et al*. demonstrated that intraperitoneal administration of 40 mg//kg PHZ for 2 days reduces hematological indices.[13] 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

Table 1: Hb level of rats before and after treatment with PHZ and after 14 days of treatment

Group	Hb level of rats		
	Before PHZ treatment	After 2 days of PHZ treatment	After 14 days of treatment
Group II			
Anemic control	13.85±0.546	8.78±0.552**	9.25±0.543*
Group III			
Standard	14.03±0.541	9.78±0.416**	13.88±0.348**
Group IV			
Test Group I	13.85±0.381	9.28±0.702**	11.4±1.363*
Group V			
Test Group II	13.96±0.598	9.25±0.653**	13.16±0.640**

Values are expressed as mean±SD followed by one-way ANOVA *P<0.05, **P<0.005, PHZ: Phenylhydrazine, Hb: Hemoglobin

Table 2: Values of RBC after 14 days of treatment

Group	RBC ($\times 10^6$/mL)
Group I	
Normal control	7.21±0.538
Group II	
Anemic control	5.15±0.602
Group III	
Standard	7.76±0.450**
Group IV	
Test Group I	6.46±0.722*
Group V	
Test Group II	7.45±0.971**

Values are expressed as mean±SD followed by students paired *t*-test *P<0.05, **P<0.005 as compared to control, RBC: Red blood cell, SD: Standard deviation

Table 3: Values of PCV and blood cell indices after 14 days of treatment

Group	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group I				
Normal control	41.83±3.48	60.91±1.74	19.11±1.930	33.03±3.583
Group II				
Anemic control	30.83±3.060	57.01±2.20	18.01±1.316	30.06±1.462
Group III				
Standard	45.66±3.32**	58.08±7.12**	17.98±1.322**	30.46±1.938**
Group IV				
Test Group I	38.83±2.85*	59.03±9.02**	17.78±2.209**	29.48±3.162**
Group V				
Test Grout II	42.66±2.732**	58.73±1.270**	17.68±1.270**	30.91±3.051**

Values are expressed as mean±SD followed by students paired *t*-test *P<0.05, **P<0.005 as compared to control, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin

Table 4: Antioxidant after 14 days of treatment

Group	GSH	SOD
Group I		
Normal control	15.21±0.426	9.43±0.637
Group II		
Negative control	11.13±0.460	5.63±0.725
Group III		
Positive control	13.28±0.541**	7.81±0.574**
Group IV		
Test Group I	12.21±0.381*	6.86±0.581*
Group V		
Test Group II	13.13±0.598**	8.31±0.598**

Values are expressed as mean±SD followed by students paired *t*-test *P<0.05, **P<0.005 as compared to control, SOD: Superoxide dismutase, SD: Standard deviation, GSH: Glutathione

leads to loss of Hb. 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 results of the study indicated that increase in the hematological indices exhibited by S. *indicum* extract may be due to vitamins and mineral contents of the seed. Administration of sesame may indicate that the plant extract has the ability to stimulate the erythropoietic factors that have a direct influence on the production of blood in the bone marrow. Erythropoietin is a maturation factor of RBC synthesis, which increases the number of erythropoietin-sensitive committed stem cells in the bone marrow, that is converted to RBCs and subsequently to mature erythrocytes. The lowest administration dose of 200 mg/kg reduced the recovery time of the blood parameters. Furthermore, the recovery was progressive such that after 2 weeks of continuous treatment, the Hb concentration and PCV were 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 400 mg/day affecting the highest change.

A significant correlation with diagnostic values has been demonstrated between RBC, Hb, PCV, and red cell indices (MCV, MCH, and MCHC) in both humans and rats.[14] Animals are similar to humans in that reduction in Hb, RBC, and PCV is indicative of anemia.[15] MCHC (MCHC - the amount of Hb per unit erythrocyte volume) often reduced in hemolytic anemia or increased in the case of massive intravascular hemolysis. MCV (MCV - average volume of the erythrocyte) is often increased in hemolytic anemia as the result of reticulocytosis. MCH (MCH - the average amount of Hb per cell) often increased in hemolytic anemia. In the present study, a marked decrease in the concentration of GSH was observed in PHZ intoxicated rats when compared to control rats. Administration of sprouted *S. indicum* L. significantly increases the levels of GSH in PHZ-intoxicated rats. There is also a marked decrease in SOD observed in negative control when compared to normal control. Moreover, the SOD levels of treated groups are elevated when compared to negative control.

CONCLUSION

The injection of PHZ to rats caused a hemolytic anemia characterized by reducing hematological parameters. The oral administration of AESI in the dose of 200 mg and 400 mg significantly increased Hb level. And also, the results demonstrated that *S. indicum* inhibits the formation of ROS in rat showing potential antioxidant effect. This result supports the traditional use of sesame in the treatment of anemia. Further investigations are needed to understand the mechanism involved in the antianemic action of *S. indicum*.

ACKNOWLEDGMENT

Authors are thankful to Medi Research Direct for their scientific support during the entire project.

REFERENCES

1. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, *et al.* A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014;123:615-24.
2. Moll R, Davis B. Iron, vitamin B12 and folate. Medicine 2017;45:198-203.
3. Stoltzfus RJ. Iron deficiency: Global prevalence and consequences. Food Nutr Bull 2003;24 4 Suppl 2:S99-103.
4. Mazed MA, Mazed S, Mohammad AM. Nutritional Supplement for the Prevention of Cardiovascular Disease, Alzheimer's Disease, Diabetes, and Regulation and Reduction of Blood Sugar and Insulin Resistance. United States Patent US 8,017,147. 2011 Sep 13.
5. Fejes S, Blázovics A, Lugasi A, Lemberkovics É, Petri G, Kéry Á. *In vitro* antioxidant activity of *Anthriscus cerefolium* L. (Hoffm.) extracts. J Ethnopharmacol 2000;69:259-65.
6. Amoo SO, Okorogbona AO, Du Plooy CP, Venter SL. *Sesamum indicum*. In: Medicinal Spices and Vegetables from Africa. Dschang, Cameroon: Academic Press; 2017. p. 549-79.
7. Silja VP, Varma KS, Mohanan KV. Ethnomedicinal plant knowledge of the mullu kuruma tribe of Wayanad district, Kerala. Indian J Trad Knowledge 2008;7:604-12.
8. Kumar M. Hepatoprotective activity of *Sesamum indicum* Linn. against Ccl4-induced hepatic damage in rats. Int J Pharm Biol Arch 2011;2:710-5.
9. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
10. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
11. Iyengar GV, Nair PP. Global outlook on nutrition and the environment: Meeting the challenges of the next millennium. Sci Total Environ 2000;249:331-46.
12. Gamit MJ, Talwelkar HS. Survey of different types of anemia. Int J Med Sci Public Health 2017;6:493-6.
13. Peter A, Christian EO, Adaobi CE. The haematinic activity of the methanol leaf extract of *Brillantasia nitens* Lindau (*Acanthaceae*) in rats. Afr J Biotechnol 2009;8:2389-93.
14. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. Afr J Biotechnol 2005;4:833-7.
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.

How to cite this article: Prasad YPS, Hari P, Shajina M, Mirshad PV, Rahiman OMF. Hematinic and antioxidant potential of aqueous extract of *Sesamum indicum* seeds against phenylhydrazine-induced hemolytic anemia in albino rats. Natl J Physiol Pharm Pharmacol 2018;8(8):1092-1096.

Source of Support: Nil, **Conflict of Interest:** None declared.