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Research Article

Evaluation of Anti-anemic Effect of Anthocyanin Rich *Oryza sativa L. indica* (Black Rice) Seeds Extract

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ABSTRACT

Oryza sativa L. *indica* (black rice) has a rich cultural history and used as a tribute food. As the outer layer of seeds contains anthocyanin, *O. sativa* L. *indica* is black. Traditionally the plant used to enhance heart health, reduces the risk of atherosclerosis, diabetes as well as allergy, control hypertension, and improves the digestive system and lipid profile. It has anti-inflammatory action, detoxifies the body, decreases cancer growth, supports in weight management, and improve memory and quality of life. Here, the methanolic extract of black rice (BRME) was evaluated for its anti-anemic effect. In study anemia was induced by administering 40 mg/kg phenylhydrazine (PHZ) for two consecutive days in rats. From day 3, animals were treated with standard drug and BRME extracts (200 and 400 mg/kg) till 15 days. The anti-anemic activity was examined by estimating different parameters like hemoglobin, red blood cell, hematocrit, reticulocytes, aspartate amino transaminase activity, alanine amino transaminase and alkaline phosphatase. The results of the phytochemical test revealed the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids, tannins, amino acid, and triterpenoids. Methanolic extract of black rice also restored the hematological parameter and biochemical parameters values near to normal levels after 13 days of treatment at 200 and 400 mg/kg. The 400 mg/kg dose shows higher anti-anemic effect compared to the lower 200 mg/kg dose. From the result, it is concluded that *O. sativa L. indica* is a valuable source of active compounds with anti-anemic properties and can be used to treat anemia.

Introduction

Anemia is a blood disorder that is characterized by a reduction in a large number of red blood cells (RBC), hemoglobin level and packed cell volume. Hemoglobin is a main element of RBC that makes the red blood cells bind with oxygen and circulate it in all the organs for important mechanisms.[1] The reduction in RBC results in impaired oxygen delivery to tissues, giving rise to physiologic consequences of tissue, hypoxia, fatigue, weakness, dizziness, headache, numbness or coldness in hands and feet, low body temperature, pale skin, rapid or irregular heartbeat, shortness of breath, chest pain and irritability. The normal level of hemoglobin is generally different for men and women. For men, anemia is typically defined as a hemoglobin level is less than 13 g/dL and in women, as hemoglobin is less than 12 g/dL.[2]

According to WHO in 2019, 27% of the population in the world have anemia *i.e.,* around in two billion peoples. Anemia is a serious global public health problem that affects young children, pregnant women, and mainly low- to middle-income populations.[3] WHO estimates that 42% of children less than 5 years of age and 40% of pregnant women worldwide are anemic. It is also estimated that one third of all women of reproductive age are anemic. In 2019, there were 1.8 billion prevalent cases of anemia across the world, with an age-standardized point prevalence of 23,176.2 per 100,000 population.[4]

Over the years, many medicines and transplant methods have been developed for the treatment of anemia and other blood related problems. The treatment includes medicines like folic acid, vitamins, hydroxyurea, chemotherapy, blood transfusion and many more. Over the past several decades,

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research has been ongoing to determine efficient naturally occurring anti-anemic agents which could overcome the life-threatening effects of anemia. The main advantage of using naturally occurring herbal medicines and plants to cure diseases is to get long-term effects with less side effects and more effectiveness.[5] This study is an attempt to explore such natural therapeutics for anemia.

Oryza sativa L. indica, commonly known as black rice belongs to the family Poaceae is consumed as a functional food due to its usefulness to health.[6] Black rice is a food that we can consume daily and is easier to consume than drugs available in markets. Various food products can be made using black rice which can be taken with no difficulties and can be really helpful in anemia related problems.[7] Black rice contains higher levels of proteins, vitamins and minerals than common white rice. *O. sativa L indica* comprises vital amino acids such as tryptophan, lysine and vitamins like folic acid and vitamin B. Black rice also has minerals such as iron, calcium, zinc, phosphorus and phosphorus and selenium.[8] It contains the highest number of antioxidants, protein and dietary fiber of all rice varieties; besides it has phenolics, flavonoids, and anthocyanins.[9] These antioxidant compounds have tremendous health benefits and can reduce the risk of developing various chronic diseases.[4]

According to the uses mentioned in traditional medicine, this rice enhances health and longevity, protects heart health, reduces atherosclerosis, controls hypertension, and improves the digestive system. It has anti-inflammatory action, reduces allergy,[10] detoxifies the body, improves lipid profile, reduces risk of diabetes, helps in weight management, reduces the growth of cancer, boosts cognitive function and increases quality of life. Hence black rice is an excellent alternative to white and brown rice, due to its nutrient density, high fiber content, and rich antioxidant content.[11]

About two billion people among the globe suffer from anemia, the most common health-related problem in developing nations. Changes in the diet, as well as iron supplements, are usually chosen in the management of disease. But oral iron therapy has numerous difficulties like inadequate absorption and less complying nature. Additionally, ingesting high amount of iron supplements causes serious health-related problems such as cancer as well as some neurogenic disorders.[12] Therefore, searching for a new entity for the treatment of anemia is needed. The drugs derived from natural sources are safer as well as cost-effective in nature. However, its anti-anemic activity has not been explored yet. Therefore, the present study was aimed to evaluate the anti-anemic effect of *O. sativa* L. *Indica* seeds.

Even the acute toxicity study of *O. sativa L indica* in animals did not show any signs or symptoms related to toxicity at the dose of 2000 mg/kg of BRB via intragastric gavage. No mortality was reported within 24 hours and even till the time to sacrifice. Significant weight loss and change in the animals' behaviors were not observed during the toxicity study. Necropsy also did not show macroscopic changes in the internal organs along with the weight of the organs of rats.[13] Therefore, in this study we selected 1/10 and 1/20 dose of safer dose and used 200 and 400 mg/kg dose the treatment.

In the present study, anemia was induced in rats except normal group by intraperitoneal administration of 40 mg/kg of Phenylhydrazine (PHZ) for 2 days. On 3rd day, phenylhydrazine treated rats' hemoglobin (Hb) level was examined and considered an anemic rat. From day 3, rats of group 3, 4 and 5 were treated with standard ferrous sulphate (75 mg/kg) and lower dose (200 mg/kg) and higher dose (400 mg/kg) of extract, respectively till 15 days. Blood samples were collected from the retro-orbital for the analysis of different hematological as well as biochemical parameters.

Materials and Methods

Materials

Phenylhydrazine hydrochloride was purchased from TCI Chemicals (India) Pvt. Ltd., ferrous sulphate ($FeSO_4$), potassium chloride (KCL), hydrochloric acid (HCL) and sodium acetate (CH_3COONa) were purchased from V. shiv chemicals, Surat (Gujarat).

Methods

Plant Collection and Authentication

The seeds of *O. sativa* L. *indica* (Black Rice) were collected from the field of local village Zhankari, district- Tapi, Gujarat. *O. sativa* L. *indica* (Black Rice) was authenticated by Dr. B. R. Patel, Associate Professor of Botany. The Patidar Gin Science College, Bardoli, Dist. Surat, Gujarat. (Authentification No: 02/2022 Botany) on date 29th January 2022.

Experimental Animal Approval

The Animal Study protocol (Protocol No. CPCSEA/SNLPCP/IAEC/22/01/128) was approved by Institutional Animal Ethics Committee (IAEC). The IAEC meeting was held on 22nd January 2022 at Shree Naranjibhai Lalbhai Patel College of Pharmacy, Umrakh, Gujarat.

Preparation of Seed Extract

The seeds of *O. sativa* L. *indica* were collected, de- husked and crushed in to coarse powder. For the extraction 10 gm black rice powder dissolved in 100 mL methanol with occasional shaking for 72 hours. After 72 hours extract was filtered and evaporated in a water bath for the complete evaporation of methanol. The extract was collected and stored in airtight glass bottle and the extraction yield was calculated.[14]

Phytochemical Screening of O. sativa L. indica Seed

The methanolic seed extracts of *O. sativa L. indica* was used for preliminary phytochemical screening for the detection of alkaloid, anthraquinone, tannin, saponins, cardiac glycoside, flavonoid terpernoid and using various phytochemical tests.[15,16]

Determination of Total Anthocyanin Content

Total anthocyanin content is determined as cyanidin 3-glucoside by pH difference method. Anthocyanin extract was dissolved in KCl buffer (0.025 M, pH 1) and sodium acetate buffer (0.4 M, pH 4.5) with a ratio of extract to buffer = 1: 5 (v / v). After 15 minutes of incubation at room temperature, absorbance of all the samples were taken at a maximum wavelength and 700 nm. Absorbance was then placed into the below formula for the determination of total anthocyanin content.

A = [(A λmx - A 700) pH = 1] - [(A λmx - A 700) pH = 4.5]

$$\text{Total Anthocyanin Content} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times b}$$

DF is the dilution factor, b is the thick solution, ε is molar absorptivity and MW is the molecular weight (cyanidin 3- glucoside 449.2 g/mole).[17,18]

Induction of Anemia

Either sex of albino wistar rats having a hemoglobin value of 11 g/dl or more were selected for study and divided then into five groups (n=6).

- Group 1: Normal rats treated with normal feed and water *ad libitum*.
- Group 2: Rats treated with 40 mg/kg phenylhdrazine at day 1 and day 2 (positive control group)
- Group 3: Rats treated with 75 mg/kg ferrous sulphate (standard drug treated group)
- Group 4: Rats treated with 200 mg/kg methanolic black rice extract (lower dose of extract)
- Group 5: Rats treated with 400 mg/kg methanolic black rice extract (higher dose of extract)

Anemia was induced in rats except normal group by intraperitoneal administration of 40 mg/kg of PHZ for 2 days. On 3rd day, phenylhydrazine treated rats' Hb level was examined and considered anemic rat. From day 3 rats of group 3, 4 and 5 were treated with standard ferrous sulfate (75 mg/kg) and lower dose (200 mg/kg) and higher dose (400 mg/kg) of extract, respectively till 15 days. Blood samples were collected from the retro-orbital on day 0 before phenylhydrazine administration as well as on day 3, 8 and 15 after phenylhydrazine injection for the estimation of various hematological parameters. The blood volume collected was 0.27 to 0.45 mL, which does not affect blood parameters. And for the analysis of biochemical parameters blood was collected on day 0 and 15.[19-21]

Statistical Analysis

The data were presented as mean ± SEM of six animals in each group. Statistical analysis was performed by Graphpad prism 7.0 software. The data were estimated by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. test. The negligible level of significance was measured at $p < 0.05$.[22]

Results

Extraction Yield

Extraction from the seeds of *O. sativa L. indica* (Black rice) was performed by the maceration method followed by the distillation method and we got an extraction yield of 11.2%

Phytochemical Screening

Different phytochemical constituents were present in the methanolic extract of *O. sativa L. indica* seed (Table 1).

Determination of Total Anthocyanin Content

The total anthocyanin content in *O. sativa L. indica* (black rice) seeds was determined using the pH differential method by UV-spectroscopy. We got the anthocyanin content in the seeds was 161.4 mg/100 g. Table 2 shows the spectroscopic reading of samples.

Hemoglobin Level

There is a significant reduction in hemoglobin level in animals after the administration of phenylhydrazine. The hemoglobin level was decreased from 13.7 to 4.2 g/DL in control group which showed that animals had anemia. After the administration of methanolic extract of *O. sativa* L. *indica* (Black rice), the hemoglobin level was increased up to 10.92 and 11.76 g/DL, respectively in 200 and 400 mg/kg of extract treated groups. In the standard drug-treated group also the Hb level was raised up to normal (Table 3).

Red Blood Cell Count

The RBC count in normal group of wistar rat was 7.6 (× 106/µL). After two days of phenylhydrazine administration, the RBC count was reduced to up to 4.96 (× 106/µL), possibly because of the destruction of RBC by oxidative stress. This

Table 1: Phytochemical screening of methanolic extract of *O. sativa L. Indica* seed

S. No	*Preliminary Phytochemical Constituents*	*BRME (Black rice methanolic extract)*
1	Alkaloids	+
2	Carbohydrates	+
3	Fats	+
4	Glycosides	+
5	Phenols	+
6	Proteins	+
7	Saponins	-
8	Steroids	-
9	Tannins	+
10	Terpenoids	+

Table 2: Determination of total Anthocyanin content in *O. sativa L. indica* (Black Rice) seeds

S. No	*Wavelength (mm)*	*Uv-spectrometer readings*	
1	520 nm	Sample-1 (pH 1)	0.83
		Sample-2 (pH 4.5)	0.32
2	700 nm	Sample-1 (pH 1)	0.067
		Sample-2 (pH 4.5)	0.089

reduced RBC count was increased about 8.01(× 106/µL) after the treatment with lower and higher doses of methanolic extract of *O. sativa L. indica* (Table 4).

Hematocrit

It is also called as packed cell volume. Hematocrit were reduced to 38.13% compared to normal rats after the induction of anemia. There was a significant increase in PCV volume after treating the animals with standard drug and 200 and 400 mg/kg of methanolic extract of black rice (BRME). After the treatment, PCV was increased up to 41.26% (Table 5).

Reticulocytes

The normal value of reticulocytes were 4–6%. After the induction of anemia, the reticulocytes were increased to 63–67%, much higher than the normal value. There was a

Table 3: Effect of methanolic extract of *O. sativa L. indica* seeds on hemoglobin level

S. No	*Groups*	*Day-0 (gm/dL)*	*Day-3 (gm/dL)*	*Day-8 (gm/dL)*	*Day -15 (gm/dL)*
1	Normal	13.75 ± 0.142	13.75 ± 0.142	13.75 ± 0.142	13.75 ± 0.138
2	Control (PHZ 40 mg/kg)	13.51 ± 0.222	7.24 ± 0.151#	5.90 ± 0.100#	4.22 ± 0.130#
3	Standard ($FeSO_4$ 50 mg/kg)	13.14 ± 0.282	8. 89 ± 0.173*	10.72 ± 0.288b	12.96 ± 0.127*
4	BRME 200 mg/kg	12.64 ± 0.417	9.02 ± 0.157*	8.94 ± 0.292b	10.92 ± 0.574*
5	BRME 400 mg/kg	12.79 ± 0.289	7.70 ± 0.440*	9.18 ± 0.388b	11.11 ± 0.371*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *p <0.05 as compared to control. # p < 0.05 as compared to normal.

Table 4: Effect of methanolic extract of *O. sativa L. indica* (Black Rice) seed on red blood cells

S. No	*Groups*	*Day-0 (10^6/µL)*	*Day-3 (10^6/µL)*	*Day-8 (10^6/µL)*	*Day-15 (10^6/µL)*
1	Normal	7.66 ± 0.240	7.65 ± 0.241	6.67 ± 0.456	7.6 ± 0.241
2	Control (PHZ 40 mg/kg)	8.17 ± 0.343	7.70 ± 0.262#	6.91 ± 0.466#	4.96 ± 0.246#
3	Standard ($FeSO_4$ 50 mg/kg)	8.08 ± 0.324	6.05 ± 0.266*	7.74 ± 0.244*	8.72 ± 0.232*
4	BRME (200 mg/kg)	7.49 ± 0.378	4.32 ± 0.228*	6.22 ± 0.345*	8.01 ± 0.161*
5	BRME (400 mg/kg)	8.01 ± 0.331	5.20 ± 0.310*	7.44 ± 0.651*	8.98 ± 0.139*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test. *p< 0.05 as compared to control. # p <0.05 as compared to normal.

Table 5: Effect of methanolic extract of *O. sativa L. indica* (Black Rice) seed on hematocrit

S. No	*Groups*	*Day-0 (10^6/µL)*	*Day-3 (10^6/µL)*	*Day-8 (10^6/µL)*	*Day-15 (10^6/µL)*
1	Normal	45.12 ± 1.055	45.12 ± 1.055	42.13 ± 1.056	45.13 ± 1.056
2	Control (PHZ 40 mg/kg)	45.93 ± 0.973	18.43 ± 0.768#	16.76 ± 0.683#	14.27 ± 0.663#
3	Standard ($FeSO_4$ 50 mg/kg)	45.95 ± 0.988	15.19 ± 1.198*	36.89 ± 0.722*	47.76 ± 0.308*
4	BRME 200 mg/kg	45.41 ± 0.723	13.46 ± 0.536*	29.18 ± 0.570*	43.68 ± 0.560*
5	BRME 400 mg/kg	45.67 ± 0.975	14.28 ± 0.979*	31.71 ± 0.443*	43.32 ± 0.566*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test. *p < 0.05 as compared to control. # p < 0.05 as compared to normal.

Table 6: Effect of methanolic extract of *O. sativa L. indica* (Black Rice) seed on reticulocytes.

S. No	*Groups*	*Day-0 (10^6/µL)*	*Day-3 (10^6/µL)*	*Day-8 (10^6/µL)*	*Day 15 (10^6/µL)*
1	Normal	4.2 ± 0.375	4.2 ± 0.375	4.2 ± 0.386	4.2 ± 0.386
2	Control (PHZ 40 mg/kg)	4.7 ± 0.367	60.13 ± 2.404#	51.55 ± 2.743#	42.39 ± 0.870#
3	Standard ($FeSO_4$ 50 mg/kg)	4.5 ± 0.372	62.12 ± 2.646*	39.090 ± 1.619*	24.91 ± 0.290*
4	BRME 200 mg/kg	4.3 ± 0.529	60.07 ± 3.226*	41.54 ± 0.581*	32.94 ± 1.027*
5	BRME 400 mg/kg	5.6 ± 0.420	59.51 ± 4.322*	42.29 ± 0.733*	30.68 ± 1.453*

Each value represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test. *p < 0.05 as compared to control. # p < 0.05 as compared to normal.

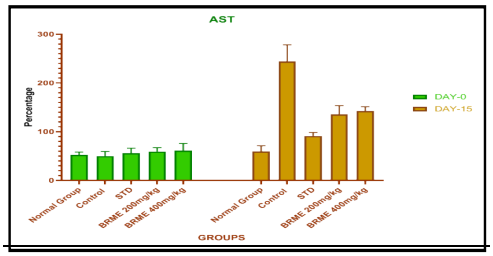

Fig. 1: Effect of methanolic extract of *O. sativa L. indica* on AST level Each bar represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one way Analysis of variance followed by Dunnett's multiple comparison test. *$p < 0.05$ as compared to control. # $p < 0.05$ as compared to Normal.

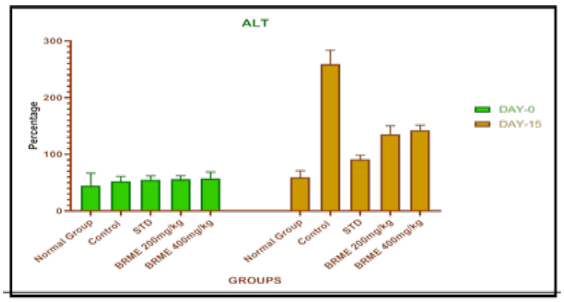

Fig. 2: Effect of methanolic extract of *O. sativa L. indica* on AST level Each bar represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test. *$p < 0.05$ as compared to control. # $p < 0.05$ as compared to Normal.

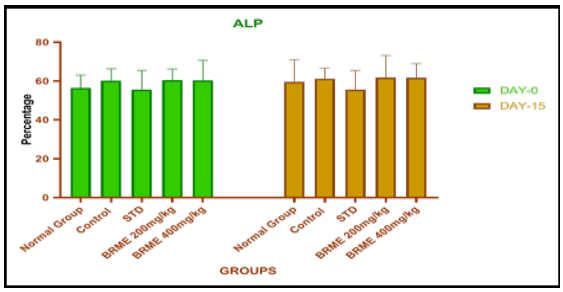

Fig. 3: Effect of methanolic extract of *O. sativa L. indica* on ALP level Each bar represents Mean ± Standard Error of Mean (S.E.M) (n=6). The statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test. *P<0.05 as compared to control. # p<0.05 as compared to Normal.

significant decrease in the reticulocyte count in animals after the treatment with standard drug, lower dose and higher dose of methanolic extract of Black rice (BRME) (Table 6).

Aspartate Amino Transaminase Activity (AST)

The effect of black rice seed extract was observed on serum AST level in all anemic rats at day 0 and 15. On day 0 the serum AST level was observed as non-significant in all the all animals of different groups. After 15 days, the control group showed significant increases in serum AST level compared to the normal group. The treatment of Black Rice seed extract at doses of 200 and 400 mg/kg showed a significant dose-dependent decrease in serum AST level compared to the control group (Fig. 1).

Alanine Amino Transaminase (ALT)

The effect of Black rice seed extract on serum ALT level in PHZ-induced anemic rats was analyzed on Day 0 and 15. The result of ALT level in all the experimental groups were not significant with each other on Day 0. But after 15 days, the control group (rats administered only PHZ) showed a significant increase in serum ALT activities compared to the normal group (rats not administered PHZ). Administration of black rice seed extract at doses of 200 and 400 mg/kg body weight caused a significant dose-dependent decrease in serum ALT level as compared with the control group (Fig. 2).

Alkaline Phosphatase (ALP)

The effect of methanolic extract of Black rice seed was studied on serum ALT level anemic rats on day 0 and day 15. But there was no significant difference in serum ALP level in all the experimental groups throughout the study period (Fig. 3).

Discussion

Anemia is an ailment that has a reduced number of RBCs and therefore decreases the ability of RBC to carry oxygen and fulfill the person's oxygen demand.[21] The main role of RBCs is to transfer oxygen as well as carbon dioxide in and outside the body. This is accomplished by using hemoglobin, a tetramer protein composed of haem and globin. Anemia damages the gas exchange capacity of RBCs by declining its amount and impairing carbon dioxide and oxygen transportation.[4]

Anemia results from one or more of the following processes: defective red cell production, increased red cell destruction or blood loss. Iron is necessary for the synthesis of hemoglobin. The critical role of hemoglobin in carrying oxygen to the tissues explains the most common clinical symptoms of anemia, which include fatigue, bounding pulses, shortening breathing, palmar pallor, and conjunctival. Anemia medically stands for lowered hemoglobin level (normal for male: 13.5–17.0 gms% and for females: 12.0w15.5 gms%), either with normal or lowered red blood cells depending upon the age and sex. Hemoglobin is the basic requirement of the body necessary for transporting oxygen throughout the body. Hemoglobin functions as the moving power station in the body. So lowered hemoglobin may cause fatigability, unusual tiredness and energy shutdown in all body parts. Anemia is not an ailment but might occur due to several illnesses. There are over 400 types of anemia, many of which are rare but, in all cases, there is lower than normal number of circulating red blood cells.[23] They are divided into three groups: (1) Anemia caused by blood loss, (2) Anemia caused by decreased or faulty red blood cell production and (3) Anemia caused by destruction of red blood cells.

Anemia is caused by factors such as low socioeconomic status, nutritional deficiencies, helminth infections and other infectious diseases, illiteracy, and blood disorders

such as sickle cell anemia. The main reasons in the people are deficiency of iron, hookworm infection, malaria and deficiency of folic acid.[24]

Black rice (*O. sativa* L. *indica)* is consumed as a functional food due to its usefulness to health. *O. sativa L. indica* is also identified as Black rice, forbidden rice, king's rice, imperial rice, heaven rice, prized rice, and purple rice. *O. sativa* L. *indica* comprises important amino acids such as tryptophan, lysine, vitamins like vitamin B and C; as well as *O. sativa* L. *indica* is a respectable origin of minerals comprising zinc, calcium, iron, phosphorus and selenium.[8] The plant has maximum antioxidants, dietary fiber and protein, anthocyanins, flavonoids and phenolics. Black rice is a good source of dietary fiber, oil, flavonoids, polyphenols and anthocyanidins. *O. sativa L. indica* comprises two main anthocyanins: peonidin-3-glucoside and cyanidin-3-glucoside. Amongst 2 anthocyanins, cyanidin-3-glucoside (C3G) is the chief anthocyanin in *O. sativa L. indica,* around of 93% of total anthocyanin content. [25, 26]

Anthocyanin is a member of flavonoids soluble in water and role as a natural pigment.[27] These pigments produce orange, purple, red and blue shades. of many of trees, vegetables and fruits. Anthocyanins are naturally occurring phenolic compounds that color many fruits (especially berries) and vegetables and have several valuable effects.[28] In black rice, pigments are located in the aleurone layer, characterized as dark purple to black in color and probably represent a mixture of anthocyanins.[29] Pigmented black rice contains about 6 times more antioxidants than brown rice. Pigmented varieties, such as black rice, have a higher content of phenolic compounds as compared to other rice varieties.

Over the years, different pharmacological models have been employed to evaluate the anemic and anti-anemic activity of herbal plants. Animal models of anemia have been proved highly effective in discovering novel treatments for anemic and blood-related symptoms and searching for clues to the underlying cause of the illness. Various *in-vivo* and *in-vitro* models are available for anemia such as Phenyl hydrazine induced anemia, aluminium chloride-induced anemia, cyclophophomide induced anemia, chloramphenicol induced anemia and aniline induced anemia.[21] But phenylhydrazine rat model is rapidly induced and dependable; thus, it is extensively used model to study antianemia drugs.[30]
Phenylhydrazine is a non-immunogenic compound that can induce hemolytic type of anemia by abolishing matured RBCs by oxidative stress, membrane phospholipids, and denaturation of red cell Hb, as well as enzymes used in energy metabolism.[31] Anemia induced by this chemical is characterized by lowered Hb, erythropenia, and reduced blood HCT levels.[32]

Earlier research recommended 40 mg/kg of intraperitoneal injections of phenylhydrazine for 2 consecutive days to persuade the anemic illness.[30] Anemia induced by PHZ causes lowered Hb level and reduced HCT levels which persisted for 8–12 days. [30] Also, PHZ makes pathological changes in kidney, heart, spleen and liver. [33] Thus, to study the antianemic effect of BRME, we analyzed the levels of Hb, RBCs and HCT throughout the experiment.

In anemia, the role of RBC gets altered which may be harmful for the ordinary operational of the body. In the present study, PHZ administration caused decline in RBCs count of blood during experimental period because interaction of PHZ with RBC produces hydrogen peroxide. It is believed that the hydrogen peroxide oxidizes vital sulfhydryl groups of enzymes, and causes peroxidation of membrane lipids and thereby induces hemolysis. Even PHZ causes selective destruction of matured RBCs through oxidative stress.[34] Therefore, the beneficial effect of BRME to increase RBCs count may be because of its capability to stop PHZ-induced hemolysis.

Hb is also a most important parameter for the evaluation of anti anemic drugs because the decreased amount of Hb causes deterioration the oxygen-carrying capacity of blood. In the present study, PHZ induction caused low level of Hb in blood throughout the experimental period. This decreased Hb level was improved by BRMC treatment. PHZ is also causes anemia by oxidative denaturation of Hb originated by free radicals. Hence, the antioxidant action of BRME might be accountable for the improve Hb level in blood. [12]

HCT, even identified as the packed cell volume, is the relation of volume of packed RBCs to the total blood volume. A lower HCT level is a pointer for anemic disorder.[12] In the study, there was a significant decrease of HCT in control group of animals may due to hemolysis. This decreased HCT level was inverted by treatment with BRME.

The treatment of BRMC at doses of 200 and 400 mg/kg showed a significant dose-dependent decrease in serum AST level as well as ALT level compared to the control group which shows improved anemic condition. But there was no significant difference in serum ALP level in all the experimental groups throughout the study period.

Conclusion

This present study shows that the methanolic extract of *O. sativa L. indica* (Black rice) increases the RBC count, hemoglobin level, haematocrit, and reticulocytes and significantly improvement in the biological parameter in phenylhydrazine induced anemic rats when compared with the untreated animals of the control group. Hence it was suggested that the methanolic extract of *O. sativa L. indica* (Black Rice) seed had a significant anti-anemic effect. This may probably due to the antioxidant property of *O. sativa L. indica* (Black Rice).

Limitations

The title of this study shows the use of anthocyanin rich extract of *O. sativa* L. *indica* (Black Rice) for the cure of

anemia. But in this study, we have used crude methanolic extract of *O. sativa* L. *indica*. Hence, this study was done using crude methanolic extract of black rice and all the results and statistical analysis are based on the crude methanolic extract of black rice.

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Conflict Of Interest

The authors hereby declare no interest in the given field.

References

1. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Annals of the New York Academy of Sciences. 2019;1450(1):15-31.
2. Teressa H. A review on major causes of anemia and its prevention mechanism. International Journal of Cell Science & Molecular Biology. 2019 Aug; 6(3): 57-62.
3. Thanuja B, Parimalavalli R. Role of Black Rice in Health and Diseases. International Journal of Health Sciences & Research. 2018; 8(2), 241-248.
4. Chandramouli B, Latha MM, Narendra K, Mallikarjuna K. Phytochemical and antimicrobial investigations of methanolic seed extract of black rice (*Oryza sativa* L.) mentioned in an ancient palm leaf manuscript (Talapatra). World Journal of Pharmaceutical Research. 2018; 7(3): 598-616.
5. Packman CH. The Clinical Pictures of Autoimmune Hemolytic Anemia. Transfusion Medicine and Hemotherapy. 2015; 42(5):317-324
6. Saha S. Black rice: the new age super food (an extensive review). American International Journal of Research in Formal, Applied & Natural Sciences. 2016 Nov; 16(1): 51-55.
7. Agrawal A, Patel A, Kala D, Patidar J, Agrawal L. Black Rice the New black gold of India. Food and Agriculture Spectrum Journal. 2021; 2(3): 1-4.
8. Kumar N, Murali RD. Black Rice: A Novel Ingredient in Food Processing. Journal of Nutrition & Food Sciences. 2020; 10(2): 771-777.
9. Peng B, Lou AQ, Luo XD, Wang R, Tu S, Xue ZY et al. The Nutritional Value and Application of Black Rice-A Review. Journal of Biotechnology Research. 2021; 7(4): 63-72.
10. Kumar R, Srivastava P, Kumari D, Fakhr H, Sridhara S, Arora N, Gaur SN, Singh BP. Rice (*Oryza sativa*) allergy in rhinitis and asthma patients: a clinico-immunological study. Immunobiology. 2007; 212(2):141-147.
11. Gani A, Wani SM, Masoodi FA, Gousia H. Whole - grain cereal bioactive compounds and their health benefits. Journal of Food J Processing & Technology. 2012; 3(3): 1 -10.
12. Sheth PA, Pawar AT, Mote CS, More C. Antianemic activity of polyherbal formulation, Raktavardhak Kadha, against phenylhydrazine-induced anemia in rats. Journal of Ayurveda and Integrative Medicine. 2021; 12(2):340-345
13. Tonchaiyaphum P, Arpornchayanon W, Khonsung P, Chiranthanut N, Pitchakarn P, Kunanusorn P. Gastroprotective Activities of Ethanol Extract of Black Rice Bran (*Oryza sativa* L.) in Rats. Molecules 2021; 26: 3812.
14. Desai T, Das SK. Effect of methanolic extract of Cissampelos pareira on Acetic acid and Dextran Sodium Sulphate (DSS) induced inflammatory bowel disease (IBD) in albino rats and mice. International Journal of Pharmacological Research. 2017; 7(10): 196-202.
15. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy and Bioallied Sciences. 2020; 12(1):1-10.
16. Khandelwal KR, Practical Pharmacognosy, 12th Edn, Nirali prakashan; Ahmedabad, India. 2004. p. 151-153
17. Ayu AC, Ida M, Moelyono M, Fakhriati SG. Total Anthocyanin Content and Identification of Anthocyanidin from Plectranthus scutellarioides (L.) R. Br Leaves. Research Journal of Chemistry and Environment. 2018; 22(1): 11-17.
18. Shehata WA, Akhtar MS, Alam T. Extraction and Estimation of Anthocyanin Content and Antioxidant Activity of Some Common Fruits. Trends in Applied Sciences Research. 2020; 15: 179-186.
19. Beshel,NF, Eyo HE, Beshel JA. Ferrous Sulphate Improves Electrolyte Levels in Phenylhydrazine Induced Hemolytic Anaemia in Wistar Rats. Scholars International Journal of Anatomy and Physiology. 2019; 2(5): 209-214.
20. Punvittayagul C, Sringarm K, Chaiyasut C, Wongpoomchai R. Mutagenicity and antimutagenicity of hydrophilic and lipophilic extracts of Thai northern purple rice. Asian Pacific Organization for Cancer Prevention. 2014; 15(21):9517-9522.
21. Sheth PA, Pawar AT, Mote CS, More C. Antianemic activity of polyherbal formulation, Raktavardhak Kadha, against phenylhydrazine-induced anemia in rats. Journal of Ayurveda and integrative medicine. 2021; 12(2):340-5.
22. Ashish, Anjali, Dixit PK, Nagarajan K. Pharmacological and Biochemical evaluation of Anti-Arthritic activity of Justicia gendarussa extract In FCA induced arthritis in wistar rats. induced arthritis in Wistar rats. International Journal of Research in Pharmaceutical Sciences. 2021; 12(3):1699-1708.
23. Soundarya N, Suganthi P. A review on anaemia – types, causes, symptoms and their treatments. Journal of science and technology investigation. 2016; 1(1): 11-17.
24. Adebayo MA, Enitan SS, Owonikoko WM, Igogo E, Ajeigbe KO. Haematinic Properties of Methanolic Stem Bark and Fruit Extracts of Ficus Sur in Rats Pre-exposed to Phenylhydrazine induced Haemolytic Anaemia. African Journal of Biomedical Research. 2017; 20(1): 85- 92.
25. Ameh SS, Aladi OF. Effect of Ethanol Extract of Moringa Oleifera Leaves in Protecting Anaemia Induced in Rats by Aluminium Chloride. Journal of Biotechnology and Biochemistry. 2018; 4(6):34-52.
26. Hu C, Zawistowski J, Ling W, Kitts DD. Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. Journal of agricultural and food chemistry. 2003; 51(18):5271-5277.
27. Pascual-Teresa SD, Sanchez-Ballesta MT. Anthocyanins: from plant to health. Phytochemistry Reviews. 2008; 7:281–299.
28. Andersen ØM. Anthocyanins. In: Encyclopedia of life sciences. John Wiley & Sons, Ltd., Chichester. 2001.
29. Zhang MW, Zhang RF, Zhang FX, Liu RH. Phenolic Profiles and Antioxidant Activity of Black Rice Bran of Different Commercially Available Varieties. Journal of agricultural and food chemistry. 2010; 58(13): 7580–7587.
30. Gheith I, El-Mahmoudy A. Laboratory evidence for the hematopoietic potential of Beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. Brazilian journal of medical and biological research. 2018; 51:1–3.
31. Harrison JH, Jollow DJ. Role of aniline metabolites in aniline-induced hemolytic anemia. The Journal of pharmacology and experimental therapeutics. 1986; 238(3):1045-1054.
32. Itano HA, Hirota K, Hosokawa K. Mechanism of induction of haemolytic anaemia by phenyl- hydrazine. Nature. 1975; 256:665–667.
33. Elaby S, Ali J. The anti-anemic effect of dried beet green in phenylhydrazine treated rats. Archives of Pharmaceutical Sciences Ain Shams University. 2018; 2:54–69.
34. Adebayo MA, Enitan SS, Owonikoko WM, Igogo E, Ko A. Haematinic properties of methanolic stem bark and fruit extracts of Ficus sur in rats pre-exposed to phenylhydrazine-induced hemolytic anaemia. African Journal of Biomedical Research. 2017; 20:85–92.

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