Research Article

Journal Of Clinical & Medical Images Case Reports

Open Access, Volume - 4

Evaluation of the effect of brown rice and white rice on protein-energy malnutrition rat models

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Received Date : December 16, 2023
Accepted Date : January 02, 2024
Published Date : January 09, 2024
Archived : www.jcmimagescasereports.org

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Abstract

Aging is a complicated process characterized by altered physiological and functional changes in the organism. This process is influenced by genetic, environmental, metabolic, dietary and other multiple factors. As the number of aged people has been increasing at an larming rate, search for anti-aging agents have reached momentum. Among them, natural agents seem most pertinent. In the present experiment, the usage of brown rice ethanolic extract on aged male Long Evans rats had been assessed through anti-oxidative potentiality, proximate composition and aged related behavioral modulation of the experimentally induced aged model rats. Compared with the mostly used white rice, the brown rice had been found containing higher amount of protein, fiber, fat, ash and antioxidants such as polyphenol, flavonoid, β-carotene, lycopene that could provide safeguard against oxidative stress induced aging processes. Also, age-onset anxiety could be ameliorated through brown rice intake. Thus, brown rice could be a natural remedy in slowing aging processes.

Keywords: Ageing, anti-oxidants, brown rice, reactive oxidant species, white rice.

Introduction

Ageing is a progressive accumulation of biological changes that result in a person's functional degeneration of organ and tissue [1, 2]. Aging can cause different type of disease such as cardiovascular, neurodegenerative, cancer, musculoskeletal, diabetes resulting from gene mutations, cellular senescence, nutritional deregulation, mitochondrial dysfunction, loss of proteostasis [3]. One type of aldohexose or monosaccharide D-galactose (D-gal) found in yogurt, butter, beets, milk, cheese, plums, honey as well as other food. The maximum daily requirement of D-gal for healthy individual is 50g, with the majority of it being digested and eliminated within 8 hours of eating from the body [1]. In general, D-gal is transformed into glucose in the presence of galactokinase and galactose-1-phosphate uridyltransferase enzyme [3]. Increased dose of D-gal can interrupt the normal body functioning which in turn result in conversion of D-gal into hydroperoxide and aldose leading to the formation of reactive oxygen species (ROS). Furthermore D-gal can form advanced glycation end product (AGE) by interacting with amino group of peptide and protein through non- enzymatic glycation [3, 4]. Various studies proposed that elevated level of ROS and AGE are related to biological aging and associated disease involving mitochondrial dysfunction, arthritis, renal failure, oxidative damage, apoptosis, atherosclerosis and neurological disorders [1, 5]. In general, there are two types of aging models. Naturally aging models and accelerated aging models. Natural aging models take a long time and cost a lot of money, accelerated aging models are chosen because they are easier to use, have a shorter study period, and have a greater animal survival rate during the experiment. Accelerated models can be created in several ways such as giving radiation, removal of thymus and D-galactose treatment. Generally D-galactose induced model is popular because it is simple to implement, has less side effects, and has a higher survival rate during the experiment [6]. Thus, in this experiment, we had prepared "aged model rats" through infusion of D-galactose. Search for effective anti-aging agents have received epoch-making interest globally. In this aspect, natural components have got momentum. There are various natural therapies for slowing down the aging process [7]. Compared to pharmaceutical medications, these natural anti-aging therapies are less expensive and safer [7]. Natural cures are an excellent way to slow down the aging process. In general, natural anti-aging therapies are made up of a variety of natural substances such as antioxidants, anti-inflammatory

Citation: Shahdat Hossain. Evaluation of the effect of brown rice and white rice on protein-energy malnutrition rat models. J Clin Med Img Case Rep. 2024; 4(1): 1606.

compounds, vitamins, and a variety of other things that can provide the greatest anti-aging treatment [8]. In Bangladesh and other Asian nations, rice is an essential and long-standing staple meal [9]. India used to eat hand-pounded rice with bran layer and germ as part of their diet [10]. The development of sophisticated milling technology, which boosted the output of head rice, has essentially replaced hand-pounded rice with milled (polished) rice. In the milling process, the bran coating of brown rice is removed, resulting in polished white rice (refined grain), which has only the starchy kernel and no bran or germ components [11]. In recent years, demands for brown rice are increasing gradually because of their enriched amount of essential component and thus they are considered more healthful [12, 13]. In this study we used BRRI [28] paddy (one of the most popular and highly consumed rice Bangladesh), in our laboratory prepared rice powder from them, analyze proximate composition, antioxidant potential and fed the rice to experimentally induced accelerated aging model rats, against normal rice, to examine its anti- aging effects. This experiment had been designed to compare and contrast the proximate composition and anti-oxidant potentiality of brown rice and conventionally utilized normal rice along with their effect on ageing realted behavioral performance through elevated plus maze (EPM) test.

Materials and methods

Collection and processing of rice samples: Both brown and white rice samples had been collected locally. Rice samples had been sun dried before being ground and pulverized, then put in enclosed Zipper bags and kept at -20°C until use. The brown and white rice powder was placed in dried bottles with the three volume of ethanol and shaken overnight by using a rotary shaker (Lab Companion SI- 300, Midwest Scientific, USA). After shaking, the liquid was collected by filtering with filter paper. A rotating evaporator at 50° C was used to concentrate the ethanol extract under decreased pressure (JP Selecta, RS 3000-V, Spain). The concentrated extract was further dried by placing it in an incubator at 37°. Dried ethanol-free extract of rice was dissolved in filtered, distilled water and sonicated within a bath sonicator (Wiseclean, Germany) to make a homogeneous solution of 10mg/1ml. This solution was then stored at -20°C and used throughout in vitro and in vivo experiments.

In vitro Experiment

Measurement of Total Polyphenol Content of Brown and White Rice Extract: The total phenolic content (TPC) of brown and white rice was determined using a colorimetric assay based on the technique described by Slinkard and Singleton with some modifications [14]. The content of total polyphenol in rice samples was determined as gallic acid equivalents using a standard curve.

Measurement of Flavonoid Content of Brown and White Rice Extract: The total flavonoid content of rice was measured using an aluminum chloride colorimetric test [15]. The content of total flavonoid in rice samples was determined as quercetin equivalents using a standard curve.

In vitro Antioxidative Activity of rice: Antioxidant activities of rice was measured by the following 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay using the method of Hasan et al. [16]. In order to calculate the percentage of DPPH radical inhibition, the following equation was used:
Percentage of DPPH radical inhibition
=(Absorbance of control-Absorbance of test sample)/(Absorbance of control) ×100

Content of β- carotene and Lycopene: Content of β-carotene and lycopene were calculated using the method of Nagata and Yamashita (1992) [17]. To determine the quantity of beta-carotene in the sample, the following formula was applied:
B-carotene (mg/100 ml) = 0.216 × A663 - 1.22 × A645 – 0.304 × A505 +0.452
A453.
Lycopene (mg/100ml) = -0458 × A663+0.204 ×A645+0.372 × A505-0.080×A453.
Finally, the amount of ß-carotene and the amount of Lycopene in rice sample were represented as ug/g.

In vivo Experimental Design

Animals: The experiment had been conducted at the laboratory of behavioral and alternative medicine studies at the department of Biochemistry and Molecular Biology, Jahangirnagar Unviersity, Dhaka, Bangladesh. Ten week old male Long-Evans's rat had been used in this investigation.Prior to treatment, all rats were acclimated to laboratory settings for one week in plastic cages with hard wood chips as bedding in a 12-hour dark-light cycle at 25 °C. All the experiments had been conducted following the ethical guidelines formulated by Bangladesh Association for Laboratory Animal Science's ethical criteria. Twenty-one rats were chosen at random and split into three groups. There are seven rats in each group, three males and four females. Group 1 is the control group, Group 2 is the brown rice group, and Group 3 is the white rice group. D-galactose (100mg/kg body weight/day) was administered subcutaneously to groups 2 and 3, whereas the control group received 0.9 percent saline. It was administered during the trial period. D-galactose (99 percent pure) was bought from Sigma-Aldrich. The following is a list of the diet components: 1. D-galactose was given subcutaneously after being dissolved in 0.9 percent saline. The weight of the rats was taken on a regular basis, and the bedding was changed at the same time. The rats were handled on a frequent basis to lessen stress and to ensure that their suffering was minimized. The duration of the experiment was 30 days and then, the rats had been sacrificed, blood, brain, liver and other organs collected, preserved at -80 °C and further experiments conducted.

Table 1: *Percent proximate composition of brown and white rice.*

Parameter	Unit	Amount in BR	Amount in WR
Carbohydrate	%	71.3	77.4
Dietary fiber	%	.8	.4
Protein	%	7.8	6.4
Total fat	%	1.1	.67
Ash	%	1.3	.5

Here, BR= Brown rice, WR= white rice.

Table 2: *The estimated mineral content in rice sample.*

Parameter	Amount in mg/ 100 gm dry powder	
	BR	WR
Na	87 ± 2.3	120 ± .56
Mg	370 ± 4	106 ± 3
K	3097 ± 7.3	2650 ± 2.5
Fe	1435 ± 6.4	1390 ± 1.3

Here, BR= Brown rice, WR= white rice.

Table 3: *Measurement of Antioxidant phytochemicals.*

Antioxidant phytochemicals	Content ug/mg of rice extract	
	BR	WR
Total polyphenol (GAE)	75.58 ± .60	37.65 ± 1.2
Total Flavonoid (QE)	62.03 ± 1.39	23.82 ± 1
β-carotene	0.050 ± 0.003	0.013776 ± 0.007
Lycopene	0.114 ± .006	0.0174 ± .002

Here, BR= Brown rice, WR= white rice.

Table 4: *Protein content of several sample of model rats.*

	NC (mg/mL)	BR+ D-gal (mg/mL)	WR+D-gal (mg/mL)
Plasma protein	8.77 ± .24	9 ± .11	8.44 ± .22
Liver WH protein	5.41 ± .28	6.06 ± .28	5.36 ± .25
Liver CF protein	8.07 ± .24	9.01 ± .21	7.76 ± .44
Hippocampal WH protein	5.55 ± .20	5.54 ± .27	5.06 ± .23

Here, WH= whole homogenate, CF= cytosolic fraction, NC= control, BR= brown rice, D-gal= D-galactose.

Procedure of Rat Dissection: All of the rats were sacrificed after the testing period (30 days), and plasma and organs were taken for biochemical examination. Ketamine injections (100 mg/kg-BW) were administered to the overnight fasting rats after 24 hours of the last treatment (Figure 12) and subsequently slaughtered.

Collection of Blood and Preparation of Serum: With a heparinized syringe, blood was drawn from the inferior vena cava

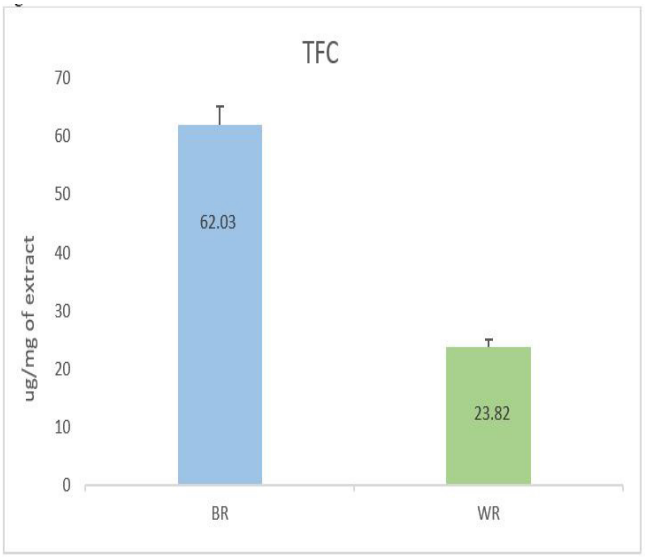

Figure 1: *Total flavonoid content (TFC) of Brown Rice and White Rice. Results are mean ± SEM. One-way ANOVA was used to analyze the data. Here, BR= brown rice, WR= white rice. Total polyphenol contents of the BR extract were 75.58 ug/mg and the WR extract were 37 ug/mg of the extract, against catechin equivalent (Figure 2).*

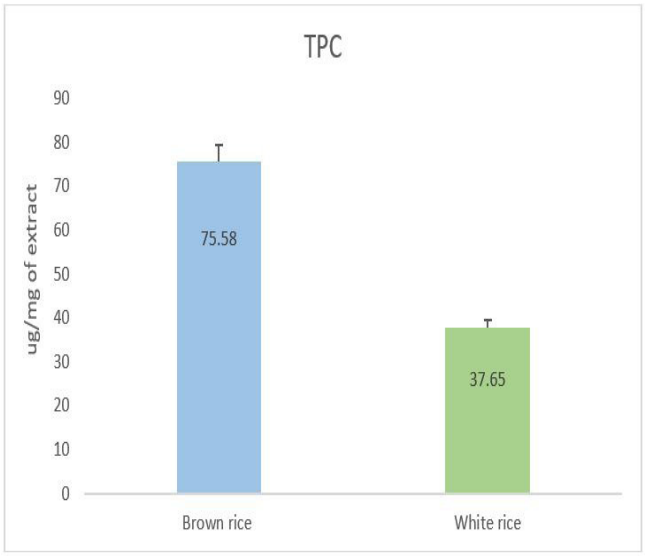

Figure 2: *Total polyphenol content (TFC) of Brown Rice and White Rice. Results are mean ± SEM. One-way ANOVA was used to analyze the data.*

right away. Half of the blood was utilized to obtain serum, while the other half was used to prepare RBCs. Half of the blood centrifuged 10 minutes at 1000 x g to separate serum from blood (Digital centrifuge, SCILAB Instruments Co. Ltd, Taiwan). And the other half mixed with Locke buffer solution and centrifuged 10 minutes at 300 x g. After discarding the supernatant, the RBCs were washed three times in Locke buffer solution. Each wash removed the buffy coat as well as a piece of the RBCs' upper coating. The remaining RBCs were tested for hemolysis right away. Sysmex XS-1000i was used to count RBCs. The serum and RBC samples were stored at -20 C.

Collection of Whole Liver and Preparation of Liver Homogenate: Following blood collection, each rat's entire liver was dissected, cleaned, and properly perfused using saline. Using

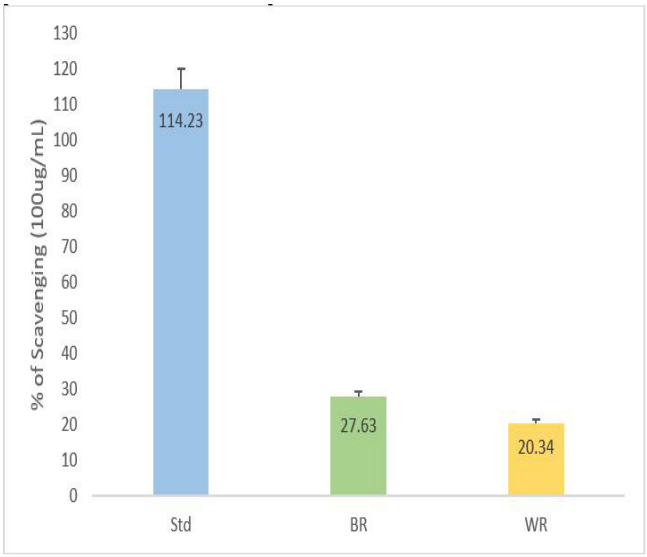

Figure 3: *Antioxidative activity of brown rice and white rice. Here, std= standard, BR= brown rice, WR= white rice.*

a Polytron tissue homogenizer, the liver was homogenized with phosphate buffer (pH 7.4) containing 1 percent phenylmethylsulphonyl fluoride (PMSF) (Kinematica, USA). The homogenates were then centrifuged at 1000 xg for 10 min to separate fragmented tissues and debris, and the resulting homogenates were stored at -20 C until assayed.

Brain Collection and Brain Tissue Preparation for Biochemical Analysis: After the blood was collected, the brains were perfused with saline (ice cold) via the left ventricle of the heart. The brain, which was supposed to be stored in formalin, was not immediately perfused. The brain was gently uncovered from the skull after the head was detached from the body (as shown in figure). Formalin was used to preserve the brain that had been harvested. After separating the brain part (hippocampus, frontal cortex, parietal cortex, brain stem), a glass homogenizer used for making whole homogenate. Phosphate buffer containing PMSF (protease inhibitor) were used for making this homogenate. The entire homogenates were centrifuged at 10000 x g for 1 hour to collect the supernatants. The samples were exposed to the assays right away and kept at -80 °C.

Lipid Peroxidation (LPO) Assay: The thiobarbituric acid reactive substances (TBARS) test of Ohkawa et al (1958) [18] had been used to determine the level of lipid peroxidation in the whole homogenate and plasma.

Total protein assay: Following the method of Lowry et al (1951) protein level in the plasma and tissues were measured [19].

Ageing related behavioral assessments

Elevated plus maze test: The anxiety like behavior was measured by using elevated plus maze [20]. The maze has two open arm and two closed arm and they shared a center platform. Each animal was placed on the middle of the platform fronting the open arm and given three minutes for their free movement. This movement was recorded by using a video recorder. 10% ethanol was used to clean the device after each session. Time spent and arm entry was calculated. The time spent in open arms was used to measure anxiety-like behavior and closed arm entry express locomotor activity.

Statistical analyses: All the experiments had been conducted in triplicate. Results had been expressed as mean±SEM. One way analysis of variance was used to analyze the data using statistical program for the social sciences (SPSS) version 20, USA.

Results

Proximate Composition: The amount of protein, carbohydrate, total fats, fiber, ash and moisture content are listed below (table 1). The results indicated that brown rice had more protein, fat, fiber, ash, and moisture than white rice. But the carbohydrate amount is higher in white rice than brown rice.

Minerals: Minerals are necessary that must be consumed in modest amounts to keep us healthy. Minerals are not produced by the body. Minerals must be received from the food and liquid we consume and drink in order to satisfy our daily demands. We calculated four significant minerals (table 2) in our sample in our study: Na, Mg, K, and Iron. The results indicated that normal rice had a greater Na level and a slightly higher iron content than germinated rice. However, the Mg and K content of germinated rice is greater than that of regular rice.

Antioxidant Phytochemicals: Total polyphenols, β-carotene, total flavonoids and lycopene were quantified in brown and white rice powders, respectively. According to the findings, brown rice has higher levels of polyphenols, flavonoids, β -carotene, and lycopene. As a result, the brow n rice had a significant quantity of antioxidant phytochemicals, as seen in the table (table 3).

Protein Estimation of Rat Sample: The amount of total protein in plasma, liver and hippocampus are listed below (table 4). The results indicated that BR+ D-gal group had more protein than WR+D-gal group.

In vitro Antioxidative Properties of Brown Rice and White Rice: Antioxidative phytoconstituents of Brown and White rice, including total polyphenols and flavonoid contents were determined in triplicate. Total flavonoid contents of the BR extract were 62.03 ug/mg and the WR extract were 23.82 ug/mg of the extract against gallic acid equivalent figure 1.

Antioxidative Activity of Brown Rice in Vitro (DPPH radical scavenging effect): Antioxidative potential of brown rice and white rice powder was evaluated with the assay of DPPH-free radical scavenging activity in vitro (Figure 3). We observed that brown rice and white rice extracts could diminish the purple color of DPPH and eliminate the absorption peak at 517 nm, suggesting DPPH-free radical scavenging activity, whereas brown rice extract had a greater activity than regular rice. The results indicate that the amount of DPPH-free radical scaveng-

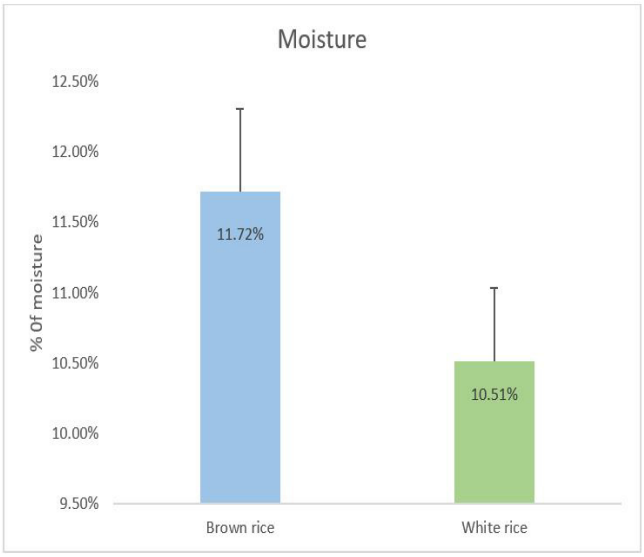

Figure 4: *The moisture contents of the BR and WR.*

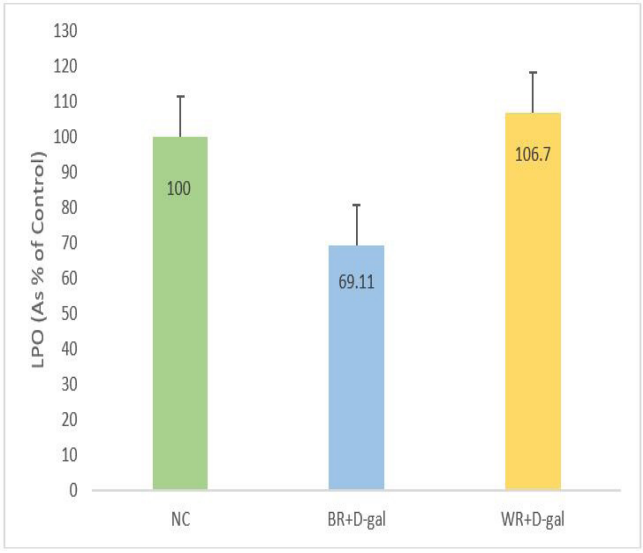

Figure 5: *Effect of dietary supplement of brown rice on the D-galactose induce hepatic LPO levels in VIVO. Results are mean ± SEM. One-way ANOVA was used to analyze the data. Here, LPO=Lipid peroxidation, NC=control, BR= brown rice, D-gal = D galactose, WR=White rice.*

Table 5: *Effects of brown rice on food intake and body weight.*

Parameter	NC	BR	WR
Food intake (g/day)	19.7 ± 1.2	17.2 ± 1.8	18.9 ± 2.1
Initial weight (g)	301.28 ± 8.75	320.14 ± 24.36	319.71 ± 24.98
Final weight (g)	344.71 ± 15.63	339.85 ± 30.78	351.85 ± 26.36
Weight gain (g)	43.42 ± 8.10	26.83 ± 6.18	32.14 ± 3.59

ing activity increased when the quantity of brown rice extract was increased. DPPH-free radical scavenging activity is expressed as percentage (%) of scavenging. These results suggest that germinated rice which contains antioxidant components, possesses considerable antioxidative potential.

Moisture content: The moisture content of the BR extract was 11.72% and that of the WR extract was 10.51% (figure 4).

Effects of Brown rice on food intake and body weight: Dietary supplement of brown rice and white rice had significant effect on food and fluid intake in either of the rat groups. Feeding of

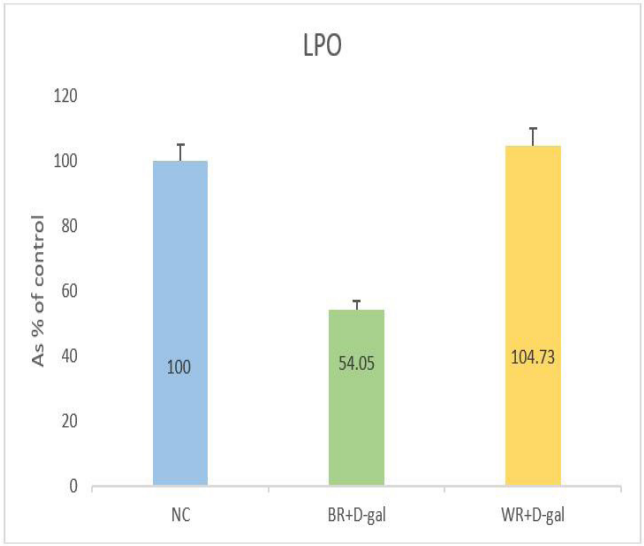

Figure 6: *Effect of dietary supplement of brown rice and white rice on the D-galactose induce hippocampal LPO levels in VIVO. Results are mean ± SEM. One-way ANOVA was used to analyze the data. Here, LPO=Lipid peroxidation. NC=control, BR= brown rice, D-gal = D galactose, WR=White rice.*

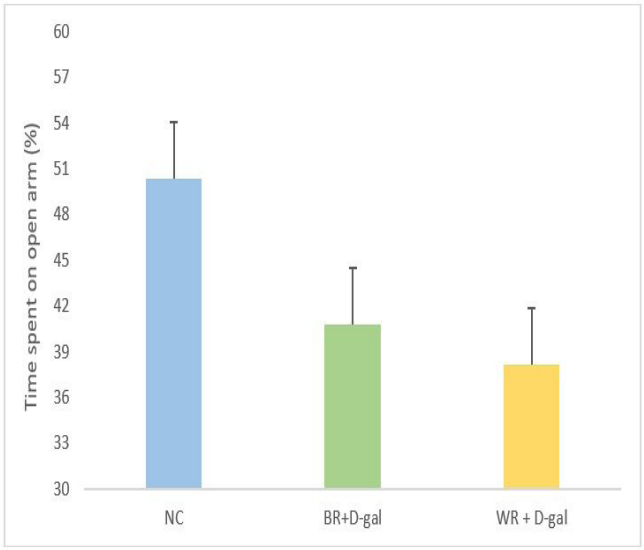

Figure 7: *Effect of Brown rice and White rice on the anxiety-like behavior of the D-galactose induced accelerated aging model rats. (A) Total time spent in the Open Arm; (B) Entry to the closed arm. Results are mean ± SEM, One-way ANOVA was used to analyze the data. NC=control, BR+D-gal=D-galactose induce brown rice fed rats, WR+D-gal= D-galactose induce White rice fed rats. There was no statistical significance in the number of entries on closed arms between the groups. (Figure 18).*

white rice significantly increased the body weight than brown rice. Brown rice significantly decreased the body weight as compared to the white rice. Results also showed that the weight reduction by brown rice is much greater than white rice fed rats. Thus, brown rice could be an effective natural agent to inhibit weight gain (Table 5).

Effect of Dietary Supplement of Brown Rice on the Hepatic LPO Levels: Lipid peroxide (LPO) level in the hepatic tissues was significantly increased in WR+D-gal rats, as compared to

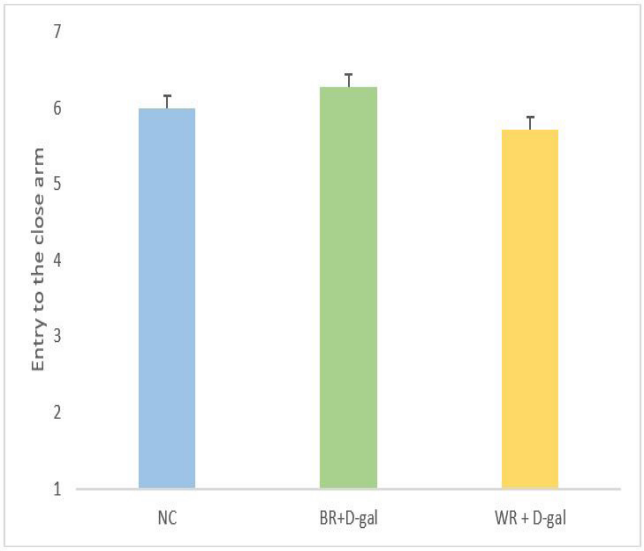

Figure 8: *Effect of Brown rice and White rice on the anxiety-like behavior of the D-galactose induced accelerated aging model rats (B) Entry to the closed arm. Results are mean ± SEM. One-way ANOVA was used to analyze the data NC=control, BR+D-gal=D-galactose induce brown rice fed rats, WR+D-gal= D-galactose induce White rice fed rats.*

those of the control (NC) rats in vivo (Figure: 5). Dietary supplement of brown rice resulted in a significant (P ≤0.05) inhibition of D-galactose induced hepatic LPO level in the BR+D-gal group rats than that of the WR+D-gal rats. The LPO level in rats fed with normal food was also lower than that of the WR+D-gal rats but not significantly. These results confirmed that BR possesses considerable anti-oxidative potential that could reduce lipid peroxidation in liver tissues even in D-galactose induce aging model states.

Effect of Dietary Supplement of Brown Rice on the Hippocampal LPO Levels: Lipid peroxide (LPO) level in the brain hippocampus was significantly increased in WR+D-gal rats, as compared to those of the control (NC) rats in vivo (Figure: 6). Dietary supplement of brown rice resulted in a significant (P ≤ 0.05) inhibition of D-galactose induced hippocampal LPO level in the BR+D-gal group rats than that of the WR+D-gal rats. The LPO level in rats fed with normal food was also lower than that of the WR+D-gal rats but not significantly. These results confirmed that BR possesses considerable anti-oxidative potential that could reduce lipid peroxidation in hippocampal tissues even in D-galactose induce aging model states.

Aging related behavioral performance of the model rats in Elevated Plus Maze: The elevated plus maze was used to assess for anxiety-like behavior. Data showed that WR+D-gal group affected anxiety- like behavior. Specifically, regarding the percentage of time spent on the open arms, WR+D-gal induce animals spent less time in the open arms than the Control group and BR+D-gal induce group animals (Figure 7).

Discussion

Rice is the main food in Bangladesh and many other countries. Most of the people consumed rice as their principal food. In this reason researchers try to evaluate the beneficial use of rice. They try to discover the proper way to consume rice and the health beneficial effect. Rice's antioxidant phytonutrients may thus have an antioxidative impact in the livers of experimental rats. Consuming brown rice may be helpful to the liver and cardiovascular system, as well as providing antioxidative protection in the livers of aged model rats. Many bioactive chemicals found in brown rice have been shown to decrease rats' lipid profiles by preventing cholesterol absorption in the intestines and consequently raising fecal cholesterol excretion. Ferulic acid is an antioxidant that aids in the absorption and breakdown of cholesterol in the liver. The bioactive chemicals may also be responsible for the reduction in total fat in the rat groups. The goal of this study was to discover the health benefits of brown rice and to assess its anti-aging potential. We chose brown rice over white rice because it includes more antioxidants [21], protein, and fiber. The capacity of the antioxidant to scavenge DPPH-free radical activity (Table 1, Figure 1, 2) clearly demonstrated its antioxidant activities. Brown rice has more antioxidant polyphenols, flavonoids, β-carotene, and lycopene than white rice (Table 1). Table 1 shows the proximate composition of brown and white rice in this investigation. The results indicated that brown rice had more protein, dietary fibers, ash and total fat content than white rice, whereas white rice had more carbohydrate content (Table 1). Grain proximate and nutrient analysis is critical in determining their nutritional importance. Because different grains are utilized as food, examining their nutritional importance can assist to understand the value of these grains. White rice has more carbohydrates than brown rice (Table 1). Because the milling procedure removes the exterior section. In addition, the ash content reduces. Higher accessible carbs and lower dietary fiber (Table 1) content, as well as their intake, result in a high glycemic response, increased insulin demand, and an increased risk of type 2 diabetes [22]. Furthermore, studies show that substituting nutritious grains, such as brown rice, for white rice lowers the incidence of type 2 diabetes. White rice has a high glycemic index due to its high carbohydrate content and low fiber content. In general, the positive effect of dietary fiber from whole grains may be mediated by a shorter transit time and perhaps a lower amount of carbohydrate absorbed, resulting in a lower insulin demand. The brown has higher moisture content (Figure 4). The moisture contents of the BR powder were 11.72% and the WR powder were 10.51% (Figure 4). White rice significantly increased body weight as compared to brown rice (Table 5). Our findings are compatible with those of Saleh et al (2019) [23]. The results also indicated that rats fed brown rice lost much more weight than rats fed white rice. As a result, brown rice may be an effective natural agent for preventing weight gain (Figure 4). We prepared an ageing model by inducing D-galactose and feeding brown rice to see if the brown rice slows down the accelerated aging. D-galactose treatment results in the generation of reactive oxygen species (ROS). Elevated ROS levels can lead to oxidative damage, inflammatory reaction, mitochondrial malfunction, and cell death. Treatment of D-galactose and its capacity to create degenerative changes in many tissues and organs. Our approach worked well in producing aged model rats. The

model rats demonstrated considerable anxious-like behavior, as evidenced by a reduction in the proportion of time spent in the open arm. In the elevated plus maze, the WR+ D-gal group showed higher anxiety like behavior (Figure 7,8). Comparing the WR+ D-gal group to the BR+ D-gal group, the WR+ D-gal group spent much more time in the closed arm. The BR+ D-gal group spent more time in the open arm. In this case our prediction was correct. Brown rice contains anti-ageing properties. Brown rice had a much stronger anti-lipid peroxidation impact than regular rice in the hepatic and hippocampus tissues of ageing model mice (Figure 5, 6). These findings support the notion that consuming brown rice may have a major hepatoprotective and neuroprotective effect. Several findings imply that oxidative stress is the main cause of hepatic fibrosis. For example, oxidative stress, which contributes to lipid peroxidation, is one of the key contributors in the development and progression of non-alcoholic steatohepatitis and liver cancer. Thus, brown rice's reduction of LPO levels in the liver and hippocampus tissues of aged model rats supports a beneficial impact.

Conclusion

In conclusion, our findings imply that brown rice has anti-aging properties. In addition, in ageing model rats, the BR may provide anti-inflammatory and anti-oxidative protection. These positive benefits were followed by a decrease in body weight growth and an increase in antioxidant content, such as polyphenols, flavonoids, β-carotenes, and lycopene. However, further study is required, particularly with human volunteers suffering from age-related illness.

Acknowledgement: Authors gratefully thank Bangladesh University Grants Committee and Jahangirnagar University for providing the grant-in-aid to conduct this study.

Conflict of interest: Authors declare no conflict of interest.

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