



# Anthocyanin-rich extract from black rice (*Oryza sativa*) ameliorates dyslipidemia and insulin resistance induced by a high-fat diet and streptozotocin in rats

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## Abstract

In Saudi Arabia, rice is a staple food and the most consumed type is white rice. Black rice (*Oryza sativa* L) is less consumed although valued for its high contents of carbohydrates, proteins, minerals, fats, fiber, and anthocyanin, which have high health benefits. In the present study, anthocyanin and other active ingredients were extracted from black rice. In an experimental trial, Rats given a high-fat diet (obese rats) orally administered the anthocyanin extract, whereas another group of rats developed diabetes mellitus and a high-fat diet (HFD) due to streptozotocin (STZ) induction. In diabetic rats, the purpose of the study was to elucidate the preventive role of black rice anthocyanin-rich extract (BRAE) against hyperlipidemia and lipid peroxidation, insulin resistance, and liver function. Our results demonstrated that BRAE significantly affected the lipid profile of obese rats. BRAE lowered the LDL and increased HDL levels as well as restored TC and TG to basal levels in both the HFD and STZ groups. This indicates an anti-dyslipidemic effect. The findings demonstrated a substantial reduction in body weight growth, insulin resistance index, total cholesterol, and triglyceride in the BRAE group relative to the HFD group. In obese rats given a high-fat diet, BRAE reduced insulin resistance but was unable to lower blood glucose. Thus, understanding black rice's phytochemical makeup and antioxidant capacity may point to black rice as a viable option for improving health. may suggest black rice as a potential candidate to promote health. The findings of this study indicate that dietary supplements containing BRAE protect obesity-associated hyperlipidemia, insulin resistance, and hepatic steatosis by altering lipid metabolism.

**Keywords:** Black rice, antioxidants, anthocyanin, diabetes, dyslipidemia, obesity

**Full length article** \*Corresponding Author, e-mail: [waheeba2003@gmail.com](mailto:waheeba2003@gmail.com)

## 1. Introduction

In Saudi Arabia, individuals from all socioeconomic groups eat rice (*Oryza sativa* L) as their main diet. However, white rice is widely consumed, brown and black varieties also find acceptance as being healthy foods. Colored rice including black rice is valued for its high

micronutrient contents including anthocyanin that gives them characteristic dark color. Among different colored rice, Black rice has the highest anthocyanin concentration, at 327.60 mg/100 g [1]. Black rice (purple) is gaining popularity due to its ability to produce Useful foods and its cultivars' genetic

diversity, which produces differences in pigmentation, nutritional content, and phytochemical characteristics. Black rice bran is rich in lipid tools and the hydrophilic chemical anthocyanin; it is a member of the same species as red and white rice (*Oryza Sativa* L. Zhang et al. [2] discovered that the anthocyanin extract from black rice is effective at emulsifying cholesterol. According to the same study, tococls block fatty acid oxidation more effectively than anthocyanins. It was stated in numerous papers that rice hulls, bran, and oils from black rice seeds might be utilized to create useful food emulsifying [3]. Additionally, black rice could be processed to make consumer-friendly products including tortillas, noodles, spaghetti, ice cream, flakes, and frankfurters. Numerous beneficial compounds and nutrient-rich components can be found in black rice [4,5]. Various anthocyanin-rich cyanidin-3-O-glucoside and peonidin-3-O-glucoside forms are among these chemicals., as well as five flavonol glycosides. According to certain research, certain components of black rice may help prevent diseases like diabetes, cancer, infections, and heart disease [5]. The existence of bioactive chemicals in colored rice variants has led to reports of their health advantages in recent years. The phytochemical components of white and black rice bran, including their anthocyanin, phenolic, and flavonoid concentrations, were assessed, as were the extracts' individual phenolic and flavonoid contents [6]. Anthocyanin is synthesized by plants and acts as an antioxidant in plants and humans consuming black rice. Anthocyanins are frequently occurring naturally as water-soluble pigments, especially in fruits and vegetables, They provide flowers, plants, and grains their striking red, purple, and blue hues [7]. Several health benefits are offered by anthocyanins, such as their capacity to scavenge free radicals and their anti-inflammatory, anti-obesity, anti-diabetic, antimicrobial, and antibacterial characteristics [8]. The study by Yao et al. [9] looked into how black rice's anthocyanins affected cholesterol levels in vitro., The 3-glucosides of cyanidin and peonidin found in black rice extract were found to dramatically reduce the absorption of cholesterol in Caco-2 cells. Shimoda and associates [10] have demonstrated that administering a single dosage of 25 mg of anthocyanin-rich black rice extract to healthy human volunteers dramatically reduced the rise in postprandial blood sugar that occurs after the individuals consume 200g of rice balls. Therefore, eating plants that naturally produce anthocyanin can help reduce the chance of developing several chronic conditions. [11]. Moko et al. evaluated the phytochemical makeup and antioxidant potential of brown, red, and black rice bran [12]. They discovered that the most phytochemical-rich black rice bran has significant antioxidant potential. Synthesis of phytochemicals in rice can be affected by climatic factors and as a result, the pharmaceutical value will change. The characterization and composition of the pigmented rice genotypes need further investigation. In Saudi Arabia, the incidence of metabolic lifestyle diseases including obesity and diabetes are on the rise. Replacing a portion of the white rice intake with black rice could be beneficial for the population. Anthocyanins are known to lower glycemic index and glycemic response aiding in the control of obesity and diabetes [13]. Additionally, it has been claimed that they lower the incidence of obesity and cancer, among other major chronic diseases [14, 15, 16]. Some studies have also found anthocyanins to be effective in ameliorating hyperlipidemia

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because of their ability to inhibit lipoprotein oxidation [17, 18]. Diets containing colored rice are healthier than those containing white rice due to the presence of anthocyanins and blunting of postprandial glucose rise [19]. The current study was planned to assess the preventive potential of anthocyanin-rich black rice extract against insulin resistance and dyslipidemia in rats that were brought on by a high-fat diet and streptozotocin, for the reasons outlined above.

## 2. Materials and Methods

### 2.1. Materials

In Buraydah, Saudi Arabia, we bought our black rice (*Oryza sativa* L.) from Tamimi Markets. Teva Pharmaceuticals in Irvine, California is the source from which we purchased streptozotocin. Tetramethoxypropane, 5,5-dithiobis 2-nitrobenzoic acid (DTND), nitro blue tetrazolium (NBT), and TBA, or thiobarbituric acid. We bought assay kits for GSH-Px, TG, TC, HDL, ALT, and AST from Bio-Merieux Laboratory Reagents and Products, France, along with blood. as well as for catalase, SOD, urea, and creatinine.

### 2.2. Animals and Experimental Design

At the Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia, forty male albino Wistar rats weighing  $120 \pm 10$  g were obtained from the Animal House of Pharmacy College. They were housed in polypropylene cages in a pathogen-free environment with a 12-hour light/dark cycle and,  $25 \pm 2^\circ\text{C}$  and 40–60% humidity. AIN-93 recommendation was followed for feeding rats a basal meal during the study period [20]. The animals were provided with a standard fed laboratory pellet diet during the acclimatization period and provided with water ad-libitum.

### 2.3. The high fat, high-cholesterol diet, or HF-Diet

Diets rich in fat and cholesterol were created; these meals consisted of 67 g of the standard diet, 1% pure cholesterol, 31.70 g of animal fat, and 0.30% bile acid [21].

### 2.4. Preparation of anthocyanin-rich extract (BRAE)

Anthocyanin-rich extract (BRAE) was prepared by acidified hydro-ethanol extraction method [22]. In a round-bottomed flask, 500 mL of an acidified hydro-ethanol combination (50% v/v) was combined with 50 g of finely powdered black rice. At  $50^\circ\text{C}$ , the extraction process was maintained in a shaking water bath. After 60 minutes, the extract was vacuum-filtered through Whatman No. 1 filter paper.

### 2.5. Design Experiments

King Abdul-Aziz City for Science and Technology (KACST), Saudi Arabia's National Committee of Bioethics (NCBE), approved the study's conduct. The NCBE's Review Board Number is 10024677, and it expires on August 12, 2024. Five groups of rats were randomly assigned ( $n = 8$ ). The first group received no additional therapy and was used

as the normal control (NC). The second group, designated as a positive control (HFD-C), was put on the HF diet for four weeks to cause obesity. They were then left to maintain the HF diet without receiving any medical attention. (HFD-C). The third group was fed an HF diet mixed with 116 mg of BRAE/100g. The fourth and fifth groups received intraperitoneal (IP) injections of STZ (60 mg /kg weight) to induce type 2 diabetes. The fourth group is fed the HF diet without treatments (STZ-C), while the fifth group is fed an HF diet mixed with 116 mg of BRAE / 100g (STZ-E). Blood glucose was measured daily for the successive seven days by tail puncture with a glucometer (Contour–Bayer, Germany). More than 250 mg/dL of blood glucose was present in the rats used in the investigation. Every rat's body weight was recorded once a week for the duration of the study. Rats were fasted for a single night after the experiment, and after being given diethyl ether anesthesia, blood was extracted via heart puncture. The blood was centrifuged for 10 minutes at 3,000 rpm following a 60-minute coagulation period at room temperature. The serum was collected and stored at -20°C before analysis.

## 2.6. Biochemical Analysis

A variety of chemical studies were performed on black rice anthocyanin-rich extracts (BRAE); the quantity of moisture, ash, protein, fat, and carbs is determined using AOAC recommendations [23]. UV-Vis spectrophotometry was used to measure the rice grains' flavonoids and anthocyanins at  $\lambda = 374$  nm and 535 nm, respectively. To ascertain the amount of flavonoids and anthocyanins, utilize formulas 1 and 2, respectively [24]. Total anthocyanins are measured in mg of cyanidin-3-glucoside equivalents (CGE) per 100 g dry weight sample (mg CGE 100 g DW), while total flavonoids are measured in mg of quercetin equivalents (CE), a plant pigment.

$$\text{Total flavonoids (TF)} = A_{374\text{nm}} \times \text{dilution factor} / 76.6$$

$$\text{Total anthocyanins (TA)} = A_{535\text{nm}} \times \text{dilution factor} / 98.2$$

The total polyphenolic content of black rice anthocyanins rich Using a spectrophotometer (Secomam, France) and the Folin-Ciocalteu assay based on the Škerget et al. [25] assay, extract (BRAE) was quantified. Using the Thomas et al. method [26], blood samples were examined for blood sugar and insulin levels. The Ike et al. method was used to assess the serum levels of total cholesterol, HDL, and triglycerides. [27] The Levey et al. methodology was used to calculate the liver enzymes (AST and ALP). [28]. Lipid peroxides were detected in serum as malondialdehyde (MDA), according to Namiduru et al. [29]. Using Trolox as a standard for the calibration curve and the Fe-EDTA and H<sub>2</sub>O<sub>2</sub> reaction, serum total antioxidant capacity was ascertained. Using the technique of Ike et al. (2016), decreased levels of glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were identified in serum and liver tissues as indicators of oxidative stress.

## 2.7. Insulin Resistance Index Model Assessment (HOMA-IR)

The HOMA-IR index was computed utilizing the formula provided by Morimoto et al. [30]. A result of <1 on the index denotes the best possible insulin sensitivity, >1.9 denotes early insulin resistance, and >2.9 denotes substantial insulin resistance.

$$\text{HOMA-IR} = \text{Fasting insulin } (\mu\text{U L}^{-1}) \times 405 - \text{Fasting glucose mg D}l^{-1}$$

## 2.8. Statistical treatment

To find statistically significant differences between the exposed and control groups, a one-way ANOVA and a two-tailed student t-test (SPSS version 20.0 for Windows) were employed. When comparing the data sets, a p-value of 0.05 or less was deemed statistically significant. Each graph was created using Origin Pro-2018 software, which is available from Origin Lab Corporation, located in Massachusetts, USA.3. 3.

## 3. Results and discussion

Table 1 displays the findings of the proximate analysis. The water contents of all the black rice samples analyzed for this study were within the allowed range of 11.47 to 13.99%. The water content of black rice was 13.8 percent, and that of white rice was the highest at 13.9%. A significant difference ( $p \leq 0.05$ ) was observed in the ash content, which represents the overall mineral content, between black rice (1.36%) and white rice (0.35%). Samples of white and black rice had varying percentages of carbohydrates (77.88% to 73.34%); The two types of rice differed significantly from one another (0.012). White rice had the highest protein level (8.18%), while the sample of black rice had the lowest protein content (5.87%). The total amount of phenolic compounds in colored rice is higher than in white rice. White rice exhibited a total phenolic range of 45.3 mg GAE/100 g dry weight, whereas black rice displayed a range of 201.5 mg GAE/100 g dry weight. Compared to white rice bran, black rice bran had an average total flavonoid concentration that was 6.7 times higher ( $p \leq 0.05$ ). Black rice contains between 1631 and 2112 mg of cyanidin-3-glucoside equivalent per 100 g of dry weight total anthocyanin content. The range of free anthocyanins' percentage contribution to the total was 99.5 to 99.9%. Black rice bran samples contained cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside, with corresponding concentrations ranging from 100.7 to 534.2 mg/100 g of DW. The levels of cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside, as well as the overall phenolic, total flavonoid, and total anthocyanin content, were found to be strongly linked with the total antioxidant activity of black rice bran.

### 3.1. Anthocyanin-rich extract's impact on body weight

The changes in body weight of animals in different groups are presented in Figure 1. It was observed that all the animals gained weight during the study period to a variant extent. The highest gain of 33% was seen in the high-fat diet-fed animals, which were countered by BRAE treatment. A

body weight gain of 13% was recorded in the HFD-E group with BRAE treatment. The reverse was observed in diabetic groups, wherein animals in the STZ-C group gained 4.3%, while the animals in the STZ-E group with BRAE treatment showed a significantly higher gain in body weight (8.4%).

### 3.2. Effect of anthocyanin-rich extract on serum lipid profile

Table 2 shows the current blood levels of total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides for the different groups. As might be expected, feeding high-fat animals streptozotocin caused their TG, TC, and other parameters to significantly (0.001) rise, and LDL levels while decreasing HDL levels. Administration of BRAE restored TC and TG to basal levels, while significantly ( $p \leq 0.05$ ) reduced LDL levels towards basal levels in both HF-diet and STZ experimental groups. The levels of HDL also increased significantly (0.004) with BRAE treatment in experimental groups. In Table 2, The mean values ( $n=8$ ) in the a, b, and c columns—which have different superscript letters—differ at  $p \leq 0.05$ . Total cholesterol (TG), triglycerides, HDL (high-density lipoprotein), LDL (low-density lipoprotein), and STZ-C (diabetic control), STZ-E (diabetic-experimental), NC (normal control), HFD-C (high-fat diet – control), and STZ-E (high-fat diet – experimental).

### 3.3. Anthocyanin-rich extract's impact on oxidative stress indicators and serum transaminases

Table 3 shows the changes in serum transaminases (AST and ALT), MDA, GSH, and total antioxidant capacity (TAC). Compared to control, HF-diet and diabetic rats had a considerable rise (0.010) in serum transaminases; BRAE therapy successfully brought these levels back to baseline. Furthermore, HF-diet and streptozotocin administration significantly (0.002) increased oxidative stress markers as indicated by decreased GSH and increased MDA levels consequently lowering TAC in HFD-C and STZ-C groupings in contrast to the NC group. Anthocyanin-rich black rice extract exhibited remarkable antioxidant activity as these effects were reversed and their levels were recovered to basal levels upon administration of BRAE to both the HFD-E and STZ-E groups.

### 3.4. Effect of anthocyanin-rich extract on oxidative stress markers in the liver

The effect of BRAE on antioxidant status in the liver is summarized in Table 4. As expected, BRAE treatment led to significant improvement in the antioxidant defenses against the pro-oxidant effects of HF diet and streptozotocin. A significant (0.003) elevation in the levels of MDA and reduction in reduced glutathione, glutathione peroxidase, catalase, and superoxide dismutase were found in the HFD-C and STZ-C groups compared to the NC group. Upon treatment with BRAE, these effects were reversed and GSH, GSH-Px, CAT, and SOD were found to reach normal basal levels in HFD-E and STZ-E groups. Similarly, MDA decreased to normal levels by BRAE in both the groups (HFD-E and STZ-E).

### 3.5. Anthocyanin-rich extract's impact on the homeostasis of glucose

Figure 2 shows the impact of BRAE on insulin, blood sugar, and the HOMA-IR index. A significant (0.001) increase in serum glucose compared to normal control was noted in all other groups and BRAE treatment did not show hypoglycemic effect in any of the groups. However, BRAE supplementation significantly (0.003) decreased circulating insulin levels in HFD-E and STZ-E groups compared to HFD-C and STZ-C groups, respectively. Insulin levels fell by 47% in 20% HFD-E and STZ-E groups respectively. It is noteworthy that, both HF diet and STZ increased circulating insulin levels compared to normal control. BRAE did not lower them to basal levels. However, BRAE supplementation decreased the resistance index to 1.21 compared to 2.18 under the category of high-fat diets. However, BRAE supplementation failed to lower the index in the diabetic group indicating the non-efficacious glucose homeostatic action of BRAE. The water content of all the black rice samples evaluated in this study fell between 11.47 and 13.99%, according to the proximate analysis of the results. One of the key factors influencing rice quality during storage is its water content. To prevent insect infestation and ensure long-term preservation, the Indonesian National Standardization Agency, or BSN, mandates that milled rice have a maximum water content of 14% [31]. It has been observed that pigmented rice has a larger mineral content than unpigmented rice [32], and the ash content of the rice samples examined here was comparable to that of the samples examined earlier by Sompong et al. [33]. The flavor of the cooked rice is correlated with its fat level. Higher-fat rice tastes better and has less starch in it. Since fat mostly accumulates in the brain, rice's fat content is affected by polishing intensity [34]. Our findings concur with those of Sompong et al. [33].

The level of protein in black rice reported in the present study is comparable with the levels reported from regions of Thailand, Sri Lanka, and China (7.16% and 10.36%). The proximate analysis of the black rice utilized in the present investigation indicated that it is of high quality and quality and is a powerful engine in the food value chain. A spectrophotometer was used to test the anthocyanin levels in black rice that had been obtained utilizing the pH difference approach. In comparison to brown and white rice, black rice has higher anthocyanin levels based on earlier studies [34, 37]. Like in most Arab countries, food consumption patterns and total daily energy expenditure (TDEE) in Saudi Arabia have changed dramatically resulting in higher consumption of fats often in the form of fast foods [38]. Because dietary antioxidants particularly anthocyanins with strong antioxidant capabilities are beneficial in the management of metabolic diseases, The current study assessed how well anthocyanin-rich black rice extract prevented insulin resistance and dyslipidemia in rats that were brought on by a high-fat diet and streptozotocin. After chlorophyll, anthocyanins are the most prevalent naturally occurring pigments in the kingdom of plants [7].

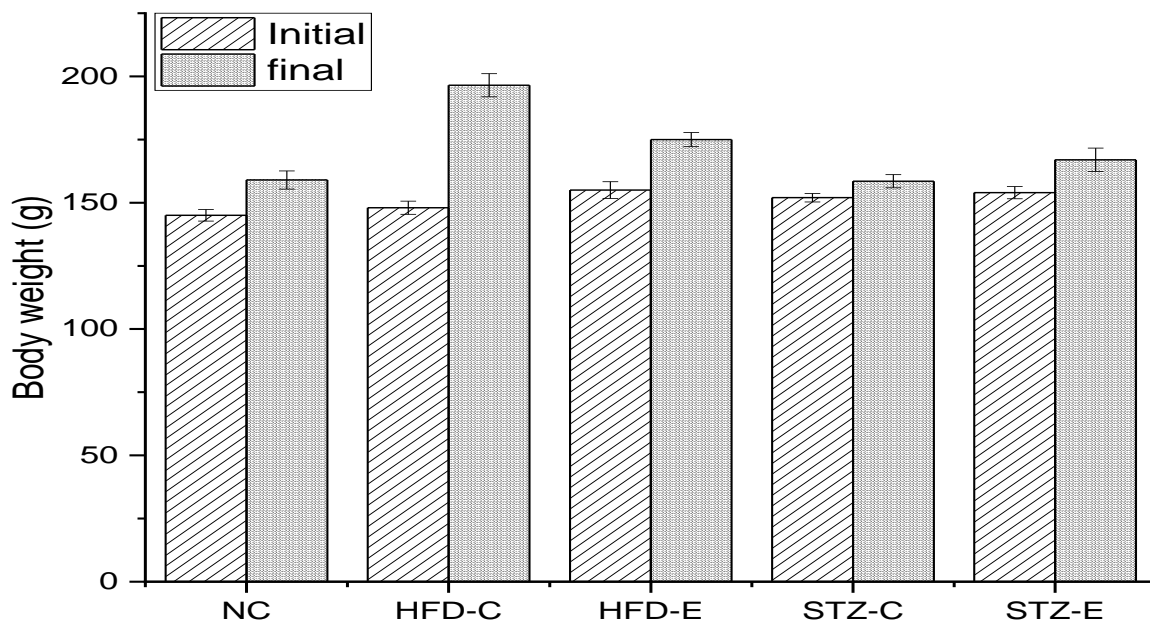
**Table 1.** Proximate composition of the white and black rice varieties

Parameters	White Rice	Black Rice
	Mean± SD	Mean± SD
Moisture (%)	11.09±0.43	13.08±0.07
Fat (%)	2.94±0.12	3.72±0.06
Protein (%)	10.18±0.31	9.87±0.4
Carbohydrate (%)	74.63±0.55	77.88±0.88
Ash (%)	1.26±0.11	1.36±0.12
Fiber (%)	3.88±0.25	4.08±0.34
Total Phenolic components (mg GAE/g DW)	1.52±0.12	6.65±0.22
Total Flavonoids components (mg QE/g DW)	0.63±0.08	9.824±0.3
Anthocyanins (mg/100g DW)	ND	256.6±7.6

**Table 2.** Serum lipid profile of different groups

	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
NC	2.46 <sup>b</sup> ± 0.17	1.12 <sup>b</sup> ± 0.01	1.24 <sup>a</sup> ± 0.06	0.99 <sup>c</sup> ± 0.04
HFD-C	3.84 <sup>a</sup> ± 0.24	2.04 <sup>a</sup> ± 0.14	0.44 <sup>c</sup> ± 0.06	2.99 <sup>a</sup> ± 0.12
HFD-E	2.62 <sup>b</sup> ± 0.80	1.16 <sup>b</sup> ± 0.14	0.86 <sup>b</sup> ± 0.04	1.53 <sup>b</sup> ± 0.16
STZ-C	3.66 <sup>a</sup> ± 0.24	2.06 <sup>a</sup> ± 0.12	0.64 <sup>c</sup> ± 0.06	2.61 <sup>a</sup> ± 0.18
STZ-E	2.54 <sup>b</sup> ± 0.06	1.19 <sup>b</sup> ± 0.11	0.85 <sup>b</sup> ± 0.06	1.45 <sup>b</sup> ± 0.18

\*The mean values (n=8) in the columns with distinct superscript letters a, b, and c differ at p≤0.05. Total cholesterol, or TG; triglycerides, HDL; high-density lipoprotein, LDL; low-density lipoprotein, and STZ-C; diabetic control, STZ-E; diabetic-experimental, NC; normal control, HFD-C; high-fat diet – control, and STZ-E; high-fat diet – experimental.



**Figure 1.** Shows how an extract high in anthocyanins affects body weight. Diabetic Control, STZ-E; Diabetic-Experimental; NC; Normal Control, HFD-C; High Fat Diet–control, HFD-E; High Fat Diet–Experimental, STZ-C.

**Table 3.** Effect of anthocyanin-rich extract on serum transaminases and oxidative stress markers

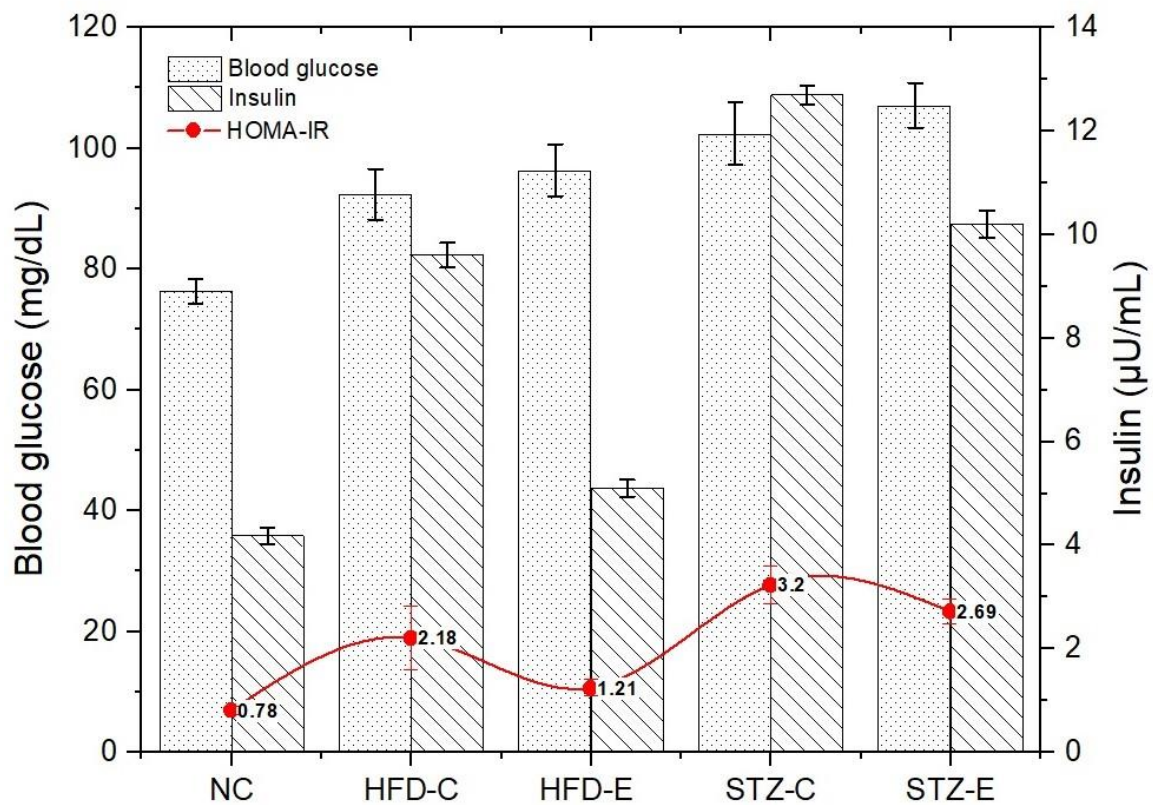
	ALT (unit/L)	AST (unit/L)	MDA (nmol/mL)	GSH (nmol/mL)	TAC (mM/L)
NC	36.46 <sup>b</sup> ± 2.42	72.56 <sup>b</sup> ± 3.64	0.24 <sup>b</sup> ± 0.06	26.16 <sup>a</sup> ± 2.12	1.82 <sup>a</sup> ± 0.06
HFD-C	68.42 <sup>a</sup> ± 1.72	102.64 <sup>a</sup> ± 3.42	0.65 <sup>a</sup> ± 0.02	10.18 <sup>c</sup> ± 2.11	0.38 <sup>b</sup> ± 0.02
HFD-E	39.22 <sup>b</sup> ± 3.48	89.38 <sup>b</sup> ± 2.82	0.25 <sup>b</sup> ± 0.04	20.24 <sup>b</sup> ± 1.32	1.62 <sup>a</sup> ± 0.08
STZ-C	64.28 <sup>a</sup> ± 2.72	101.24 <sup>a</sup> ± 3.06	0.71 <sup>a</sup> ± 0.08	11.16 <sup>c</sup> ± 1.82	0.41 <sup>b</sup> ± 0.06
STZ-E	38.36 <sup>b</sup> ± 4.32	87.42 <sup>b</sup> ± 3.36	0.28 <sup>b</sup> ± 0.06	21.62 <sup>b</sup> ± 2.18	1.58 <sup>a</sup> ± 0.06

\*Mean values (n=8) with different superscript letters a, b, c, in columns differ significantly at  $p \leq 0.05$ . NC; normal control, HFD-C; high-fat diet – control, HFD-E; high-fat diet – experimental, STZ-C; diabetic control, STZ-E; diabetic-experimental, ALT; aspartate aminotransferase, ALT; alanine aminotransferase, MDA; malonaldehyde, GSH; glutathione, TAC; total antioxidant capacity.

**Table 4.** Effect of anthocyanin-rich extract on oxidative stress markers in live

	MDA (nmol/g tissue)	GSH-Px (U/mg protein)	CAT (U/g protein)	SOD (U/g protein)	GSH (μmol/g tissue)
NC	58.19 <sup>b</sup> ± 4.32	3.32 <sup>a</sup> ± 0.16	254.3 <sup>a</sup> ± 42.2	2.12 <sup>a</sup> ± 0.14	12.36 <sup>a</sup> ± 1.26
HFD-C	84.16 <sup>a</sup> ± 5.24	1.82 <sup>b</sup> ± 0.22	126.0 <sup>b</sup> ± 24.4	1.34 <sup>b</sup> ± 0.06	7.28 <sup>b</sup> ± 1.42
HFD-E	63.18 <sup>b</sup> ± 4.26	3.12 <sup>a</sup> ± 0.28	228.4 <sup>a</sup> ± 28.6	2.16 <sup>a</sup> ± 0.18	14.28 <sup>a</sup> ± 1.84
STZ-C	76.28 <sup>a</sup> ± 6.82	1.68 <sup>b</sup> ± 0.24	154.8 <sup>b</sup> ± 8.3	1.44 <sup>b</sup> ± 0.16	8.26 <sup>b</sup> ± 1.46
STZ-E	64.18 <sup>b</sup> ± 3.32	3.02 <sup>a</sup> ± 0.18	246.6 <sup>a</sup> ± 18.3	2.02 <sup>a</sup> ± 0.14	13.66 <sup>a</sup> ± 1.24

\* The mean values (n=8) in the columns with different superscript letters (a, b, and c) indicate significant differences at p≤0.05 for the following: NC; normal control; HFD-C; high-fat diet – control; HFD-E; high-fat diet – experimental; STZ-C; diabetic control; STZ-E; diabetic-experimental; MDA; malonaldehyde; GSH-Px; glutathione peroxidase; CAT; catalase, SOD; superoxide dismutase, GSH; glutathione



**Figure 2.** Effect of anthocyanin-rich extract on glucose homeostasis.

\*Values are mean ± SD of triplicate determinations (n=8). NC; normal control, HFD-C; high-fat diet – control, HFD-E; high-fat diet – experimental, STZ-C; diabetic control, STZ-E; diabetic-experimental, HOMA-IR; homeostatic model assessment for insulin resistance index.

In addition to their color, anthocyanins have been linked to several biological activities, such as anti-inflammatory, anti-cancer, anti-hyperlipidemic, antimutagenic, and anti-obesity [39]. Though more than 300 distinctive anthocyanins reportedly occur in nature [40], their bioavailability is poor (approximately 1.8%) in the human gut [41]. However, the unabsorbed anthocyanins are hydrolyzed by colonic bacteria to yield smaller phenolic compounds and phenolic acids, which are absorbed through colonic mucosa [42]. Furthermore, Anthocyanins unquestionably play a function in promoting human health. [41]. Therefore, it is important to study the health benefits of dietary anthocyanins derived from staple food sources to optimize their utilization as functional foods. In this direction, the current study demonstrated that BRAE derived from black rice restores elevated triglycerides and cholesterol levels in a high-fat diet to normal levels. but not glucose levels in streptozotocin-induced diabetic rats. The extracts also raised HDL levels and lowered the levels of LDL, AST, and ALT toward basal levels. These findings are consistent with several past in vitro and in vivo investigations [43, 19, 44]. The observed phenomenon is attributed to the excellent antioxidant activity of BRAE as indicated by lowered oxidative stress in BRAE-treated groups in both HFDs. BRAE reduced malondialdehyde levels in both serum and liver homogenate and increased the levels of reduced glutathione, glutathione peroxidase, superoxide dismutase and, catalase, to varying levels in both HFD. These observations are also in good agreement with earlier studies, wherein fruit anthocyanins increased the levels of glutathione peroxidase and superoxide dismutase in experimental animals [45, 46]. Several studies have ascribed anthocyanin with excellent antidiabetic properties mediated through inhibition of carbohydrate hydrolyzing enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) thereby lowering blood glucose, augmenting insulin secretion via increased pancreatic  $\beta$ -cell proliferation, diminishing proinflammatory cytokines and insulin resistance in different experimental models [47, 48]. In a similar study, anthocyanin-rich extract from black rice lowered hyperlipidemia and insulin resistance in obese rats [6, 17]. However, in the current study, such an effect was not observed on glucose homeostasis. BRAE failed to lower blood glucose or insulin resistance in both high-fat diet-fed rats and streptozotocin-induced diabetic rats. This observation is contrary to the earlier reports on anthocyanins in general. The possible reason for such adverse observation could be the structural diversity of anthocyanins. Structural diversity determines their bioavailability, individual sensitivity, and rapid biodegradation characteristics [41]. Therefore, further controlled clinical trials are warranted to establish the efficacy of black rice anthocyanins about their hypolipidemic and antidiabetic effects.

#### 4. Conclusions

The findings of the study demonstrate that anthocyanin-rich extract from black rice possesses a strong antioxidant effect when incorporated into the diet. It is effective in treating high-fat diet-induced dyslipidemia, as seen by decreased serum levels of LDL, triglycerides, and cholesterol and elevated levels of HDL and antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase. Contrary to what was expected, the *Alfawzan et al., 2024*

extract proved to be non-efficacious in either lowering blood glucose or insulin resistance in any of the supplemented groups. It can be concluded that anthocyanin-rich extract from black rice improves antioxidant defenses and could be beneficial in hyperlipidemic conditions.

#### Author Contributions

O.A.: Data curation, Formal interpretation, and approach. HMM: ideation, verification, composition—analysis, and editing. S.A.: Validation, Writing—original draft. F.A.: Data curation, Formal analysis, Software. M.S.A.: Data curation, Investigation, Methodology, Writing—original draft. H.A.A.: Formal analysis, Investigation, Project administration. W.E.A.: Conceptualization. Project administration, Visualization. W.E.A.: Methodology, Software, Writing: proofreading, editing, and review. Formal analysis, methodology, data curation, and A.M.E. Each author has reviewed the published version of the manuscript and given their approval. The authors express their gratitude to Qassim University's Deanship of Scientific Research for providing money for the publishing of this study.

#### Institutional Review Board Statement

King Abdul-Aziz City for Science and Technology, KACST, Kingdom of Saudi Arabia's National Committee of Bioethics (NCBE) approved this study, and it was carried out with Review Board Number: 10024677.

#### Data Availability Statement

The raw data and materials used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

By the format needed, the writers declare that they have no conflicts of interest. The writers have no affiliations or business dealings that could provide a conflict of interest when it comes to writing this study.

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