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Evaluation of the Anti-diabetic Activities of Colored Rice Varieties in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

The study was done to evaluate the replacement of white rice with other varieties of pigmented rice in a streptozotocin (STZ) -induced type 1 diabetic rat model (T1DRM). Application was carried out for a month in the form of ethanol extracts delivered via oral intubation or supplementation of whole-grain rice as 20% of the feed. The antioxidant activities of different colored rice varieties were measured, with the highest activity recorded in brown rice, followed by black, and then red rice. The results obtained for the T1DRM indicated that there had been a gain in body weight in all rats that received rice supplementation, with the highest seen in groups given diets supplemented with 20% red rice, while the lowest was in those supplemented with white rice. It was noted that the black and brown rice, either in extract form or 20% whole rice supplementation (P <0.05) led to significant hypoglycemia. The supplementation of black rice extract led to a significant decrease in the levels of AST at (P <0.01) and ALT, ALP at (P <0.05). However, the supplementation of 20% whole black rice resulted in a significant reduction in the level of AST (P <0.05) and a significant increase in the activity of pancreatic antioxidant enzymes. The supplementation of black, brown, or red rice extracts resulted in leucopenia (P <0.05), neutrophilia (P <0.05), and lymphocytosis (P <0.05) compared to the T1DRM group. Finally, it is clear from the results that the substitution of whole-grain pigmented rice for white rice may play a role in lowering the risk of diabetes. The current study recommends the consumption of whole-grain pigmented rice, as it exhibits efficient antidiabetic and antioxidant activities. Further extensive research is needed to explore the use of pigmented rice as a supplement in the management of diabetes in humans.

Keywords: Antioxidant, Colored rice, Diabetic rat model, Leukogram, Streptozotocin

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INTRODUCTION

The complexity of the relationship between inflammatory and stress biomarkers and the pathogenesis of diabetes has not been extensively studied in detail in terms of food used as a nutraceutical in modern therapy. Diabetes mellitus (DM) is classified as a metabolic disease and its prevalence is regarded as high among the worldwide population [1]. According to the World Health Organization (WHO), a total of 170 million people of different ages (4-5% worldwide) have suffered from DM since the beginning of the century, and the number is expected to reach up to 438 million (7.7%) by 2030 [2]. Therefore, development of new therapeutic strategies to eliminate and/or decrease the progression of diabetic complications is critically needed. There are many risk factors associated with DM type 1, one of which is dietary influence [3]. Previous studies have shown that the prevalence of both types of diabetes has increased rapidly among the Saudi population and stands at 30%, indicating a daunting public health challenge [4, 5]. The Kingdom of Saudi Arabia (KSA) has been recently listed by the International Diabetes Federation among the top 10 countries worldwide with a high percentage of diabetes (16% in 2011, projected to be 20% by 2030) [6]. According to the WHO [7], 22% of males and 21.7% of females aged 25 in the Saudi population were hyperglycemic.

Diabetes mellitus is a disorder affecting the body's metabolism of lipids, carbohydrates, and proteins and exhibiting characteristic hyperglycemia. The connection is well established between DM and other disorders like renal failure, blindness, circulatory disease, retinal disease, kidney disease, and neuropathies. Although DM can lead to mortality [8], the control of serum glucose and lipid concentrations reduces the complications. For these reasons, in the treatment of diabetes, the objective is metabolic control.

The role of oxidative stress biomarkers in augmenting DM complications is well documented. This includes the increase in oxygen free radicals induced by hyperglycemia, followed by increased protein and lipid oxidation [9]. The Islet β-cells are strongly affected by oxidative stress due to a reduction in endogenous antioxidants [10]. Biomarkers related to oxidative stress are revealed in the form of reduced antioxidant enzyme activities [10].

Rice is the most popular cereal in developing countries, including KSA. About 50% of the world population consumes rice as the basic source of starch and carbohydrates. In the Asian community, the high percentage of rice production (95%) and consumption is well documented [11]. In addition to common polished (white) rice, there are colored varieties (black, brown, and red) [12]. Some types of colored rice are not commonly consumed. The colors in the rice may be attributed to the precipitation of a high concentration of anthocyanin pigment in the integument of the rice. The largest number of phytochemicals was found in black rice, followed by brown and red-colored rice [13]. Thus, the introduction of colored rice to the Saudi Arabian diet is essential because of its high nutritional value. About, 35% of the recommended daily dietary minerals can be provided by

50 g of black rice. The high mineral content of black rice may be a result of the milling process [13].

In comparison to white rice, black rice is superior in terms of phenolic compounds [14]. The estimated phenolic content has been previously described as nearly four times greater than in white rice [15]. Compared to white and brown rice, red and purple rice also exhibited elevated total phenolic content, as well as flavonoids and antioxidant properties [16, 17, and 18]. The capacity of the phytochemicals presents in rice to interfere with the absorption of cholesterol and platelet aggregation has been described [19]. Black rice is known to have health-promoting compounds including antioxidants and is used in the management of diseases with oxidative stress such as cancers [20]. This rice is rich in essential components, especially iron and vitamin E, and also has a high fiber content [13]. The antioxidant property of the phenolics present in black rice has beneficial effects on blood coagulation and tumor pathogenesis [21].

Concerning research related to colored rice and diabetes, recent studies [22, 23] have shown that colored rice, especially black rice, could reduce the blood glucose levels of rats to 125 mg/dL, and elevate the levels of blood urea nitrogen to 33 mg/dL in diabetic rats with nephric complications. The authors concluded that the antidiabetic agent in black rice was a result of its high flavonoid content and its effect on beta-cell regeneration, which enabled the secretion of insulin. The stimulation of insulin release by flavonoids was due to the increase in Ca2+ concentrations in the islet of Langerhans cells [24]. In order to develop colored rice, from black or purple to brown and red, a high percentage of anthocyanin is concentrated in the rice integument. Although a few studies have reported on the anti-diabetic agents of brown rice [25, 26], only one study has looked at the relation between brown rice consumption and the risk of diabetes [27].

Studies that examine the effects of whole-grain rice consumption on the complications and metabolic homeostasis disturbance of DM are lacking. Thus, this work was carried out to assess the replacement of white rice with colored rice (black, red, and brown) in streptozotocin (STZ)-induced diabetic rats. Application was carried out in the form of ethanolic extracts administered by oral intubation or supplementation of whole-grain rice as 20% of the feed. Indicators such as weight gain, glucose levels, tissue antioxidant defences, liver and kidney function tests, and hemogram and leukogram findings were assessed.

MATERIALS AND METHODS

Ethical statement

The current work was permitted through the Animal Ethics Committee of Qassim University (QU - No.5341-cavm-2018-1-14-S**)**. The experiments were consistent with the guides recognized by the International Animal Ethics Committee and were carried out according to local laws and regulations.

Hundred and ten male Wistar rats (130±15 g body weight) were reared in the experimental animal unit. Animals were kept in control conditions of temperature, humidity, and light/ dark cycle (25°C, 55%, and 12:12 h respectively). The commercial diet was obtained from the General Company of Feed Silo and Powder Mint. The diet was formulated to furnished requirements [28] and was delivered with water *ad-libitum*. The experimental conditions were done parallel to the guidelines for animal care and ethics as recommended by Scientific Research Dean. After acclimatization, the animals were prepared for the Type 1 Diabetes Rat Model (T1DRM).

Induction of T1DRM

T1DRM was performed by intraperitoneal injection (i.p) of rats with 75 mg/kg Streptozotocin (STZ - Santa Cruz, Germany). All rats were given 5% glucose during the following day in drinking water due to the destruction of the β-cells of the pancreas by STZ. Puncture of the tail vein was done to obtain a drop of blood for measuring glucose levels with Blood Glucose Monitoring System Glucometer (One-Touch Basic; MedNet GmbH 48163 Munster, Germany). After three days from STZ treatment, rats that had reached hyperglycemic plateau over 250 mg/dl were involved in the study while rats with lower or normal glucose levels were excluded from the study.

Preparation of colored rice extracts

The colored or pigmented rice (black, red, and brown) and white rice were first grounded to small particles and weighed. The extraction was done by mixing 100 g of each fine rice particles into acidified 1000 ml of ethanol at (50% w/w) ethanol: H_2O according to [29]. The extraction process was carried out in a water bath at 50°C for 60 minutes [30, 31]. Air-dried powder of each grain (100 g) was mixed well in one-liter distilled water and kept for one day at room temperature. The solution was filtered using muslin material or Whattman filter No. 1. The filtrate was then collected in pre-weighed tubes after centrifugation at 3000 rpm for 30 min [32].

Antioxidants activity of rice

0.1 g of each rice extract was stirred for 3 minutes in 10 ml 50% aqueous ethanol at 25,000 rpm using a homogenizer (IKA, Germany). Extracts were then centrifuged at 3000 rpm for 15 min and the supernatants were used for further analyses [33].

Determination of total phenolic content (TPC)

The TPC was measured following the Folin-Ciocalteu method using gallic acid as the standard. A 500 µL diluted Folin-Ciocalteu reagent (Merck, Germany) was added to 100-µL aliquot of plant extract for oxidation. The mixture was neutralized with 1 ml sodium carbonate (7.5%, w/v) after 5 min, and incubated for 120 min before reading absorbance at 765 nm. TPC is expressed as mg gallic acid equivalents (GAE) per 100 g DW of samples [34].

Assay of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) activity

The DPPH was measured following the method of Brand-Williams et al. [35]**.** Two ml of methanolic DPPH solution (40 mg/L) was mixed with 100 µl sample extract, incubated in the dark at 25°C for 30 min, and the absorbance of the solution was read at 517 nm.

Assay of 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) activity

The ABTS assay method of Re et al. [36] was modified to determine antioxidant activity using Trolox as the standard. ABTS was created by oxidizing 7 mM ABTS with 2.45 mM potassium persulfate to produce radical cations. The solution was kept overnight before use. The ABTS solution was diluted with distilled water. For assays, 1 ml ABTS cation solution was mixed with 100 µL sample and the reduction in absorbance at 734 nm was measured.

Experimental design and sampling

Hundred and ten rats were divided into eleven groups, ten rats each. Rats received the treatment as follows; Group (1): healthy control rats fed a commercial diet with no supplementation, Group (2): type 1 diabetic rats' model (T1DRM) kept as a positive control. Group (3): T1DRM received long-acting insulin daily for month. Each rat received 4 IU/100 g of body weight per day (100 IU/mL Insulin Lantus; Sanofi-Aventis, France) via subcutaneous

(SC) injection. Groups (4, 5, 6, 7): T1DRM gavaged with a white, black, red, or brown rice ethanolic extract 100 mg/kg orally for month, respectively. Group (8, 9,10,11): T1DRM received food containing 20% white, black, red or brown rice daily for month, respectively. Body weights were recorded weekly for each group throughout the experimental period.

Blood samples were collected from each rat via the inner canthus of the eye using capillary tubes under mild ether anesthesia every week for 30 days. Part of the blood samples was used for extraction of serum by centrifugation at 2500 r.p.m for 15 min. Collected sera were labeled and deep- frozen for pending analysis. Another blood sample were collected in heparinized tubes (Becton Dickinson, NJ, USA) for use in hematological parameters. At the end of the experimental period (4 weeks), all rats were fasted overnight, anesthetized, bled from the inner canthus, and sacrificed. Whole blood samples were analyzed in Beckman Coulter Clinical Chemistry AU analyzer (Abaxis, CA, USA). Body weight (BW), and body weight gain (BWG) and BWG % were measured weekly. The serum parameters were measured via an automated biochemical analyzer (Abaxis, CA, USA.) to evaluate the glucose**,** urea, creatinine (CREA), uric acid, aminotransferases (ALT and AST) and alkaline phosphatase (ALP).

The hemogram included erythrocytes count (RBC), hemoglobin concentration (Hb), hematocrit values (HCT), platelets count (PLT) and erythrocyte indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Measurements related to leucogram included total (TLC) and differential leukocyte number [lymphocyte (LYM), monocyte (MON), neutrophil (NEU) and eosinophil (EOS)].

Antioxidant activity of pancreatic tissue

The pancreas specimens were dissected from each rat and washed with cold saline and homogenized with ice-cold Tris-HCl (0.1M, pH 7.4) in a ratio of 1 w: 10 v [37]. The homogenates were stored at -20°C until use. The suspended mixture was centrifuged at 2500 r.p.m for 15 min at 4 °C in a cold centrifuge. The obtained supernatant was used for the assay of Glutathione peroxidase (GSH-Px, BIODIAGNOSTIC Kits, CAT. No 2524), Superoxide dismutase (SOD, BIODIAGNOSTIC Kits, CAT. No. 2521) and Catalase (CAT, BIODIAGNOSTIC Kits, CAT. No. 25 17). Measurement of protein content of the supernatants was done [38]. Enzymes activities were expressed in terms of U/mg protein.

Statistical analysis

Obtained results were intended for statistical analysis by SPSS 14 version software. The significant differences between groups was done using one-way analysis of variance (ANOVA). In the status of significant differences, the Student-Newman-Kuels test was performed. All data were recorded on an individual basis. Duncan's New Multiple Range test was applied to determine significant differences (P <0.05, P *<0.01* and P *<0.001*). Data were presented as means ± standard error (SE).

RESULTS

There was gain in body weight in all the groups after finishing the experiment (Table 1). The obtained body weight gain in the T1DRM group was significantly reduced compared to their counterparts in the healthy control group (P<0.05). The body weights of the insulin treated group and all colored rice supplemented groups were higher than the T1DRM group in general (Table 1). However, the highest groups were those supplemented with either 20% red rice (40.43 ± 1.47 g/4 w), 20% black rice (37.43 ± 1.29 g/4 w) or red rice extract (33.32 ± 1.14 g/4 w), while the lowest groups were those supplemented with the white rice either as extract (20.11±2.27 g/4 w) or whole rice (26.15±1.86 g/4 w).

Table 1: The effects of colored rice supplementation via intubation (extracts) or 20% in food (whole rice) on body weight gain (BWG) in type 1 diabetic rat model (T1DRM)

Groups / Parameters	Initial body weight (g)	Final body weight (g)	Body weight gain (g/4 w)	Body weight gain/loss (%)
Healthy control	181 ±3.11	212 ±3.54	30.54 ±1.37	16.79%
T1DRM	186 ±2.44	187 ±4.65	2.43* ±1.45	1.30%
T1DRM + insulin	185 ±1.51	222 ±3.54	40.44[a] ±2.63	21.62%
T1DRM + white rice extract	182 ±4.34	202 ±4.11	20.11 ±2.27	10.99%
T1DRM + black rice extract	179 ±3.28	212 ±3.54	30.43[a] ±1.49	16.76%
T1DRM + brown rice extract	183 ±2.41	199 ±4.65	21.32 ±2.54	11.48%
T1DRM + red rice extract	186 ±3.09	210 ±5.64	33.32[a] ±1.14	17.74%
T1DRM + 20% white rice	177 ±4.54	201 ±5.11	26.15[a] ±1.86	14.69%
T1DRM + 20% black rice	183 ±2.35	219 ±5.72	37.43[a] ±1.29	20.22%
T1DRM + 20% brown rice	186 ±5.33	228 ±5.18	33.18 ±1.97	17.74%
T1DRM + 20% red rice	177 ±1.17	211 ±3.54	40.43[a] ±1.47	22.60%

Values in the same column with the mark (*) of the diabetic group were significantly different from the value of the healthy group at (P< 0.05). Values of the treated groups with the liters ([a]) were significantly different from the value of the diabetic group at (P< 0.05). Mean ± Standard error (SE).

The liver and kidney function parameters were affected in STZ induced T1DRM relative to the healthy control group (Table 2). Significant hyperglycemia was noted (P<0.01) along with a significant elevation in urea, uric acid and CREA (P<0.01) in STZ induced T1DRM relative to the control group. In addition, a significant increase was showed in ALT, AST and ALP (P<0.05) in STZ induced T1DRM relevant to the healthy group. Insulin administration significantly restored the glucose levels as well as the kidney and liver functions by decreasing the measurable items. The levels of glucose, urea, uric acid and CREA and activities of ALT, AST ALP were significantly decreased (P<0.05) compared to the T1DRM group. Supplementation with colored rice extracts via intubation or 20% in food significantly restored the glucose, kidney and liver function parameters except for the white rice

that didn't show any significant effect. The supplementation of the black rice as extract or 20% whole black rice resulted in a significant hypoglycemia (P< 0.001). Besides, the black rice extract significantly decreased the levels of uric acid (P< 0.01), ALT (P< 0.05), AST (P< 0.01) and ALP (P< 0.05), while the supplementation of 20% whole black rice resulted in a significant reduction in the level of CREA and AST (P< 0.05). We noticed that the brown rice supplementation led to a significant decrease in blood glucose level either as extract (P< 0.001) or 20% whole rice in food (P<0.05). The levels of CREA, uric acid, and ALP were significantly reduced following supplementation with the brown rice extract. Furthermore, 20% whole brown rice supplementation in food resulted in a significant decline in the levels of urea (P< 0.05), CREA (P< 0.01), ALT, AST, and ALP (P< 0.05). The supplementation of the red rice resulted in a significant hypoglycemia either as extract (P< 0.001) or 20% whole rice in food (P<0.05). A significant decline was also noticed on the levels of urea (P< 0.05), CREA (P 0.01), AST and ALP (P< 0.05). On the other hand, supplementation with 20% whole red rice in food resulted in a significant decrease in the levels of urea, uric acid and ALP (P< 0.05) and CREA (P< 0.01).

Table 2: The effects of colored rice supplementation via intubation (extracts) or 20% in food (whole rice) on glucose level and hepatic & renal bio-indices in T1DRM

Groups / Parameters	Glucose (mg/dl)	Urea (mmol/l)	Creatinine (umol/l)	Uric acid (umol/l)	ALT (U/l)	AST (U/l)	ALP (U/l)
Healthy control	122.43 ±4.52	7.86 ±1.17	39.43 ±3.11	86.61 ±6.93	91.34 ±2.87	163.45 ±5.02	214.55 ±4.51
T1DRM	410.72** ±3.11	13.21** ±1.88	60.56** ±4.99	112.43** ±8.11	111.44* ±4.76	193.34* ±4.87	276.67* ±5.88
T1DRM + insulin	101.53c ±2.43	8.95a ±1.54	44.48a ±4.48	89.18a ±4.70	88.32a ±3.51	156.65a ±4.04	248.03a ±5.11
T1DRM + white rice extract	397.49 ±4.50	11.34 ±1.52	52.48 ±3.56	101.55 ±3.65	112.45 ±3.08	191.45 ±5.71	279.55 ±6.12
T1DRM+black rice extract	132.66c ±3.55	9.33 ±1.88	47.51 ±2.14	72.80b ±5.12	79.34a ±4.72	155.23b ±4.65	241.12a ±6.04
T1DRM+brown rice extract	242.99c ±3.81	9.49 ±1.11	36.22c ±3.06	88.61a ±5.09	98.45 ±3.09	171.23 ±6.34	234.76b ±7.49
T1DRM + red rice extract	238.83 c ±4.60	7.51a ±2.61	39.37b ±1.23	80.34 ±4.11	81.34a ±4.67	163.53 ±5.11	245.66a ±10.7
T1DRM + 20% white rice	382.75 ±3.22	10.12 ±1.23	55.81 ±3.19	101.56 ±5.22	90.12 ±5.84	179.34 ±4.62	265.11 ±8.32
T1DRM + 20% black rice	137.55c ±5.12	9.29 ±1.09	47.70a ±3.63	96.28 ±4.09	98.34 ±4.11	166.34a ±3.99	262.67 ±6.92
T1DRM + 20% brown rice	333.87a ±4.09	7.63a ±2.02	33.35b ±2.34	90.61 ±4.77	80.65a ±2.29	158.32a ±5.09	249.45a ±6.99
T1DRM + 20% red rice	241.41b ±3.88	8.54a ±1.22	37.53b ±2.04	84.80a ±3.70	91.38 ±3.03	179.23 ±4.91	258.52a ±7.54

Mean ± Standard error (SE); Values in the same column of the T1DRM group were significantly different from the value of the healthy control group at P< 0.05 (*) and P< 0.01(**). Values of the treated groups were significantly different from the value of the T1DRM group at P< 0.05 (a), P< 0.01 (b) and P< 0.001 (c). T1DRM: type 1 diabetic rat model.

The hemogram was affected in T1DRM relative to the healthy control group (Table 3). In TIDRM, the RBC count, Hb concentration, HCT (P <0.05), MCHC and PLT count (P <0.01) were significantly lower than the healthy group. However, a significant increase was noticed in Hb concentration, HCT and PLT count (P<0.05) after insulin supplementation comparing to TIDRM. Hemogram parameters were restored after the supplementation with the colored rice extracts. Supplementation of STZ-induced diabetic rats with black, brown, or red rice extracts resulted in a significant increase in RBC count, Hb concentration, HCT, and PLT count (P <0.05) comparing to T1DRM group. Moreover, supplementation of 20% black or brown rice showed similar results where RBC count, Hb concentration and PLT count were significantly elevated compared to the T1DRM group. In addition, we observed a significant increase in RBC count, Hb concentration, HCT, and PLT count (P <0.05) in the 20% red rice group relative to the T1DRM group.

The leukogram was affected in the T1DRM group relative to the healthy control group (Table 4). In STZ induced T1DRM, while there was a significant elevation in WBC count and NEUT % (P< 0.05), LYMP % was significantly lowered (P< 0.05) comparing to healthy group. These results were restored after the supplementation of insulin where WBC count and NEUT % significantly decreased (P< 0.05) and LYMP % was significantly increased (P< 0.05) relative to the T1DRM group. Similarly, the supplementation of black, brown or red rice extract resulted in a significantly lower WBC count and NEUT% (P< 0.05) and a significant increase in LYMP% (P< 0.05) comparing to STZ induced T1DRM. However, the supplementation of 20% black rice led to a significant decrease only in WBC count relative to the T1DRM group (Table 4).

Table 3: The effect of colored rice supplementation via intubation (extracts) or 20% in food on hemogram of T1DRM

Groups / Parameters	RBC (10^6/Ul)	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^3/UL)
Healthy control	6.65 ±0.32	14.43 ±1.32	41.66 ±2.11	61.34 ±2.64	21.33 ±2.56	32.01 ±3.87	48.66 ±4.44
T1DRM	4.11* ±1.43	9.32* ±1.99	31.54* ±2.09	55.54 ±4.08	18.21 ±4.55	19.32** ±2.36	25.87** ±3.22
T1DRM + insulin	6.32 ±1.98	14.41[a] ±1.78	41.57[a] ±5.34	58.33 ±3.88	22.35 ±3.35	23.45 ±2.87	41.71[a] ±2.10
T1DRM + white rice extract	4.43 ±1.77	10.22 ±3.67	32.65 ±4.56	60.54 ±4.14	21.50 ±1.43	21.63 ±2.65	29.38 ±4.65
T1DRM + black rice extract	6.99 [a] ±0.49	14.94[a] ±1.56	44.97[a] ±3.51	60.23 ±1.65	23.65 ±2.22	26.66 ±1.34	51.41[b] ±5.43
T1DRM + brown rice extract	6.43 [a] ±0.45	14.65[a] ±1.91	42.65[a] ±3.50	61.12 ±5.23	22.57 ±1.45	29.12[a] ±2.32	47.04[a] ±3.97
T1DRM + red rice extract	6.35 [a] ±1.56	15.38[a] ±2.18	42.43[a] ±2.82	62.54 ±1.34	21.84 ±1.33	31.58 ±1.89	55.54[a] ±3.65
T1DRM + 20% white rice	4.37 ±1.43	11.65 ±2.56	33.36 ±3.10	61.33 ±4.87	20.34 ±1.22	30.76 ±1.32	33.27 ±5.78
T1DRM + 20% black rice	6.47 [a] ±1.07	15.35[a] ±3.00	38.45 ±2.14	61.19 ±2.43	20.33 ±1.87	28.45 ±1.67	50.61[a] ±4.66
T1DRM + 20% brown rice	7.15 [a] ±1.11	15.19[a] ±2.56	39.24 ±3.11	62.65 ±3.10	20.12 ±2.87	29.65 ±3.43	51.28[a] ±2.87
T1DRM + 20% red rice	6.36[a] ±1.08	15.65[a] ±2.91	41.25[a] ±2.15	59.62 ±2.56	20.76 ±1.66	31.73 ±2.65	45.39[a] ±4.65

Mean ± Standard error (SE); Values in the same column of the T1DRM group were significantly different from the value of the healthy control group at P< 0.05 (*) and P< 0.01(**). Values of the treated groups were significantly different from the value of the T1DRM group at P< 0.05 ([a]) and P< 0.01 ([b]). T1DRM: type 1 diabetic rat model, RBC: erythrocytes number, Hb: hemoglobin concentration, HCT: hematocrit value, PLT: platelets number, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Table 4: The Effects of colored rice supplementation via intubation (extracts) or 20% in food (whole rice) on leukogram of T1DRM

Groups / Parameters	WBC (10^3/Ul)	NEUT (%)	LYMP (%)	MONO (%)	EOS (%)
Healthy control	6.43 ±1.32	24.66 ±2.45	69.34 ±4.54	5.11 ±0.45	4.30 ±0.65
T1DRM	11.44* ±1.22	33.54* ±2.45	51.33* ±3.04	4.99 ±0.55	5.66 ±1.20
T1DRM + insulin	6.34[a] ±1.43	23.34[a] ±2.76	70.55[a] ±5.33	4.48 ±0.12	5.34 ±0.25
T1DRM + white rice extract	11.54 ±0.94	34.39 ±1.54	59.73 ±3.65	5.76 ±0.43	5.33 ±0.56
T1DRM + black rice extract	6.33[a] ±1.04	24.83[a] ±1.03	68.37[a] ±2.65	5.53 ±0.65	6.23 ±1.33
T1DRM + brown rice extract	6.58[a] ±1.44	21.22[a] ±2.43	77.63[a] ±3.11	5.57 ±1.03	3.56 ±0.65
T1DRM + red rice extract	5.63[a] ±0.32	22.47[a] ±1.55	69.22[a] ±2.17	5.52 ±1.11	4.75 ±0.26
T1DRM + 20% white rice	10.17 ±0.76	33.64 ±2.54	62.58 ±5.43	4.10 ±0.43	3.09 ±0.45
T1DRM + 20% black rice	5.50[a] ±0.56	35.44 ±3.54	61.96 ±2.65	4.32 ±0.77	4.58 ±0.65
T1DRM + 20% brown rice	5.87 ±1.23	33.71 ±1.23	65.01 ±3.66	5.13 ±0.45	5.64 ±1.54
T1DRM + 20% red rice	5.44 ±1.08	39.48 ±1.37	67.34 ±1.76	5.48 ±0.11	4.44 ±1.20

Mean ± Standard error (SE); Values in the same column of the T1DRM group were significantly different from the value of the healthy control group at P< 0.05 (*) and P< 0.01(**). Values of the treated groups were significantly different from the value of the T1DRM group at P< 0.05 ([a]) and P< 0.01 ([b]). T1DRM: type 1 diabetic rat model, TLC: total leukocyte count, LYM: lymphocyte, MON: monocyte, NEU: neutrophil, EOS: eosinophil.

The antioxidant enzymes activities in serum of the different groups were measured (Table 5). A significant decline in the activities of the serum GSH-Px (P < 0.01), SOD and CAT (P < 0.05) were noticed in the T1DRM group compared to the healthy group. A significant elevation in the activities of the serum SOD (P < 0.05), GSH-Px and CAT (P < 0.01) of T1DRM+ insulin group compared to the STZ induced T1DRM group. However, treatment with the black rice resulted in a significant increase in serum GSH-Px (P < 0.01) and SOD (P < 0.05) as noticed in the T1DRM+black rice extract group compared to the STZ induced T1DRM group. Treatment with brown rice extract resulted in a significant elevation in the serum GSH-Px (P < 0.05) only as recorded in the T1DRM+brown rice extract group compared to the STZ induced T1DRM group. On the other hand, supplementation of 20% black rice in food significantly elevated GSH-Px (P < 0.01), SOD and CAT (P < 0.05) activities as shown in the T1DRM+20% black rice group relevant to the STZ induced T1DRM group. Moreover, supplementation of either 20% brown rice or 20% red rice in food resulted in a significant increase in GSH-Px and CAT (P < 0.05) as shown in the T1DRM+20%

brown rice and T1DRM+20% red rice groups compared to the STZ induced T1DRM group.

Table 5: The effect of colored rice supplementation via intubation (extract) or 20% in food (whole rice) on antioxidant enzymes of pancreatic tissue of T1DRM

Groups / Parameters	GSH-Px (U/mg protein)	SOD (mU/mg protein)	CAT (U/mg protein)
Healthy control	478.49 ±8.32	2.35 ±0.31	38.57 ±2.11
T1DRM	366.22** ±7.10	1.76* ±0.23	27.05* ±2.06
T1DRM+ insulin	500.28[b] ±11.34	2.67[a] ±0.41	41.79[b] ±3.28
T1DRM + white rice extract	337.51 ±9.29	1.83 ±0.22	31.41 ±2.51
T1DRM+black rice extract	519.55[b] ±8.34	2.55[a] ±0.16	38.89 ±2.92
T1DRM+brown rice extract	457.26[a] ±9.26	1.945 ±0.34	30.04 ±3.16
T1DRM + red rice extract	407.10 ±10.32	1.88 ±0.27	30.74 ±2.90
T1DRM + 20% white rice	353.83 ±7.33	1.49 ±0.31	26.54 ±3.14
T1DRM + 20% black rice	512.57[b] ±6.10	2.95[a] ±0.18	38.93[a] ±1.28
T1DRM + 20% brown rice	425.55[a] ±4.82	2.15 ±0.22	38.12[a] ±1.51
T1DRM + 20% red rice	421.50[a] ±6.11	2.23 ±0.19	35.87[a] ±2.55

Mean ± Standard error (SE); Values in the same column of the T1DRM group were significantly different from the value of the healthy control group at P< 0.05 (*) and P< 0.01(**). Values of the treated groups were significantly different from the value of the T1DRM group at P< 0.05 ([a]) and P< 0.01 ([b]). T1DRM: type 1 diabetic rat model, GSH-Px: glutathione peroxidase, SOD: superoxide dismutase, CAT: catalase.

The antioxidant capability of the colored rice (brown, black and red) was determined (Table 6). TPC activities were 1023.333, 911.2121 and 579.3939 in brown, black and red rice, respectively. DPPH% was 67.85714, 56.94165 and 25.80483 in brown, black and red rice respectively, while ABTS% activities were 85.80645, 70.32258 and 18.3871 in brown, black and red rice respectively.

Table 6: Antioxidant capacity of the different colored rice extracts

Rice type / Parameters	TPC (GAE)	DPPH %	ABTS%
Brown	1023.333 ±6.060606	67.85714 ±3.06841	85.80645 ±0.645161
Black	911.2121 ±12.12121	56.94165 ±0.603622	70.32258 ±3.870968
Red	579.3939 ±25.75758	25.80483 ±0.050302	18.3871 ±2.258065

TPC: total phenolic content; GAE, gallic acid equivalents, DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, ABTS: 2, 2'-Azino- Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid.
Mean± Standard deviation (SD).

DISCUSSION

The complexity of the relationship between health biomarkers and pathogenesis of diabetes has not been investigated in detail in terms of food used as a nutraceutical in modern therapy. Due to the high cost of modern medications, non-traditional treatments including medicinal plants have become extremely popular. Thus, the present study was conducted to assess the effects of replacing white rice with varieties of colored rice (black, red, and brown) in the diet of streptozotocin (STZ)-induced diabetic rats. Application was carried out in the form of ethanolic extracts administrated via oral intubation or supplementation of whole-grain rice as 20% of the feed, followed by assessment of some indicators including body weight gain, glucose levels, antioxidant defences, liver/kidney function, and hemogram and leukogram findings.

To develop the type 1 diabetic rat model (T1DRM) in this study, the rats were injected with STZ, a broad-spectrum antibacterial agent that acts as a strong cytotoxic glucose equivalent. The STZ changes the DNA and causes nuclear weakness through activation of cellular-damaging pathways mediated by the reactive oxygen species (ROS). This leads to pancreatic β-cell obliteration and results in the withdrawal of insulin and the induction of DM [39]. Various studies have used different dosages of STZ to induce DM in rats. The single dose of STZ recommended by several studies ranged between 50 mg/kg body weight [40], 70 mg/kg body weight [41], and up to 150 mg/kg body weight [42]. The dose used in the present study to induce DM was 70 mg/kg body weight. The body weight gain in the T1DRM at the end of the experimental period was significantly reduced compared to that of the healthy group. Moreover, the body weight values of the insulin-treated group were higher than in the T1DRM group. These data are comparable to those in the study of Scridon et al. [43] that indicated less body weight gain in the diabetic rats, even with increased consumption of food and water.

The glucose level was affected in the STZ-induced T1DRM relative to the control group and significant hyperglycemia was noted. This finding corresponds with that of a previous study performed by Akbarzadeh *et al*. [44]. In line with a previous work [45], the rats were considered diabetic if serum glucose levels surpassed 250 mg/dL. The targeting of the β-cells by STZ was mainly due to its uptake via the GLUT2 glucose transporter and the following drug accumulation events [46].

The parameters related to the liver and kidney functions were affected in the STZ-induced T1DRM relative to the control group. A significant increase was showed in the urea, uric acid, and CREA levels in the STZ-induced T1DRM compared to the normal group. In addition, a significant increase was observed in the ALT, AST, and ALP in the STZ-induced T1DRM group. Insulin administration significantly restored the kidney and liver functions by decreasing the measurable parameters. It was demonstrated earlier that in DM, chronic hyperglycemia can induce hepato renal dysfunction in addition to pancreatic β-cell dysfunction [47]. The kidney and liver dysfunction in DM may be caused by the decrease in oxidative enzymes and hepatocellular fat accumulation [48], which can lead to a significant increase in the activities of serum hepatic transaminases including AST, ALT and ALP. Many studies have shown that a single dose of streptozotocin can cause a rise in ALT and AST levels [40, 42 and 49]. Moreover, significant renal dysfunction, nephropathy, and renal impairment have also been reported in DM [43].

In the T1DRM, the hemogram was affected, with the Hb concentration and the HCT, MCHC, RBC, and PLT counts significantly lower than in the healthy group. However, a significant increase was noticed in the Hb concentration and the HCT and PLT counts after insulin supplementation. In the STZ-induced T1DRM, compared to the healthy group, there was a significant rise in the neutrophilia (NEUT)% and leukocytosis (LEUK)%, while the lymphocytosis (LYMP)% was reduced. These parameters were restored after the supplementation of insulin. Anemia has been previously documented as a common complication in diabetic animals and humans [50]. A study by Agrawal et al. [51] reported that accumulation of glucose inside the cells might lead to cell membrane deformity followed by hemolysis and a reduced cell number. Low erythropoietin synthesis due to renal dysfunction was found as another cause of anemia in DM [52]. Erythrocytes were previously described as being the first cells affected in diabetic patients [53]**.** The findings in the leukogram reported in the present study correspond to previous observations [54]**.** Yakhchalian et al. [55] reported that the diabetic group had significantly elevated leukocytosis compared to the normal control group. The NEUT% was also elevated in the diabetic group. They also documented the apparent mechanism of leukocytosis as the oxidative stress that occurs in DM.

Concerning the effect of different types of colored rice in the present study, body weight gain was observed in all rats supplemented with rice. The highest gain was in those groups supplemented with 20% red rice, 20% black rice, or red rice extract, while the lowest gain was seen in the groups supplemented with white rice, both extract and whole rice. These data confirmed the role of colored rice in weight management. Colored rice is considered a functional food because of its antioxidant properties, which are essential for optimal health and well-being [56]. Regarding the glucose level, supplementation with colored rice extracts via intubation or 20% of the feed significantly restored the glucose level, except for the white rice, which exhibited no significant effect. Our data indicated that the red and brown rice, in particular, led to a significant decrease in the blood glucose level, either as an extract or as 20% whole rice supplementation. The variation in the color of the rice is due to the anthocyanin compounds that produce red, purple, and black pigments in plants. These pigments provide antioxidant and radical scavenging activities and decrease hyperglycemia in diabetic mice [23]. Rice also contains flavonoids that are known for their ability to lower the glucose level through the release of insulin from the beta cells. This is accomplished by increasing Ca2+ in the Langerhans islet cells [13] or stimulation of the glucose uptake into the peripheral tissues [57]. Our data showed that supplementation of black rice as 20% of the feed resulted in a significant elevation of GSH-Px, SOD, and CAT.

Administration of colored rice extracts via intubation or 20% supplementation in the feed significantly restored the kidney and liver function parameters, except for the white rice, which exhibited no significant effect. Black rice extract, in particular, significantly led to a decrease in the levels of uric acid, ALT, AST, and ALP. The supplementation of 20% whole black rice resulted in a significant decrease in the levels of CREA and AST. The CREA, uric acid, and ALP levels were also significantly reduced by brown rice extract supplementation. However, 20% whole brown rice supplementation resulted in a significant decline in the levels of urea, CREA, ALT, AST, and ALP. On the other hand, the supplementation of 20% whole red rice resulted in decreases in urea, CREA, uric acid, and ALP. Anthocyanins, especially cyanidin-3-glucoside (C3G), have been shown to reduce the inflammation of adipose and liver steatosis in diabetic rats [58, 23]. Based on previous studies, natural medicinal components derived from plants have been proposed as valuable alternatives in the management of hepatotoxic conditions [59]. Significantly, the health-promoting effects of the components of colored rice and its antioxidant capacity have been consistently recognized. In addition, a number of studies have reported its ability to protect the liver from damage [60]. Hou et al. [61] found that the addition of black rice to the diet was shown to alleviate the causes of fatty liver disease, signifying its direct effect on liver health. This finding was attributed to its antioxidant capacity to prevent liver toxicity and to improve liver function. In addition, black rice was reported to increase fatty acid metabolism and reduce the risk of hyperglycemia and cholestrolemia [61].

In the present study, hemogram parameters were restored after the supplementation of extracts from different types of colored rice. Supplementation of black, brown, or red rice resulted in a significant increase in the RBC and PLT counts, Hb concentration, and HCT% compared to the T1DRM group. However, the supplementation of 20% whole black or brown rice showed the same results, in which the Hb concentration and the RBC and PLT counts were significantly more elevated than in the T1DRM group. In addition, relative to the T1DRM group, a significant increase was observed in the RBC and PLT counts, Hb concentration, and HCT% after the 20% red rice supplementation. The significant effect of different varieties of colored rice on the hemogram may have been due to the iron and polyphenol content in addition to the antioxidant capacities. Yodmanee et al. [62] noted that darker-colored-grain had higher contents of iron and polyphenol as well as antioxidant capacities than light-colored grain. Other studies have also reported higher total phenolics in red and purple rice than in light-colored rice [17, 18].

The supplementation of black, brown, or red rice extract resulted in a significant lowering of the WBCs and NEUT% and increase in the LYMP% compared to the STZ-induced T1DRM. However, the supplementation of 20% black rice resulted in a decrease in WBCs relative only to the T1DRM. The anti-inflammatory impact of colored rice was previously documented as having a strong effect in

diabetic rats due to its high phenol content. Mira, et al. [15] reported that the total phenolic content was four times higher in colored rice than in white rice.

CONCLUSION

Finally, the results of the current study suggest that the replacement of whole-grain pigmented rice for white rice may help to lower the risk of DM complications. The current study recommends the consumption of pigmented rice, as it has been shown to exhibit good antidiabetic activity in experimental animals. Hence, due to its antioxidant content, pigmented rice is an exceptional alternative to white rice. Further extensive research is needed on the use of pigmented rice supplementation to manage the complications of this disease in humans and for it to be considered as a functional food because of its nutraceutical value.

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AUTHOR CONTRIBUTIONS

TIA and AAZ contributed to conceptualization, project Administration. TIA contributed to data curation and funding acquisition. SMA, SAA and FAMA shared in formal analysis, investigation and methodology. TIA and AAZ participated in validation**,** visualization and writing the original draft preparation. SMA, SAA and FAMA participated in reviewing and editing. All authors read and approved the final manuscript for publication.

CONFLICT OF INTEREST

The data and research results are honest, and the authors confirm that there are no conflicts of interest in this work.

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