Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Antioxidant extract of black rice prevents renal dysfunction and renal fibrosis caused by ethanol-induced toxicity

Extrato antioxidante de arroz-preto previne disfunção renal e fibrose renal causada pela toxicidade induzida pelo etanol

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Abstract

This study was conducted to evaluate the protective role of extracted natural antioxidants from black rice and their effect on kidney failure and renal cirrhosis caused by ethanol-induced toxicity. Antioxidant activity in terms of total phenol content, flavonoid compounds and anthocyanin, as well as antioxidant capacity, was determined in an extract of black rice. The findings noted that the black rice extract contained high amounts of antioxidant activity and capacity. Total phenolic compounds from black rice extract were fractionated using HPLC and the results showed that ferulic, sinapic, ascorbic, salicylic and coumaric acids were the highest in the extract. Biological experiments were performed on male albino adult rats (40 animals, 10 rats for each group), divided into four groups. After five weeks, kidney functions and protein fractions were assessed. In addition, superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) enzyme activities were determined in all groups. The results found that kidney function, total protein, albumin and globulin were affected by renal dysfunction and renal fibrosis in the positive control (PC), whereas groups 3 and 4 noted an improvement in renal function nearly or equal to the healthy rats which were fed on a basal diet. Furthermore, the PC group showed significantly decreased levels of enzymatic antioxidants, namely SOD and GSH with a concomitant elevated MDA level compared with those in the negative rats fed on a basal diet. Groups 3 and 4 also reported improvements in enzyme activity. These results were further supported by histopathological findings which revealed a curative effect in groups 3 and 4, which avoided renal dysfunction and renal fibrosis from ethanol-induced toxicity. From the results, it can be said that the black rice extract with the highest amounts of antioxidants led to improvements in all parameters, especially kidney function, total protein, albumin, and globulin, in addition to enzyme activity. Therefore, black rice can be recommended as a benefit to general health.

Keywords: antioxidant-black rice, renal dysfunction, renal fibrosis.

Resumo

Este estudo foi conduzido para avaliar o papel protetor dos antioxidantes naturais extraídos do arroz-preto e seu efeito na insuficiência renal e cirrose renal causada pela toxicidade induzida pelo etanol. A atividade antioxidante em termos de teor de fenóis totais, compostos flavonoides e antocianinas, bem como a capacidade antioxidante, foi determinada em um extrato de arroz-preto. As descobertas observaram que o extrato de arroz-preto continha grandes quantidades de atividade e capacidade antioxidante. Os compostos fenólicos totais do extrato de arroz-preto foram fracionados por HPLC e os resultados mostraram que os ácidos ferúlico, sinápico, ascórbico, salicílico e cumárico foram os mais elevados no extrato. Experimentos biológicos foram realizados em ratos adultos albinos machos (40 animais, 10 ratos para cada grupo), divididos em quatro grupos. Após cinco semanas, as funções renais e frações proteicas foram avaliadas. Além disso, as atividades das enzimas superóxido dismutase (SOD), glutationa (GSH) e malondialdeído (MDA) foram determinadas em todos os grupos. Os resultados mostraram que a função renal, proteína total, albumina e globulina foram afetadas por disfunção renal e fibrose renal no controle positivo (CP), enquanto os grupos 3 e 4 observaram uma melhora na função renal quase ou igual aos ratos saudáveis que foram alimentados em uma dieta básica. Além disso, o grupo PC apresentou níveis significativamente diminuídos de antioxidantes enzimáticos, ou seja, SOD e GSH com um nível concomitante de MDA elevado em comparação com aqueles nos ratos negativos alimentados com uma dieta basal. Os grupos 3 e 4 também relataram melhorias na atividade enzimática. Esses resultados foram ainda apoiados por achados histopatológicos que revelaram um efeito curativo nos grupos 3 e 4, que evitou a disfunção renal e fibrose renal por toxicidade induzida pelo etanol. A partir dos resultados, pode-se dizer que o extrato de arroz-preto com maior quantidade de antioxidantes levou a melhorias em todos os parâmetros, principalmente função renal, proteína total, albumina e globulina, além da atividade enzimática. Portanto, o arroz-preto pode ser recomendado como um benefício para a saúde geral.

Palavras-chave: arroz-preto antioxidante, disfunção renal, fibrose renal.

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Received: March 9, 2022 – Accepted: July 22, 2022

1. Introduction

Rice (*Oryza sativa Linn.*) is one of the main cereal crops, having great significance to more than half of the world (Clampett et al., 2002; Hansakul et al., 2011). It has a greatly significant role in diet and health because it contains bioactive compounds like minerals, vitamins and polyphenols, and a lot of antioxidative phytochemicals and anthocyanins (Clampett et al., 2002; Lum and Chong, 2012). Antioxidant activity has characteristics including being non-toxic. Thus, they have sufficiently great health effects and it are utilised as an additive in the food industry (De Pascual-Teresa et al., 2002; Kong et al., 2003).

Black rice (*Zizania aquatica*) is a type of rice that contains anthocyanin pigments. It has high levels of nutritious chemicals and amino acids. It has plenty of health benefits, such as natural antioxidation, anti-inflammatory properties, lipid oxidation, lowering sugar levels in the blood and anti-cancer effects (Kumar and Murali, 2020). Purple rice is a source of natural anthocyanin compounds for consumers who include rice as part of their basic diet. Anthocyanin is a major antioxidant compound which scavenges the free radicals that cause cellular damage in humans. The benefits to human health are apparent against both chronic and non-chronic diseases (Yamuangmorn and Prom-u-Thai, 2021).

Black rice (BlR) is particularly high in phytochemicals, anthocyanin colours, protein, and vitamins. BlR's capacity as an antioxidant is well recognised (Sompong et al., 2011). Antioxidants are essential for improving memory and boosting the immune system. According to Choi et al. (2007), coloured rice bran pigments have been shown to block allergy responses in vitro. Peonidin, peonidin 3-glucoside, cyanidin 3-glucoside, and other significant anthocyanins of black rice have been shown to inhibit the invasion of cancer cells, according to Chen et al. (2006). According to Ichikawa et al. (2001), BlR are effective and two times stronger when it comes to the antioxidant properties of blueberries.

Ethanol plays a significant role in fat oxidation, which may be due to its solubility in water and fat. Thus, it can diffuse across the gastric membrane and appear in the urine (Abraham et al.., 2002). Oxidative stress from alcohol may be due to ethanol.

The kidney is a significant organ that has not only excretory functions but also other functions, like the production of substances that activate enzymatic reactions, immunisation and others. Ethanol and its metabolites pass through the kidneys and are excreted in the urine and its rise the content of than that of the blood and the liver. The kidney is a vital organ that is mostly excluded in chronic alcoholics who do not have hepatorenal syndrome. However, regular consumption of alcohol can cause kidney damage (Chander et al., 2003). Experimental research in animals has observed that ethanol promoted fatty acid oxidation by renal microsomes and peroxisomes and influenced the activities of some renal lysosome hydrolyses. Chronic ethanol intake leads to lowered antioxidant defence. Therefore, chronic alcohol consumption may directly or indirectly accelerate the oxidation mechanism, ultimately leading to cell death and tissue damage (Olayinka and Ore, 2012).

Oxidative stress is the main pathway of alcohol-induced kidney injury. Oxidative stress is the term used to characterise an imbalance that favours oxidants and/or unfavourable antioxidants, which can lead to damage (Aly et al., 2010). It is assumed that observed renal failure is only secondary to deficiencies in hepatic function. Thus, a potentially contributing role for concurrent events is ignored in view of the marked increase in lipid accumulation in ethanol-fed mice, the observed renal hypertrophy in autopsy material from alcoholics with mild liver disease, and recent evidence that ethanol is toxic to a diversity of excess liver tissues (Alcohol & Alcoholism, 1972; Van Thiel et al., 1975).

According to epidemiological research, the antioxidant component content of rice may have a role in the low occurrence of several chronic illnesses in rice-eating parts of the world. Rice contains phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol, and phytic acid, all of which have antioxidant properties. For instance, black rice variations showed the greatest antioxidant activity among the four types of rice classified by colour, followed by purple, red, and brown rice varieties. It is obvious that rice should be ingested whole grain or with bran in order to increase the intake of antioxidant chemicals (Goufo and Trindade, 2014).

Therefore, the present study aims to extract natural antioxidants from black rice, then examine their preventive effect against renal dysfunction and renal fibrosis caused by ethanol-induced toxicity. In addition, the effect of antioxidants on oxidative enzymes was determined and the kidney was histologically examined.

2. Materials and Methods

2.1. Black rice extract

Black rice was requested from the Agriculture Research Centre, Giza, Egypt. The extraction was performed on 100 g of black rice using ethyl alcohol (150 ml) for 24 h. The solvent was twice extracted, collected and kept at 4 °C until use.

2.2. Estimating antioxidant activity from the black rice extract

The total phenolic content was determined as gallic acid equivalents (GAE mg/g of dry weight) according to Qawasmeh et al. (2012).

The total flavonoids from the black rice extract were measured according to Eghdami and Sadeghi (2010). The results were expressed in terms of milligrams of quercetin equivalents (QEmg/g of dry weight).

Anthocyanin was determined as mg cyanidin chloride equivalent (mg CCE) per g dry weight according to Kim et al. (2008).

2.3. Determining antioxidant capacity

The scavenging effects on radical ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] of the black rice extract were determined as mg Trolox equivalent (TEAC/g) according to Chalermpong et al. (2012).

The ferric reducing antioxidant power (FRAP) of the extract was determined as mg Fe2SO4 equivalents/g according to Suwannalert et al. (2010). The lipid peroxidation inhibition was determined as the percent of linoleic acid peroxidation= (1-A532 of sample/A532 of control) × 100, according to Chalermpong et al. (2012).

2.4. Quantitative and determination of phenolic compounds by HPLC

According to the procedure described by Goupy et al. (1999), phenolic compounds were identified in black rice by HPLC as follows: Samples weighing 5 gm were combined with methanol, centrifuged at 10,000 rpm for 10 min, and the supernatant was filtered through a 0.2 m Millipore membrane filter. Then, 1-3 ml of the filtrate was collected in a vial for injection into HPLC. Hewlet Packared (series 1050) is outfitted with an ultraviolet (UV) detector set at 280 nm, an auto-sampling injection, a solvent degasser, and a quaternary HP pump (series 1050). In order to inject phenolic acid standards into HPLC, they were first dissolved in a mobile phase. Using data from Hewlett-Packard software, retention duration and peak area were utilised to calculate the concentration of phenolic chemicals.

2.5. Biological methods

Male albino rats (40 rats weighing 170 ± 2g, 10 rats for each group) were used and maintained at the animal house of the Institute for Researcher and Medical Consolation (IRMC, Dammam, Saudi Arabia). Rats were fed on basal diet ingredients like corn oil (10%), vitamin premix (1%), mineral premix (1%), oyster shell (1%), bone meal (2%), salt (0.25%) and non-nutritive cellulose (5%). Test protein-casein (10%) and corn starch (70%) were added according to Pell et al. (1992) for seven days. The rats were divided into 4 groups, 10 rats in each:

G1: negative control (NC) fed on basal diet.

G2: PC was given ethyl alcohol orally (6 mg/kg bw/day).

G3: treated with ethyl alcohol (6 mg/kg bw/day) + black rice ethyl extract (100 mg/kg bw/day).

G4: treated with ethyl alcohol (6 mg/kg bw/day) + black rice ethyl extract (200mg/kg bw/day).

After 5 weeks, blood samples were collected and centrifuged to obtain the serum.

Institutional Review Board (IRB): 2015-10- 239.

2.6. Biochemical assays in serum

Serum kidney functions, protein fractions and the lipid peroxidation as malondialdehyde (MDA) were determined by kits.

The plasma superoxide dismutase (SOD) was measured according to Ohkuma et al. (1982). Glutathione (GSH) was tested according to Ellman (1959). The results were expressed as µmol/g tissue.

2.7. Renal histology

After five weeks, the rats' kidneys were treated with buffered formalin and placed in paraffin with a melting point at 55–57 ºC. Three to four-micrometre sections were cut and stained with haematoxylin-eosin and Masson's trichrome stains. Then, it was examined under a light microscope.

2.8. Statistical analysis

The variances between groups were estimated using analysis by variance (ANOVA), LSD test and the significant was given at P ≤ 0.05. The analysis was conducted using the ANOVA procedure of the Statistical Analysis System.

3. Results and Discussion

3.1. Antioxidant content and capacity of black rice

The antioxidant content was determined in the black rice ethanol extract. The data are tabulated in Table 1. The results revealed elevated levels of phenolic acid (250.30 mg GA/g). The flavonoid compounds were recorded as 12.57 mg Q/g and anthocyanin was 395.76 mg cyanidin chloride/g). The black rice ethanol extract was found to be rich in flavonoid compounds and phenolic acids. Anthocyanins are a water-soluble antioxidant. The purple-coloured pigments are very nutritious (Saeed and Saeed, 2020).

Furthermore, the same table explains the total antioxidant capacity for the black rice ethanol extract is necessary to know their free radical scavenging ability. The antioxidant properties assessed were regarding ABTS, FRAP and lipid peroxidation (MDA).

The black rice ethanol extract showed the highest in the ABTS assay. The Trolox equivalent was 350.38 ±

Table 1. Antioxidant activity and capacity of BREE.

Antioxidant activity	BREE	Antioxidant capacity	BREE
Total phenolic acids mg GAE /g of dry weight	250.52 ± 6.38	ABTS mg Trolox equiv/g	350.38 ± 8.29
Total flavonoid mg QE /g dry weight	12.57 ± 1.25	FRAP mg FeSO4equiv/g	42.12 ± 2.73
Anthocyanin mg CCE/g dry weight	359.76 ± 7.16	Lipid peroxidation (%)	95.64 ± 4.68

Values are means ± SD (n = 3).

16.16 mg Trolox equivalent/g of extract. Moreover, the FRAP had the highest Fe2SO4equivalents of black rice, at 42.12 mg of Fe2SO4equivalent/1 g. In addition, the lipid peroxidation (MDA) assay results indicated that the black rice was recorded as 95.46% of inhibition/1 mg of ethanol extract. Most plant extracts that have antioxidant activities as a result of the content of various phenolic compounds. These compounds usually contain at least one hydroxyl substituted aromatic ring system that can readily be an essential mechanism of antioxidants (Kovacs and Keresztes, 2002; Bilto et al. 2012). Moreover, Ben Hassine et al. (2021) reported the total phenolic content was highly correlated with ABTS activities. Therefore, this study has clarified the significance of the content of phenolic acids as a reducing agent, which may be the reason for their strong electron-donating abilities.

3.2. Fractionation and quantification of phenolic acids extracted from black rice by HPLC

Total phenolic compounds were fractionated from black rice extracted using HPLC and the results in Table 2 are illustrated that the ferulic, sinapic, ascorbic, salicylic, and *P*-coumaric acids were the highest amounted by 69.43, 31.68, 17.38, 15.29 and 10.71 mg/100g, respectively. Meanwhile, the vanillic, syringic, P-hydroxybenzoic, and caffeic acids were medium amounted in black rice extract (6.50, 3.15, 2.14, and 1.83 mg/100g, respectively). Moreover, gallic, and chiorogenic were the lowest phenolic compounds amounted in black rice extract.

Ferulic acid (56–77% of total phenolic acids) is the most prevalent phenolic acid found in black rice whole grain, followed by P-coumaric acid (8–24%), sinapic acid (2–12%), gallic acid (1-6%), protocatechuic acid (1-4%), p-hydroxybenzoic acid (1-2%), vanillic acid (1%), and syringic acid (1%). Caffeic, chlorogenic, cinnamic, and ellagic acids are minor components that make up less than 1% of the total phenolic acids. This placement is in line with the metrics provided by Goufo et al. (2014).

Table 2. Phenolic acids content extracted from black rice by HPLC.

Phenolic acids compounds	Concentration mg/100g	Concentration %
Ascorbic acid	17.38	10.92
P-Cumaric acid	10.71	6.73
Ferulic acid	69.43	43.64
P-Hydroxybenzoic acid	2.14	1.35
Vanillic acid	6.50	4.09
Gallic acid	0.82	0.52
Caffeic acid	1.83	1.15
Chlorogenic acid	0.17	0.04
Syringic acid	3.15	1.98
Sinapic acid	31.68	19.91
Salicylic acid	15.29	9.61
Total	159.10	99.94

According to Sumczynski et al. (2016), there are noticeable variations in the kinds and quantities of phenolic chemicals present in various rice cultivars. Numerous elements, including as harvesting and planting techniques, growing environments, variety, the ripening process, storage, and the extraction process, have an impact on the synthesis of phenolic compounds in grains.

It has being investigated if polyphenols can treat or prevent renal problems (Noce et al., 2020). The capacity of these substances to regulate redox and inflammatory pathways fundamentally underpins the interest in them. According to scientific data, inflammation and reactive oxygen species (ROS) are important factors in the pathophysiologic processes of renal disorders. According to Daenen et al. (2019), oxidative damage is linked to a variety of renal impairments, including acute renal failure, obstructive nephropathy, and chronic renal failure. The kidney is a particularly sensitive organ to ROS assault (Ling and Kuo, 2018). Therefore, nutritional and pharmaceutical anti-inflammatory and antioxidant therapies might lessen kidney damage (Kalantar-Zadeh et al., 2021).

3.3. Effect of black rice extract on renal functions

This study concentrates on the effect of chronic ethanol consumption and black rice ethanol extract as a kidney protection factor, in addition, the determination of kidney function-related parameters is shown in Table 3.

The positive group of rats exposed to ethanol had the highest urea and creatinine compared to the different groups. Although Monder et al. (1989) found that alcohol consumption was significantly connected to lowered kidney function, more research observed that the ingestion of 5 mg/kg body weight/day of ethanol for two months in male rats increased kidney functions (Motilva et al., 1994). On other hand, uric acid concentration was significantly reduced in the PC compared to the NC (Table 2). However, the two groups of black rice ethanol extract showed significantly decreased urea and creatinine, and elevated concentrations of uric acid compared with the PC. High doses of the black rice ethanol extract produced results close to the NC in urea, creatinine, and uric acid, respectively. When the rats were given ethanol orally (1.6 mg/kg body weight/day) for 12 weeks, the results demonstrated that the serum urea and creatinine increased significantly (Ameer et al., 1996). The measurement of serum creatinine is the method most frequently used to assess renal function. However, a significant rise in urea and creatinine levels might signal renal failure and the subsequent retention of urea and creatinine in the blood (Amin et al., 2016). In mammals, the main byproduct of protein catabolism is urea, which serves as the body's main transporter for the removal of toxic ammonia. Greater serum urea levels subsequently result in higher serum ammonium levels. Additionally, elevated urea levels are associated with nephritis, renal ischemia, and generalised obstruction of the urinary system; hence, urea measurement is very helpful for monitoring kidney function (Taki et al., 2005).

3.4. Influence of black rice extract on serum protein fraction on ethanolic administrated rats

The effects of the experiments with ethanol and black rice ethyl extract (BREE) on the total protein, albumin, globulin and albumin/globulin (A/G) ratio in rats are shown in Table 4. The results illustrated a significant decrease in the tested parameters compared to the NC group. This general increase in all parameters was ameliorated by treatment with BREE, especially when treated with BREE at 200mg/kg body weight. Its results were close to the NC group. The A/G ratio highly increased in the PC compared to other groups; on other hand, BREE at 200mg/ kg body weight gave better results compared to BREE at 100mg/ kg body weight. The higher total protein level in the treatment groups may be due to the BREE and the rich amounts of antioxidant activity (Nout et al., 1995), leading to increased amino acid absorption in intestinal tissues and increased protein synthesis (Ritchie et al., 1999). The albumin level was elevated in the treatment groups, probably due to the anti-inflammatory activity of the BREE (Minaiyan et al., 2014). The greatest globulin contents may have been caused by the immune-stimulating activity of the bioactive molecules contained within BREE (Tian et al., 2016).

3.5. Influence of BREE on enzyme activities on ethanolic administrated rats

The effect of chronic ethanol feeding on the content of MDA, GSH and SOD and on the usage of the BREE is tabulated in Table 5. The group as control positive group, which was treated with ethanol, recorded reduced levels for GSH, SOD and MDA compared to the control healthy

Table 3. Influence of BREE on urea, creatinine, and uric acid in ethanolic administrated rats.

Treatment	Urea mg /dL	Creatinine mg / dL	Uric acid mg/ dL
G1 Negative control(NC)	25.07c ± 0.07	0.84c ± 0.00	1.89a ± 0.00
G2 Positive control(PC)	64.84a ± 0.23	2.32a ± 0.07	0.72c ± 0.13
G3(100 mg/kg bw black rice ethyl extract (BREE)	39.32b ± 0.20	1.31b ± 0.03	1.31b ± 0.20
G4(200 mg/kg bw black rice ethyl extract BREE)	25.58c ± 0.16	0.88c ± 0.02	1.79a ± 0.09
Least Significant Difference (LSD) Test	0.52797	0.119	0.108

Values are means ± SD (dev for 10 rats in each group). Values that have a different superscript letter (a, b, c,) differ significantly with each other ($p \leq 0.05$).

Table 4. Influence of black rice ethyl extract (BREE)on protein fractions in ethanolic administered rats.

Treatment	Protein g/ dL	Albumin g/ dL	Globulin g/ dL	A/ G Ratio
G1 Negative control(NC)	8.68a ± 0.10	5.088a ± 0.071	3.60a ± 0.10	4.27d ± 0.15
G2 Positive control(PC)	4.87c ± 0.09	1.882c ± 0.061	2.99b ± 0.12	17.22a ± 0.28
G3(100 mg/kg bw black rice ethyl extract (BREE)	6.90b ± 0.18	3.300b ± 0.098	3.60a ± 0.24	14.94b ± 0.23
G4(200 mg/kg bw black rice ethyl extract BREE)	8.53a ± 0.09	4.947a ± 0.057	3.59a ± 0.09	8.98c ± 0.23
Least Significant Difference (LSD) Test	0.252	0.153	0.310	0.470

Values are means ± SD (dev for 10 rats in each group). Values that have a different superscript letter (a, b, c, d) differ significantly with each other ($p \leq 0.05$).

Table 5. Influence of black rice ethyl extract (BREE) on enzymes activity in ethanolic administrated rats.

Treatment	Malondialdehyde (MDA) nmol/mL	Glutathione (GSH) mmol/g	Superoxide dismutase (SOD) U/mL
G1 Negative control(NC)	25.40c ± 0.17	14.850a ± 0.146	5.35a ± 0.18
G2 Positive control(PC)	55.23a ± 0.19	7.970c ± 0.056	1.97c ± 0.08
G3(100 mg/kg bw black rice ethyl extract (BREE)	35.05b ± 0.25	11.100b ± 0.105	3.26b ± 0.13
G4(200 mg/kg bw black rice ethyl extract BREE)	26.13c ± 0.09	14.368a ± 0.210	5.00a ± 0.04
Least Significant Difference (LSD) Test	0.384	0.295	0.251

Values are means ± SD (dev for 10 rats in each group. Values that have a different superscript letter (a, b, c,) differ significantly with each other ($p \leq 0.05$).

rats. These results were confirmed by Jang et al. (2012) and Al-Jameel and Abd El-Rahman (2018). The amount of GSH, SOD and MDA was 7.970 ± 0.056 mmol/g, 1.97d ± 0.08U/ml, and 55.23 ± 0.19 nmol/ml after 5 weeks, respectively. Table 4 demonstrates that the increases in the groups that were given BREE at 100 and 200 mg/kg bw in GSH and SOD levels compared with the PC group for GSH, and SOD were 11.100c ± 0.105 and 14.368b ± 0.210mmol/ml, and 3.26c ± 0.13, and 5.00b ± 0.04U/ml at 100 and 200mg/kg body weight, respectively. These results are in agreement with Al-Jameel and Al-Namshan (2017) and Al-Jameel and Abd El-Rahman (2018), who reported that taking ethanol extract of black rice with ethanol had a significant effect on the non-enzymatic and enzymatic antioxidant activities close to the levels of the control group.

The level of MDA was reduced significantly in the groups that were treated with BREE. There were no significant differences ($P \leq 0.05$) between the NC group and the group given BREE at 200mg/kg body weight in all oxidative stress parameters (MDA, GSH and SOD). The decrease in GSH and SOD and increase in MDA could be due to the ineffective scavenging of ROS which may be involved in the oxidative disruption of enzymes (Jayaraman et al., 2009), which demonstrates that the extract is a powerful antioxidant (Arulmozhi et al., 2012). Moreover, the antioxidant and radical scavenging properties of the extract may be due to the high polyphenol content (Zhang et al., 2010). Hou et al. (2010) observed that ethanol taken orally may be the reason for a sharp elevation in MDA and a reduction in GSH levels in rats, whereas the BREE contained anthocyanin which decreased MDA formation. Moreover, SOD and GSH were significantly lower in the PC rats compared to the NC rats. The BREE-treated groups (100 and 200 mg/kg body weight) significantly attenuated the SOD and GSH to levels close to normal (Al-Jameel and Al-Namshan, 2017; Al-Jameel and Abd El-Rahman, 2018).

3.6. Histological experimental of kidney

Renal parenchyma tissues of the control rats were normal and were like those described in previous literature for mammalian kidneys, including rats (Figure 1, shape. 2). Distal tubules showed unstained cells with dark-spotted nuclei. The tubules have extended lumina and contain glass moulds (Figure 1, shape 1). A marked increase in the grade of distal tubular dilatation was observed in group 2 (PC) (Figure 2, shape 2) compared to NC and other groups.

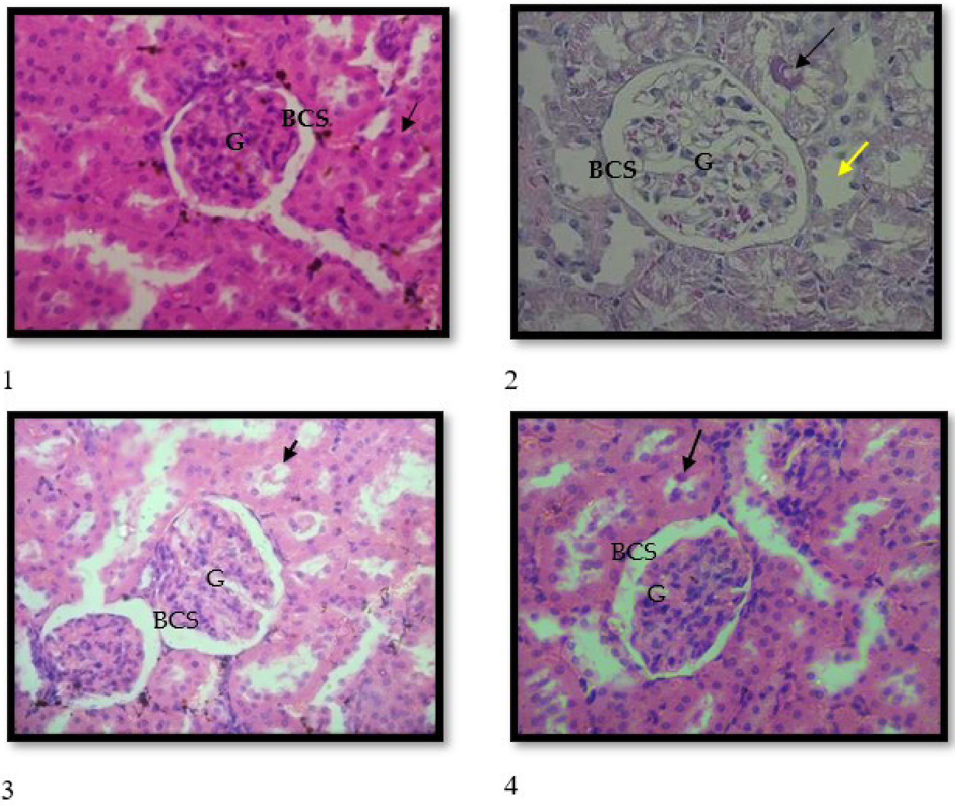

Figure 1. Histopathological changes of kidney sections of 1) NC (Normal Control group) Control section of kidney showing cortical parenchyma to consist of dense rounded structures, the glomeruli (G), surrounded by narrow Bowman's capsular spaces (BCS); 2) PC (Positive Control group) showing glomeruli with mild mesangial proliferation (G), moderate degree of chronic interstitial inflammatory infiltrate and tubular epithelial cells focal degeneration, Cloudy swelling tubular cells with narrow (arrow) or obliterated (yellow arrow); 3) 100 mg/kg bw BREE showing mild interstitial inflammation in the interstitium (arrow); 4) 200 mg/kg bw BREE showing normal appearance of glomerular capillary tuft (G) and Bowman's capsule basement membrane (BCS) and a clear improvement in the general shape of tubes and cells. H&E (Mag. X400).

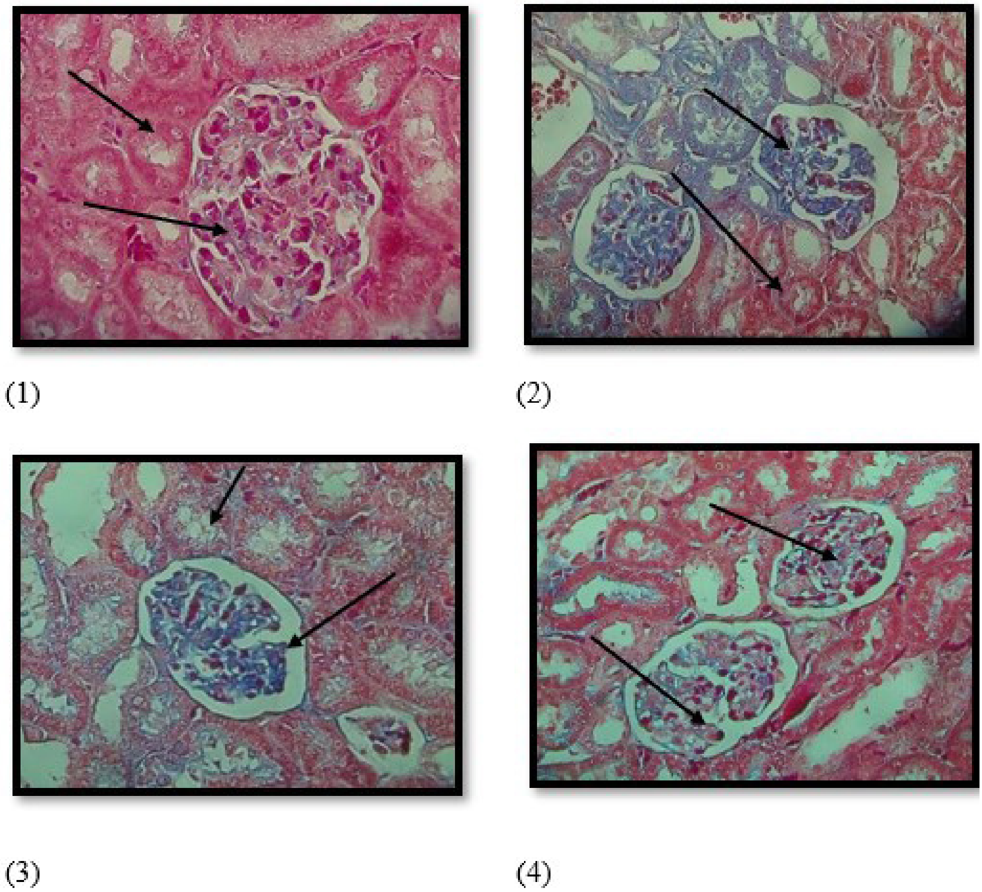

Figure 2. Histopathology of nephropathy in 1) NC (Normal Control group) showing minimal amount of collagen around renal tubules, capillary tuft and Bowman's capsules of glomerulus; 2) PC (Positive Control group) showing an increase of the collagen fibers around Bowman's capsule, capillary loops and convoluted tubules of glomerulus and both necrotic and apoptotic changes in the renal tubules; 3) 100 mg/kg bw BREE showing mild to moderate increased collagen fibres; 4) 200 mg/kg bw BREE. Showing a little amount of collagen similar or close to NC. (Masson's Trichrome × 400).

The kidney vessels at the cortical-spinal junction of the PC group observed massive degeneration of vascular elements and perivascular edema (Figures 1 and 2). Histological features in the kidneys were present bilaterally and were normal in all groups. Muna and Aticka (2009) noticed histological influences of kidneys in rats as a control positive and verified that the main microscopic alteration was hyperplasia of the epithelial cells lining the kidney tubules, with demineralisation and necrosis of some tubules. In addition, lead damages membrane-associated enzymes that lead to renal tubular injury (Plumlee, 2004).

On histological examination, the sagittal section of the kidneys of the control group observed highly cortical renal corpuscles, which consisted of rings of glomerular capillaries, surrounded by Bowman's capsules, with an intracapsular space. The cortical region indicated a cross-section of proximal and distal convoluted tubules lined with simple cuboidal epithelium (Figure 2, shape 1). The PC group presented with manifestations of distension, severe degeneration and contraction of glomeruli, Bowman's capsules and related tubule structures, edema of renal tubules, and urinary space elevation in renal tissue compared to the NC (Figure 2, shape 2). Figure 2, shapes 3 and 4 show a significant improvement appears compared to the PC. The biggest advantage of black rice is that it contains antioxidants.

4. Conclusions

BREE contained high amounts of phenolic acid (250.30 mg GA/g). The flavonoid compounds totalled 12.57 mg Q/g and anthocyanin was 395.76 mg of cyanidin chloride/g. Total phenolic compounds from black rice extract were fractionated using HPLC and the results showed that ferulic, sinapic, ascorbic, salicylic and coumaric acids were the highest in the extract. The group of rats with toxicity induced by ethanol were treated with ethyl alcohol by using 6 mg/kg bw/day with BREE (100 and 200mg/kg body weight/day), to improve the kidney function, total protein, albumin and globulin. In addition, enzyme activity prevents renal dysfunction and renal fibrosis caused by ethanol-induced toxicity. The results of the renal histological examination confirmed the previous findings.

Acknowledgements

The author was grateful for the assistance of Prof. Soheir, N. Abd El-Rahman the Head Field Crop Technology Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

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