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PREVENTIVE EFFECT OF BLACK RICE ETHANOLIC EXTRACT AS NATURAL ANTIOXIDANTS ON SOME HEAVY METALS INDUCED DISORDERS IN EXPERIMENTAL ANIMAL

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ABSTRACT

Purpose: The present study investigated the protective effect of black rice ethanolic (60 and 80%) extract (BREE) on Drinking Water Heavy Metals (DWHM) induced liver injury, renal damage and alteration of lipid profile in male rats.

Design and methodology: Total phenols and total flavonoids in BREE were determined by HPLC. The study was conducted on 36 male Wistar rats weighing 193–195 g, the animals were divided into six equal groups. The first group was given Distilled Water (DW) and used as a control group (NC). The second group was administered DWHM (Mn = 1000 ppm; Hg = 10 ppm; Pb = 100 ppm, Ni = 100 ppm, Fe = 1200 ppm) and used as Positive Control (PC). The other groups of rats were administered BREE 60 and 80% (1 mg/1 ml) in DW and BREE 60% and 80% (1 mg/1 ml) in DWHM. Blood and tissue samples were collected after eight weeks. Lipid profile, hepatic markers, renal markers, Fasting Blood Sugar (FBS) and CA19.9 as tumour marker for (GIT) were determined. Also, histological changes in kidney tissue were studied.

Findings: The results revealed that the rats treated with DWHM showed a significant ($p \leq 0.05$) increase in levels of LDL-C, TG, FBS, ALT, AST, urea, creatinine, uric acid and CA19.9 and significant ($p \leq 0.05$) decrease in HDL-C level. Heavy Metal (HM) intoxication induces some pathological alterations in the

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kidney as degeneration and large lumen. The rise in serum hepatic enzymes, LDL-C, TG, FBS, urea, creatinine and uric acid and histopathological changes were significantly attenuated by BREE 60 and 80%. Moreover, the level of serum HDL-C in BREE 60 and 80% (1 mg/ 1 ml DWHM) groups showed a significant ($p \leq 0.05$) increase as compare with PC.

Research implications: The current results as certained the beneficial effects of BREE in controlling HMs induced disorders and the protection of liver and kidney against HMs intoxication in male rats.

Keywords: black rice; ethanolic extract; heavy metals; HMs; drinking water.

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INTRODUCTION

Water is considered one of the clear signs of prosperity, health, serenity, beauty, artistry, purity and many other attributes. Therefore, water contamination with Heavy Metals (HMs) has become the prime focus of environmental scientists (Hamby, 1996). HM in water may be derived from both natural and anthropogenic sources (Chanpiwat et al., 2010; Muhammad et al., 2010). It may contaminate the surface water and groundwater resulting in deterioration of drinking and irrigation water quality (Krishna et al., 2009), and considered as severe pollutants owing to their toxicity, persistence and bioaccumulative nature in environment (Pekey et al., 2004).

Metallic compounds on land and water pose potential health hazard not only to livestock and wild life but also to fishes, birds, mammals and even to human beings (Atef, 2011). Continuous environmental and occupational heavy metals exposure can lead to chronic nephropathy. However, many experimental studies showed that several HMs caused renal failure associated with severe histopatholgical and physiological alterations (Kutlubay and Oğuz, 2007; Suradkar et al., 2009; Soudani et al., 2010). High concentrations of manganese (Mn), and lead (Pb) are considered highly toxic for human and aquatic life (Ouyang et al., 2002). Also, the Ni-sulfate and Ni-chloride ingestion can cause severe health problems, including fatal cardiac arrest (Knight et al., 1997). The complete blood count is candetect many abnormalities including stress and toxic metal burden (Flaiban et al., 2008). Enzyme leakage into the blood following tissue damage can predict the site of tissue damage (Murray et al., 2010).

Rice (*Oryza sativa* Linn.) is the most important cereal crop in the world, either directly as human foods or indirectly as animal feeds. Phytochemicals are bio-active compounds that include phenylpropanoids, tannins, lignins, γ-oryzanol, tocotrienols, tocopherols, phenolics compounds and flavonoids. Most common groups of phenolic compounds are flavonoids which are water-soluble plant pigments with many colours (Hansakul et al., 2011). Phenolic compounds and flavonoid contents are potential antioxidative phytochemicals that can act as metal ion chelators, free radical scavengers and reducing agents thus offer human health benefits, which also can be found in pigmented rice (Lum and Chong, 2012; Srisawat et al., 2010). Additionally, pigmented rice extracts have been reported to effectively decrease inflammation and oxidative stress as well as atherosclerotic lesions (Xia et al., 2003). Black glutinous rice is the most famous one, generally used as an ingredient in snacks and desserts. Wholegrain pigmented rice has been categorised as one of the potent functional foods since it contains high amounts of phenolic compounds, especially anthocyanins in pericarp (Abdel-Aal et al., 2006; Yawadio et al., 2007). The pigment from black rice contains two major anthocyanins: cyanidin-3-glucoside andpeonidin-3-glucoside (Abdel-Aal et al., 2006; Hu et al., 2003). Anthocyanins can decrease the risk of coronary heart diseases, inflammatory process and atherosclerosis through their anti-oxidant, anti-platelet and anti-inflammatory activities (Hu et al., 2003; Xia et al., 2003). Phenolic antioxidants from plant materials, solvent extraction has mostly been used to obtain the phenolic fraction due to its simplicity and low cost. Organic solvents commonly used for the extraction include absolute methanol, ethanol and acetone (Sun and Ho, 2005; Yawadio et al., 2007; Yu et al., 2002). The mixtures of those organic solvents with water were also widely used (Awika et al., 2004; Nam et al., 2006; Pȇrez-Jimȇnez and Saura-Calixto, 2005). However, most studies on anti-oxidant activity of pigmented rice extract did not report details on the optimisation of the solvent extraction process. Moreover, no published data on the application of black rice ethanolic extract as an anti-oxidant to protect from HM toxicity. Therefore, our study was conducted to evaluate the effects of sub-chronicoral HMs (Mn, Hg, Fe, Ni, Pb) intoxication on blood indices, CA19.9 tumour marker, liver and kidney injuries of adult rats and efficacy of black rice ethanolic extract in reducing these effects.

MATERIALS AND METHODS

Materials

Black rice grains was obtained from Field Crops Research Institute. Adult male albino rats were obtained from animal house, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. All kits were purchased from Bio diagnostic Company, Dokki, Giza, Egypt. All other chemicals used were purchased from Algomhorya company, Giza, Egypt.

METHODS

Black rice ethanolic extract

Black rice was ground by blender (Waring 2–1 Laboratory Blender – The Lab Depot Inc). Then, a weighed portion (100 g) of black rice was extracted with 250 ml of ethyl alcohol 60 and 80% for 24 hr. The extract of ethyl alcohol was filtered through What man No. 4 filter paper. Each solvent extraction was repeated three times and each of the extract solution was combined and dried under vacuum rotary evaporator. All dried extracts were kept in the freezer (−20°C) until used.

High-performance liquid chromatography

Agilent 1100 HPLC equipped with multiwavelength detector set at 280 nm for determination of phenols/compounds, 330 nm for determination of flavonoids/compounds, degazer, Auto-sampler, qauternerypumb and column compartment set at 35°C. The column was used to fractionation these compound zorbox ODS 5 μm 4.6 × 250 mm. Flow rate of the mobile phase was set at 1 ml/min (Pirjo et al., 2000; Pascale et al., 1999).

Animals and experimental design

Eight-week-old male Wistar rats weighing 193–195 g were used. The animals were kept at constant room temperature (25°C) with 12 hr of light/dark cycles. All animals received normal rat chow and had access to Distilled Water (DW) *ad libitum* during the acclimatisation period. The individual animal body weight was recorded weekly throughout the experiment. We had six groups comprising of four groups each for (BREE at 60 and 80%) and (Mn, Hg, Fe, Ni, Pb + BREE 60 or 80%) the other groups include the control (positive and Negative Control (NC)). The basal diet prepared according to (AOAC, 2005); corn starch 70%, casein 10%, corn seed oil 10%, salts mixture 4%, vitamins mixture 1% and cellulose 5%.

Preparation of nutrient substance and HMs

Exposed groups received DW that contained 100 ppm lead acetate, 1000 ppm manganese chloride, 100 ppmnical chloride, 1200 ppm iron chloride and 10 ppm mercury chloride + 1 mg/ml water BREE 60 or 80%. The percentage (%) mixture was mirrored to the recommended daily dose for this nutrient substance (Yantasee et al., 2003).

Experimental protocol

All group received Basel diet according to AOAC (2005). Group 1 was fed normal rat (NC) received DW and either one (group 2) received Drinking Water Heavy Metals (DWHM) HMs solution (Mn = 1000 ppm; Hg = 10 ppm; Pb = 100 ppm, Ni = 100 ppm, Fe = 1200 ppm). Groups 3 and 4 were fed rat chow BREE 60% (1 mg/1 ml DW) and BREE 60% (1 mg/1 ml DWHM), Groups 5 and 6 were fed rat chow BREE 80% (1 mg/1 ml DW) and BREE 80% (1 mg/1 ml DWHM). All administrations were through the oral route. Total feed consumption was weighted, fresh feed was provided every day and total body weight of the animals was recorded at the beginning and during the experimental period. Blood samples were collected from the orbital plexus by mean of heparinized capillary glass tubes according (Schermer, 1967). Each sample was placed into a dry clean centrifuge tube and centrifuged 1500 × g for 30 min at 4°C to obtain serum.

Biochemical assays

Hematologic indices were determined according to standard methods (Hoffmann and Janssen, 2002). Tests included counts of Red Blood Cells (RBCs), White Blood Cells (WBCs) and PLT and the concentrations of hemoglobin (Hb) and hematocrit (Ht). All indices were measured according to the manufacturer's recommendations of the commercial hematology kits (Diamond Diagnostics, USA) using the fully automated hematology analyzer, Abbott Cell-Dyn 4000 (Abbott Diagnostics, Santa Clara, California, USA), as an experimental device.

TC, TG, HDL and LDL assays

Total Cholesterol (TC) was determined according to the method described by Allain et al. (1974) and triglycerides was determined according to the method described by Fossati and Prencipe (1982). High Density Lipoprotein Cholesterol (HDL-C) was determined according to the method described by Lopez-virella et al. (1977) and Low-Density Lipoprotein Cholesterol (LDL-C) levels were calculated for serum samples using the formula of Friedewald et al. (1972).

ALT and AST assays

Serum transaminasess AST and sALT (Aspartatetransferase and Alaninetransferase) were measured colorimetrically according to the method described by Reitaman and Frankel (1957).

Urea, Creatinine and Uric acid assays

Serum urea was determined according to Fawcett and Soctt (1960) and creatinine was determined according to the method of Barthes et al. (1972). Also, serum uric acid was measured by a modified carbonate-phosphotungstate method (Henry et al., 1957).

Determination of Fasting Blood Sugar (FBS)

FBS of the rats was measured at intervals using a glucometer with strips (Prestige IQ ® blood monitoring system, AR-Med LTD, Runny Mede Malthouse, Egham TW209BD, UK). A drop of blood is placed on the strip and the appropriate blood sugar concentration is displayed on the glucometer screen after 10–50 sec. The glucometer employs glucose oxidase principle for blood glucose measurement (Trinder, 1969).

Tumour markers

CA19.9 was measured in the serum using a commercially available immunometric assay kit (Immulite, DPC).

Histopathological examination

For microscopic evaluation, livers and kidneys were fixed in 10% neutral phosphate buffered formalin solution. Following dehydration in an ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5 μm were stained with hematoxylin-eosin (H-E). A minimum of 10 fields for each liver and kidney slide were examined and assigned for severity of changes by an observer blinded to the treatments of the animals.

STATISTICAL ANALYSIS

Results were expressed as mean SEM. The intergroup variation was measured by one way Analysis of Variance (ANOVA) followed by Fischer's LSD test. Statistical significance was considered at ($P \leqslant 0.05$). The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

RESULTS AND DISCUSSION

Environmental pollution by HMs and heavy metals toxicity are serious problems in most countries Worldwide. In Egypt pollution by HMs is considered one of the most dangerous hazards affecting the country. The pollution with HMs has increased in Egypt because of increases in population (90 million), industries, number of agricultural projects

Table 1 Total Phenols and Total Flavonoids components in BREE analysed using HPLC

Total phenols		*Total flavonoids*	
Compounds	*mg/100 g*	*Compounds*	*mg/100 g*
Benzoic	2.397	Rosmarinic	0.134
Cinnamic	1.795	Hispertin	2.276
Pyrogallal	3.677	Kampferol	0.331
Protocatchuoic	3.898	Quercetin	6.721
Hydroytyrozol	2.331	Luteolin	0.219
Gallic	0.574	Apignen	0.429
Coumarin	4.214	Naringin	1.824
Salycilic	7.797	Narenginin	1.309
Ferulic	0.013	Quercetrin	3.592
Vanilic	1.181	Hypersoide	0.363
Caffeic	0.761	Rutin	6.565
Catechein	4.743	Hisperdin	2.278
Catechol	0.587	–	–
Ellagic	2.158	–	–
Cholorogenic	5.091	–	–
Caffein	1.146	–	–
Total	42.36 (mg/100 g)	Total	26.04 (mg/100 g)

and other activities along the Nile Delta. Also, levels of chromium, copper, vanadium, cadmium, nickel, lead, arsenic, manganese, titanium and antimony were higher than those considered safe for the general population. Therefore, the incidence of breast cancer attained about 37.5% of total cancer cases among Egyptian females and is considered the fourth cause of death (Elattar, 2005). Additionally, Darwish et al. (2015) indicated that liver and kidney most affected by toxic HMs compared to muscles.

So that, a comprehensive strategies to address the problem of HMs pollution in Egypt are urgently needed. And thus could be achieved via developing a plan for prevention and control this disorder. For example using some plants for the prevention, especially for people with hepatic and renal diseases who have a greater risk. In our area of research we tested the preventive effect of black rice ethanolic extract as natural anti-oxidants on some HMs.

Data in Table 1 shows Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) in black rice extract analysed using HPLC. The results indicated that TPC and TFC were 42.36 and 26.04 mg/100 g, respectively. Reports also show that the quantity of TPC in Malaysia rice ranged from 22.59 to 329.53 mg/kg (Lum and Chong, 2012). Moreover, China black rice variety contained the highest TPC than red and white rice from all the solvents extraction (hexane, methanol, ethanol-water (70:30 v/v) and water at room temperature) (Chanida et al., 2013). Muntana and Prasong (2010) told that high phenolic compounds can be found in pigmented rice such as red and black rice. TFC can be observed the same result in all rice varieties. Samples extracted with 70% ethanol (at 25°C) explained that the TFC range from 16.98 to 158.47 mg/kg and black rice varieties had the highest TFC than red and white rice varieties (Chanida et al., 2013; Melissa and Enio, 2011). In the same rice variety, the 70%

Table 2 Effects of BREE on Body Weight of Control and DWHM Administrated

Treatments	*Body weight*			
	Initial (g)	*Finial (g)*	*Gain (g)*	*Daily gain(g)*
NC	193.8 ± 3.114[a]	215 ± 3.317[a]	21.2 ± 3.421[d]	0.36 ± 0.0547[d]
PC (DWHM)	195.8 ± 2.387[a]	118.6 ± 3.361[d]	277.2 ± 3.193[a]	21.29 ± 0.0651[a]
60% BRE	194.2 ± 2.683[a]	212.6 ± 3.209[a]	218.4 ± 4.669[b]	20.3 ± 0.0707[b]
60% BRE + (1 mg/1 ml DWHM)	194.8 ± 3.492[a]	168.6 ± 3.577[b]	226.2 ± 5.403[c]	20.43 ± 0.0836[c]
80% BRE	193.8 ± 3.898[a]	216.4 ± 2.881[a]	22.6 ± 3.361[d]	0.38 ± 0.045[d]
80%B RE + (1 mg/1 ml DWHM)	193.8 ± 3.493[a]	205.2 ± 2.280[a]	11.6 ± 2.509[d]	0.19 ± 0.055[d]
LSD at 5%	4.2028	4.0897	5.0695	0.08298

Note: Values are mean (dev for 8 rats in each group. Compared with control group: $P \leqslant 0.05$ Compared with PC group: $P \leqslant 0.05$.

Table 3 Effect of BREE on hemogram in the Serum of Control and DWHM-Administered Rats

Treatments	*Hb(g/dl)*	*Ht (%)*	*RBCs(10^9 L^{-1})*	*WBCs(10^6 L^{-1})*	*Plet(10^9 L^{-1})*
NC	12.33 ± 0.252[c]	40.66 ± 0.208[b]	4.233 ± 0.251[b]	6.303 ± 0.274[b]	1648.3 ± 17.15[a]
PC	6.3 ± 0.1[e]	47.76 ± 0.3055[a]	3.200 ± 0.361[a]	7.67 ± 0.213[a]	653.3 ± 25.17[b]
60% BRE	12.73 ± 0.208[b]	39.83 ± 0.611[b]	4.266 ± 0.351[b]	6.323 ± 0.155[b]	1638.7 ± 33.56[a]
60% BRE (1mg/1ml DWHM)	9.53 ± 0.288[a]	44.33 ± 0.208[c]	4.266 ± 0.115[b]	6.553 ± 0.393[b]	1441 ± 42.67[b]
80% BRE	12.93 ± 0.152[c]	40.86 ± 0.873[b]	4.100 ± 0.264[b]	6.43 ± 0.199[b]	1642.7 ± 27.23[a]
80% BRE (1mg/1ml DWHM)	12.2 ± 0.173[cd]	40.93 ± 0.568[b]	4.166 ± 0.321[b]	6.613 ± 0.271[b]	1663.3 ± 30.55[a]
LSD at 5%	0.3655	0.9301	0.5153	0.4665	54.1043

Note: Values are mean (dev for 8 rats in each group. Compared with control group: $P \leqslant 0.05$ Compared with PC group: $P \leqslant 0.05$

ethanol extracts contained more TPC and TFC compared to water (at 25°C) and hot water (at 50°C) extracts (Chanida et al., 2013). Ethanol can effectively increase permeability of cell wall thus facilitate efficient extraction of polar sub-stances (Anwar and Przybylski, 2012).

Table 2 shows the initial and final body weights in control and experimental animals. During the course of present investigations. It was observed that the control body weight, BREE 60% (1 mg/1 ml DW) and BREE 80% (1 mg/1 ml DWHM) treated groups have increased progressively, contrary, in PC (DWHM) treated rats, results revealed were decrease in body weight gain compared to the control. This reduction in weights might be due to low food consumption and reduction in protein levels (Costa et al., 1994).The decreased of body weight in our study is in good agreement with some previously published articles by Youcef et al. (2014) they reported that $HgCl_2$ (0.5 mg/kg body weight i.p) treated rats were significantly decreased in body weight gain by −4.93% as compared to the control after two weeks. Djemli et al. (2012) indicated that Ni (800 mg/l Ni as NiSO4 6H2O) – treated animals were significantly decreased ($p < 0.001$) in body weight gain as compared to the control group (−46%). As the nickel ions have a higher affinity for proteins and amino acids and have shown to produce oxidation of proteins in cells (Costa et al., 1994).

Hematologic changes in NC and PC rats and rats administration of BREE 60, 80% (1 mg/1 ml DW) and BREE 60, 80% (1 mg/1 ml DWHM) are shown in Table 3. Data indicated insignificant changes in the levels of WBCs in PC. Interestingly, RBCs, Hb and Plet of PC rats were significantly ($p \leqslant 0.05$) lower (3.2, 6.3 g/dl and 653.3), respectively than NC (12.33 g/dl and 1648.3), respectively. While, Ht was significantly ($p \leqslant 0.05$) increased in PC (47.76%) than NC (40.66%). On the other hand, BREE 60, 80% (1 mg/1 ml DWHM) treated groups significantly alleviates the RBCs, WBCs, Hb, Plet and Ht to near normal levels (Table 3), which is similar to the previous study (Khadiga et al., 2011). Sub-chronic lead intoxication caused as light increase in the number of RBCs (Iavicoli et al., 2003; Jacob et al., 2000). Iavicoli et al. (2003) observed that small increase of blood Pb was associated with increased RBC count and also increased Hct levels, but in our study, Hb and Hct were reduced despite of high RBC count in lead treated rats. It may be due to low hemoglobin production because of lead induced disturbance of hemebiosynthesis (Wildman et al., 1976). Low Hb level might result in reduced oxygen transfer by RBCs, which was

Table 4 Effect of BREE on Lipid Profile Markers and FBS in the Serum of Control and DWHM-Administered Rats

Treatments	*TC (mg/dl)*	*HDL (mg/dl)*	*LDL (mg/dl)*	*TG (mg/dl)*	*FBS(mg/dl)*
NC	185.3 ± 2.50[b]	133.7 ± 3.214[a]	51.60 ± 4.932[b]	77.66 ± 0.577[e]	101 ± 1.0[c]
PC	242 ± 5.1[a]	47.33 ± 1.527[c]	194.67 ± 0.577[a]	152.7 ± 3.055[a]	135 ± 1.0[a]
60% BRE	184.3 ± 3.094[b]	134.7 ± 1.527[a]	49.60 ± 2.00[b]	79.00 ± 2.645[e]	101.3 ± 1.15[c]
60% BRE + (1 mg/1 ml DWHM)	194 ± 3.605[ab]	114.3 ± 2.886[b]	75.70 ± 2.516[b]	95.33 ± 1.527[c]	113 ± 1.645[b]
80% BRE	186.6 ± 4.61[b]	136 ± 2.645[a]	50.60 ± 1.00[b]	77.33 ± 0.555[e]	99.66 ± 0.578[cd]
80% BRE + (1 mg/1 ml DWHM)	194.0 ± 2.0[ab]	133.3 ± 0.577[a]	56.70.33 ± 2.516[b]	80.66 ± 0.527[e]	98 ± 1.00[cd]
LSD at 5%	3.9603	4.0219	4.7254	3.4577	1.7814

Note: Values are mean (dev for 8 rats in each group. Compared with control group: $P \leqslant 0.05$ Compared with PC group: $P \leqslant 0.05$.

compensated by increased number of these cells. Also, tissue hypoxia is a possible mechanism for high production of RBCs in moderate lead poisoning. The same effects on blood indices that were observed previously in other researches (Mohammad et al., 2007).

Table 4 shows the levels of total cholesterol, triglycerides, HDL, LDL and FBS in NC and PC rats and rats administration of BREE 60, 80% (1 mg/1 ml DW)and BREE 60, 80% (1 mg/1 ml DWHM). TC significantly ($p \leqslant 0.05$) increased in PC. While, the level of HDL-C in serum significantly ($p \leqslant 0.05$) decreased and LDL-C significantly ($p \leqslant 0.05$) increased compared to NC. On the other hand, no significantly ($p \leqslant 0.05$) effect of TC, HDL-C and LDL-C in groups administration of BREE 60, 80% (1 mg/1 ml DW)and BREE 60, 80% (1 mg/1 ml DWHM), respectively. Table 4 also shows the results of TG. The data indicate significantly ($p \leqslant 0.05$) increased in PC group compared to NC. While, the groups administration of BREE 80% (1 mg/1 ml DW) and BREE 80% (1 mg/1 ml DWHM) gave the best results compared to NC. FPS significantly ($p \leqslant 0.05$) increased in PC group compared to NC (Table 4). While, BREE 60, 80% (1 mg/1 ml DWHM) gave results near to NC. Black rice is rich in polyphenolics as antioxidant, which might be involved in protection against cardiovascular risk (Manach et al., 2005), and might protect blood vessels against the deleterious consequences of oxidant stress associated with many cardiovascular risk factors. Additionally, antioxidant can decrease the circulating LDL and oxidation of membrane lipids and their deleterious consequences in endothelial cells. Also, specific structures of polyphenols can, independently from their antioxidant properties, for improve the endothelium function and inhibit angiogenesis and migration and proliferation of vascular cells (Stoclet et al., 2004). Our results agree with Jerzy et al. (2009) they indicated the levels of LDL cholesterol were significantly ($p < 0.05$) lower in the plasma of rats fed with black rice in the presence of cholesterol and bile and in those fed with diets containing cholesterol and black rice fraction (BRF). The plasma triacylglycerol level was significantly ($p < 0.05$) decreased in rats fed with the BRF as compared with the level in rats on control diets. The atherogenic index (ratio of HDL to total cholesterol) was significantly higher ($p < 0.05$) in rats fed with 3% BRE compared with the control group.

The results in Table 5 indicated that treatment with DWHM caused a significant ($P \leqslant 0.05$) increase in the activities of AST and ALT as compared to the NC . This increase may be due to the hepatic damage resulting in increased release of functional enzymes from biomembranes or its increased synthesis (Pari and Prasath, 2008). BREE 60% (1 mg/1 ml DWHM) and BREE 80%

Table 5 Effect of BREE on Hepatic Markers in the Serum of Control and DWHM-Administered Rats

Treatments	*ALT (U/L)*	*AST (U/L)*
NC	36.33 ± 1.527^{b}	26.00 ± 1.732^{c}
PC	61.66 ± 2.886^{a}	47.33 ± 2.081^{a}
60% BRE	36.00 ± 2.645^{b}	26.00 ± 1.605^{c}
60% BRE + (1 mg/1 ml DWHM)	34.66 ± 1.527^{b}	31.00 ± 1.00^{b}
80% BRE	36.00 ± 2.645^{b}	25.96 ± 1.527^{c}
80% BRE + (1 mg/1 ml DWHM)	35.66 ± 1.155^{b}	27.33 ± 3.214^{bc}
LSD at 5%	3.8658	4.2348

Notes: Values are mean (dev for 8 rats in each group. Compared with control group: $P \leqslant 0.05$ Compared with PC group: $P \leqslant 0.05$.

(1 mg/1 ml DWHM) treatment did not show any significant alteration in ALT activity. However, the combined treatment of BREE with DWHM results in recovery in AST activity as compared to the NC (Table 5). These findings are in accordance with Hoda et al. (2011, 2012). Mixture of HMs (Pb, Hg, Cd and Cu) induced significant increases of plasma ALT and AST. The same observations were noted in several experimental investigations on animals exposed to Pb (Liu et al., 2010) and Hg (Bashandy et al., 2011).

The levels of urea, creatinine and uric acid in serum of NC and experimental rats are shown in Table 6. DWHM administration caused a severe increase of serum urea, creatinine and uric acid ($P \leqslant 0.05$). Pretreatment of rats with BREE 60% or 80% (1 mg/ml DWHM) reduced the formation of urea, creatinine and uric acid of serum. Many studies conducted on experimental animals intoxicated with Pb, Hg, Cd, Cu and other HMs showed a significant enhancement of blood urea, creatinine and uric acid concentrations, and renal histological alterations (Al-Madani et al., 2009; Brzoska et al., 2003; Chen et al., 2006a, b; Missoun et al., 2010). Atef (2011) studied the effect of some HMs intoxicated on mice chronically and shows a decline obvious in kidney function this is confirmed by the promote of plasma urea, creatinine and uric acid levels and histopathological alterations.

Table 7 shows the concentrations of CA19.9 tumor marker of GIT in NC and PC rats and rats administration of BREE 60 and 80% (mg/1 ml DW) and BREE 60 and 80% (mg/1 ml DWHM). The results showed that significantly ($p \leqslant 0.05$) increased in PC (17.43 U/ml). On the other hand, the CA 19.9 was not detected in NC, BREE 60 and 80% (mg/1 ml DW), but BREE 60 and 80% (mg/1 ml DWHM) were reduce the CA19.9 to 7.51 and 4.62 (U/ml), respectively. These results may be due to poly phenolic, flavonoid and anthocyanins in black rice, which might be involved in protection against tumor these results agreed with (Chen et al., 2006a,b).

The results mean that, the group's treatment with 60 and 80% BREE 60 and 80% (mg/1 ml DWHM) had health promotion of rats by 56.92 and 73.61%, respectively. The results show indicates to BREE lead to improve against toxic effect of HMs induced.

Histopathological examination of rat kidney

HMs can induce severe kidney damage. The kidney samples of DWHM-treated rats showed some conspicuous histopathological changes in

Table 6 Effect of BREE on vRenal Markers in the Serum of Control and DWHM-Administered Rats

Treatments	*Urea (mg/dl)*	*Creatinine (mg/dl)*	*Uric acid (mg/dl)*
NC	39.33 ± 1.527^{b}	0.766 ± 0.0577^{c}	2.10 ± 0.12^{a}
PC	54.00 ± 2.00^{a}	1.533 ± 0.577^{a}	4.20 ± 0.30^{b}
60% BRE	40.33 ± 1.527^{b}	0.833 ± 0.057^{c}	1.93 ± 0.057^{a}
60% BRE + (1 mg/1 ml DWHM)	40.00 ± 2.00^{b}	1.166 ± 0.152^{b}	2.06 ± 0.152^{a}
80% BRE	39.66 ± 2.516^{b}	0.861 ± 0.055^{c}	2.03 ± 0.208^{a}
80% BRE + (1mg/1ml DWHM)	39.33 ± 3.214^{b}	0.833 ± 0.057^{c}	2.07 ± 0.152^{a}
LSD at 5%	3.9335	0.1623	0.2444

Notes: Values are mean (dev for 8 rats in each group. Compared with control group: $P \leqslant 0.05$ Compared with PC group: $P \leqslant 0.05$.

Table 7 Effect of BREE on Tumor Marker in the Serum of Control and DWHM-Administered Rats

Treatments	*CA19.9 (U/ml)*
NC	0.0 ± 0.0
PC	17.43 ± 0.862[a]
60% BRE	0.00 ± 0.00
60% BRE + (1 mg/1 ml DWHM)	7.51 ± 0.00
80% BRE	0.00 ± 0.00
80% BRE + (1 mg/1 ml DWHM)	4.62 ± 0.00
LSD at 5%	1.3820

Notes: Values are mean (dev for 8 rats in each group. Compared with control group: $P \leqslant 0.05$ Compared with PC group: $P \leqslant 0.05$.

the kidney. The distal and collecting convoluted tubules of the kidney undergo degeneration and have a large lumen due to hypertrophy (Figure 1b) these changes are in agreement with (Muna and Aticka, 2009). Also, degeneration of tubular epithelium are seen in the kidney tissue (Figure 1b). These morphological changes could be due to rapid selective accumulation of HMs in the cytosolic fraction of proximal tubular epithelium (Hascheck and Rausseaux, 1998). It was found that administration of BREE 60% (1 mg/1 ml DWHM) exhibited higher protective effects against HMs when compared to DWHM administrated rats (Figure 1c), while BREE 80% (1 mg/1 ml DWHM), BREE 60% alone and BREE 80% alone administrated rats showed histological pattern to near normalcy (Figure 1d, e and f).

The results of the present work showed that the cortex is more affected than the medulla due to long-term treatment with heavy metals. This could be partly due to uneven distribution of heavy metals in the tissue of the kidney where about 90% of the total renal blood flow enters the cortex via the bloodstream. Accordingly, a relatively high concentration of these metals might reach the cortex via the bloodstream than that would enter the medulla. Muna and Aticka (2009) studied the histological effects caused by lead kidney of rats and investigated that the main microscopic change were noticed in this study enlargement of epithelial cells lining renal tubules (proximal tubules), with hyalinization, necrosis of some tubules. Also lead damage membrane associated enzymes such as sodium – potassium pump which result in renal tubular injury (Plumlee, 2004). Exposure to high lead levels can produce renal tubular damage, it has been suggested that renal damage could be related to cumulative lead dosage (Gidlow, 2004). The glomerular tuft degeneration within 4 weeks treated group with 4 mg may be due to filtration of lead across the glomeruli (Sagheb et al., 2002). The inflammatory reaction including hemorrhage and inflammatory cell infiltration which we noticed in renal tissue of both treated groups (Muna and Aticka, 2009).

CONCLUSION

Our data indicate that BREE has a protective action against HM-induced toxicity as evidenced by decreased levels of lipid profile, ALT, AST, urea, creatinine, uric acid, FBS and CA19.9 and elevated levels of HDL-C in serum, which is probably due to its antioxidant properties. BREE plays a beneficial role in the treatment of HM induced tissue damage, which indicates the therapeutic values of black rice. In addition to their potential role in prevent cardiovascular disease. The obtained results are very promising but not yet insufficient and its early to suggest that the regular use of the BREE prevents cancer or it is recommended for routine use. Further

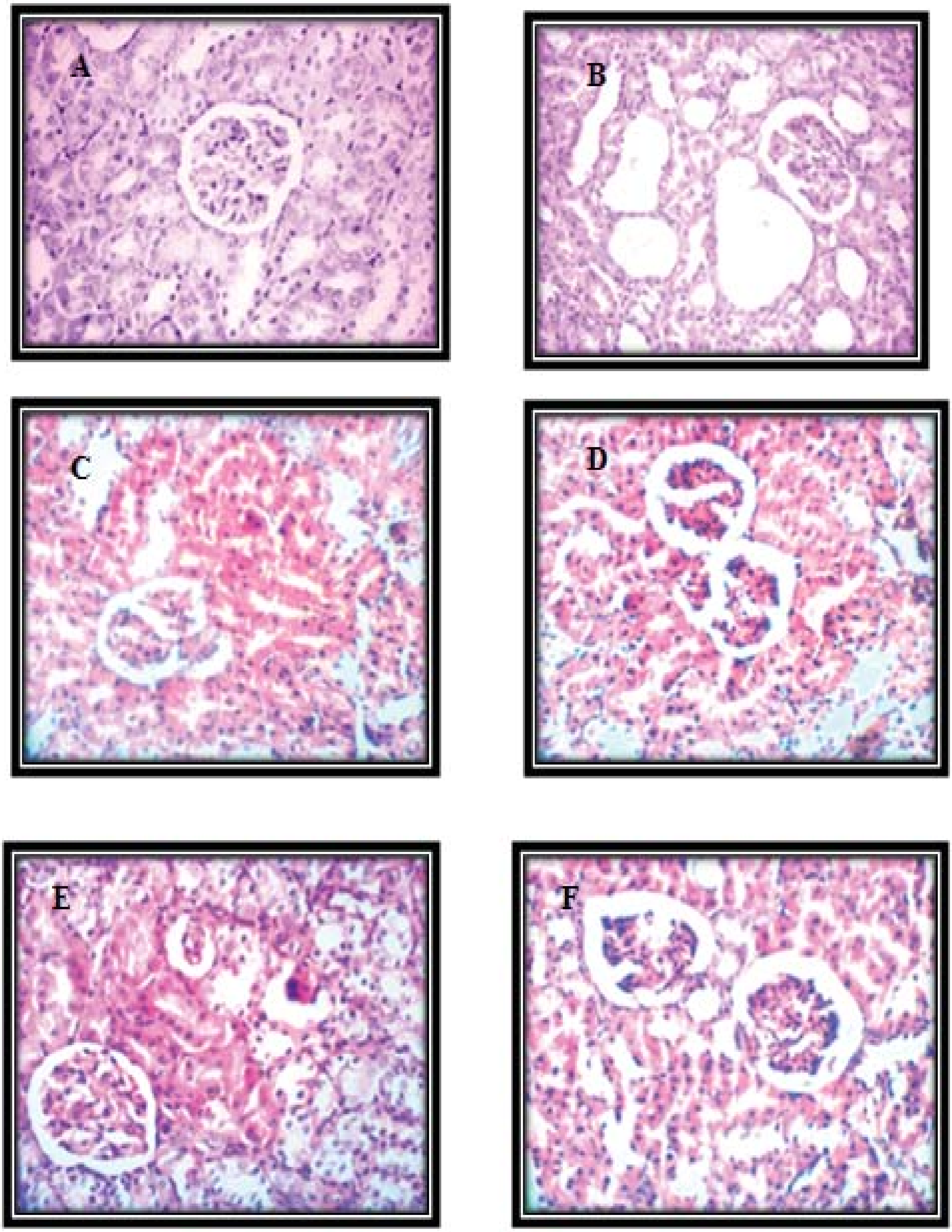

Figure 1 Photomicrographs of kidney section in control and experiment rats (H & E 400)

investigation should be done in large scale, to clarify the toxic effects of HMs and the potential protective role of BREE against them.

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