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ARTICLE

6-Gingerol-Rich Fraction from *Zingiber officinale* Prevents Hematotoxicity and Oxidative Damage in Kidney and Liver of Rats Exposed to Carbendazim

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ABSTRACT. Ginger (*Zingiber officinale*) is a globally marketed flavoring agent and cooking spice with a long history of human health benefits. The fungicide carbendazim (CBZ) is often detected in fruits and vegetables for human nutrition and has been reported to elicit toxic effects in different experimental animal models. The present study investigated the protective effects of 6-Gingerol-rich fraction (6-GRF) from ginger on hematotoxicity and hepatorenal damage in rats exposed to CBZ. CBZ was administered at a dose of 50 mg/kg alone or simultaneously administered with 6-GRF at 50, 100, and 200 mg/kg, whereas control rats received corn oil alone at 2 mL/kg for 14 days. Hematological examination showed that CBZ-mediated toxicity to the total white blood cell (WBC), neutrophils, lymphocytes, and platelets counts were normalized to the control values in rats cotreated with 6-GRF. Moreover, administration of CBZ significantly decreased the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase as well as glutathione level in the livers and kidneys of rats compared with control. However, the levels of hydrogen peroxide (H_2O_2) and malondialdehyde were markedly elevated in kidneys and livers of CBZ-treated rats compared with control. The significant elevation in the plasma indices of renal and hepatic dysfunction in CBZ-treated rats was confirmed by light microscopy. Coadministration of 6-GRF exhibited chemoprotection against CBZ-mediated hematotoxicity, augmented antioxidant status, and prevented oxidative damage in the kidney and liver of rats.

KEYWORDS. carbendazim, hematology, kidney, liver, oxidative stress, rats, 6-Gingerol-rich fraction

INTRODUCTION

Carbendazim (methyl 2-benzimidazole carbamate, CBZ, Figure 1(A)) is a broad spectrum benzimidazole fungicide widely used in veterinary and agriculture. It is

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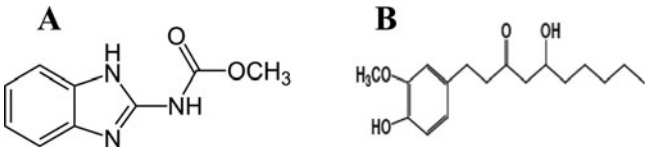

FIGURE 1. Chemical structures of tested compounds (A) carbendazim and (B) 6-Gingerol.

currently used in the control of foliar diseases on arable crops such as cereals, sugar and fodder beet, flowers and flower bulbs, and fruits (EFSA, 2010; Tortella et al., 2013). CBZ is also used as a preservative in paint, papermaking, and in the leather industry. Moreover, CBZ is a major degradation product of other fungicides such as benomyl and thiphanate-methyl (Mazellier et al., 2002). There is a growing concern about the impact of CBZ on the environment and man. For instance, incorrect application of CBZ could adversely affect nontarget organisms. Human exposure to CBZ could be through direct routes including oral, inhalation and dermal, whereas indirect routes include ingestion of contaminated fruits and vegetables, drinking water, residential, and occupational exposure (Boobis et al., 2008; Bakırcı et al., 2014). Previous studies have demonstrated that CBZ impaired liver and kidney functions (Akbarsha et al., 2000; Muthuviveganandavel et al., 2008) and imposed toxic effects on hematopoiesis and metabolism in rats (Selmanogălu et al., 2001; Zubrod et al., 2014). CBZ adversely affected reproductive and endocrine systems in various mammals via microtubule disturbances during cell divisions and oxidative stress (Adedara et al., 2013; Pacheco et al., 2012; Rama et al., 2014).

Zingiber officinale Roscoe (family, Zingiberaceae), commonly called ginger, is a widely cultivated crop with a long history of human health benefits. The powdered rhizome is an important commodity in the market throughout the whole world due to its use as a flavoring agent and cooking spice. Moreover, ginger has been used in traditional oriental medicine to treat gastrointestinal disorders including stomachaches, abdominal spasm, nausea, and vomiting (Langner et al., 1998; White, 2007). The interest in the human health benefits of ginger is on the increase owing to the presence of active principles such as gingerols, shogaols, paradols, and zingerones. 6-Gingerol (1-[4'-hydroxy-3'-methyoxyphenyl]-5-hydroxy-3-decanone, Figure 1(B), which is the most pungent of all the homologs of gingerols has being reported to exhibit anti-inflammatory, anticancer and antioxidant properties (Dugasani et al., 2010; El-Ghorab et al., 2010; Surh et al., 1999; Surh, 2002;). However, as one of the principal bioactive components in ginger, the effect of 6-Gingerol on fungicide CBZ remains unknown.

The present study aimed at investigating the role of 6-Gingerol in modulating the toxicity of CBZ in the blood, kidney, and liver. Hence, endpoints including hepatic and renal functional parameters, oxidative stress indices, hematological parameters, and histology of the kidney and liver were evaluated in Wistar male rats.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid, thiobarbituric acid, 1-chloro-2,4-dinitrobenzene, 5',5'-dithiobis(2-nitrobenzoic acid), hydrogen peroxide (H_2O_2), xylenol orange, and reduced glutathione were sourced from Sigma Chemical Company (St. Louis, MO, USA). All other reagents were of analytical grade and were procured from the British Drug Houses (Poole, Dorset, UK).

Authentication and Preparation of 6-Gingerol-Rich Fraction

Ginger rhizomes were purchased from a local vendor in Bodija Market, Ibadan, Nigeria. The rhizomes were authenticated by Mr. Dunatus Esimekhuai at the Department of Botany, University of Ibadan, Ibadan, where a sample (voucher specimen number UIH-22390) was deposited in the herbarium. Briefly, the fresh rhizomes were rinsed with distilled water, sliced, air dried, and powdered. The powder was percolated in ethanol and allowed to stand for 72 hours. This process of extraction was repeated twice and the extract was collected, filtered, and concentrated under vacuum using rotary evaporator at 45°C. The crude ethanol extract was coded FA001.

Column Chromatography

The column chromatography was carried out according to previously published procedure **(**Almada da Silva et al., 2012**)** with some modifications. Briefly, 50 g of FA001 was dissolved in ethyl acetate, absorbed over silica gel (70-230), defatted with n-hexane and isocratically eluted with n-hexane and ethyl acetate (1:1) resulting in 10 fractions (250 mLs each). Fractions 1–6 were rich in 6-Gingerol when compared on thin-layer chromatography plate with the standard 6-Gingerol (Natural Remedies Ltd., Bangalore, India). The fractions possessing the same Retardation or Retention factor (Rf) value with the standard were pooled and concentrated at reduced pressure to give a liquid residue containing about 53.6% of 6-Gingerol which was termed 6-Gingerol-rich fraction (6-GRF). Stock solution of 6-GRF (100 mg/mL) was prepared fresh every other day with corn oil during this investigation.

Animal Model

Fifty adult male Wistar rats (10 weeks old; 176 ± 4 g) obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were used for the present study. They were housed in plastic cages placed in a well-ventilated rat house, provided rat pellets and water ad libitum and subjected to natural photoperiod of 12-hr light: 12-hr dark cycle. The experimental protocol were carried out after approval and in accordance with the guidelines set by the University of Ibadan Ethical Committee, which conformed to the acceptable guidelines on the ethical use of animals in research (PHS, 1996).

Experimental Protocol

The rats were assigned randomly to five groups of ten rats per group and were treated for 14 days as follows: Group I rats received corn oil alone at 2 mL/kg and

served as control. Group II rats were orally treated with CBZ dissolved in corn oil at 50 mg/kg alone. Group III rats were orally cotreated with CBZ and 6-GRF at 50 mg/kg (6-GRF1). Group IV rats were orally cotreated with CBZ and 6-GRF at 100 mg/kg (6-GRF2). Group V rats were orally cotreated with CBZ and 6-GRF at 200 mg/kg (6-GRF3). The dose of CBZ (50 mg/kg) was chosen from previously published study (WHO, 1974) whereas 50, 100, and 200 mg/kg 6-GRF and the duration of treatment were selected from our preliminary studies (data not shown). Twenty-four hours following the last treatment, blood was collected from retro-orbital venous plexus before the animals were sacrificed by cervical dislocation. The kidneys and livers were immediately excised, rinsed with ice-cold phosphate-buffered saline, weighed, and processed for biochemical and histological analysis.

Hematological Parameters

K_2 ethylene-diaminetetraacetic acid (EDTA)-added whole blood samples were analyzed by Sysmex Automated Hematology Analyzer (KX-21, Kobe, Japan) for red blood cell (RBC), hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC, lymphocytes, neutrophils, monocytes, and platelet counts.

Plasma Biochemistry

Plasma samples were separated from blood cells by centrifugation at 3,000 *g* for 10 minutes. Subsequently, the plasma activities of aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and the levels of bilirubin, urea and creatinine were measured using commercially available kits obtained from Randox Laboratory Limited (UK).

Renal and Hepatic Antioxidant Status

The kidney and liver of the animals were homogenized in 50 mM Trisaminomethane hydrochloride (Tris–HCl) buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10,000 *g* for 15 minutes at 4°C. The supernatant was subsequently collected for the estimation of protein concentration by the method of Lowry et al. (1951). Superoxide dismutase (SOD) activity was assayed according to the method described by Misra and Fridovich (1972). Catalase (CAT) activity was determined using hydrogen peroxide as a substrate according to the method of Clairborne (1995). Glutathione-S transferase (GST) was determined by the method of Habig et al. (1974). Glutathione peroxidase (GPX) activity was determined according to the method of Rotruck et al. (1973). Reduced glutathione (GSH) was measured at 412 nm according to the method described by Jollow et al. (1974). Hydrogen peroxide (H_2O_2) generation was determined according to the method of Wolff (1994). Lipid peroxidation was determined as malondialdehyde (MDA) according to the method described by Farombi et al. (2000) and expressed as micromoles of MDA per milligram protein.

Microscopic Examination of Kidney and Liver

Biopsies from each liver and kidney were fixed in 10% formalin and processed for histology according to Songur et al. (2003). Briefly, the tissues were fixed in 10%

phosphate buffer formalin (PBF) for three days. The PBF solution was changed daily to minimize shrinkage and prevent the occurrence of artifacts. After dehydration procedures, the samples were blocked in paraffin. Sections of 4–5 μm were cut by a microtome and stained with hematoxylin and eosin. All slides were coded before examination under a light microscope (Olympus CH; Olympus, Tokyo, Japan) by pathologists who were blinded to control and treatment groups. Kidney and liver histology was evaluated and histopathological alterations were scored according to standardized method (Selmanogălu et al., 2001). Photomicrographs were taken with a Sony DSC-W 30 Cyber-shot (Sony, Tokyo, Japan).

Statistical Analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Bonferroni's test for post hoc comparisons using SPSS for Windows (version 17). Results are presented as mean $\pm$ standard deviation. $p < .05$ was considered statistically significant.

RESULTS

Body Weight Gain and Relative Organ Weights

The animals remained in relatively good health during the period of experiment without showing any treatment-related clinical signs. The data on the body weight gain and the relative organ weights presented in Figure 2 show that while there was no treatment-related effect on the body weight gain and kidney weight, the relative weight of the liver was significantly ($p < .05$) increased in the rats exposed to CBZ alone when compared with the control rats. However, the relative weight of the liver was restored to near control following treatment of 6-GRF at all the doses tested.

Hematological Parameters

Table 1 shows the erythrocyte parameters in the control, CBZ-treated, and rats cotreated with CBZ and 6-GRF for 14 days. The result revealed there was no treatment-related effect on the RBC count, Hb level, PCV, MCV, MCH, and MCHC when compared with the control. Table 2 revealed the effects of CBZ exposure and 6-GRF administration on the differential WBC and platelet counts in the experimental rats. Exposure to CBZ resulted in a significant decrease in the total WBC count and lymphocytes but significantly increased neutrophil counts when compared with the control. Moreover, platelets count was markedly decreased in rats exposed to CBZ alone in comparison with those of control. Interestingly, the total WBC, neutrophil, lymphocytes and platelets counts were normalized to the control values in rats cotreated with 6-GRF. Administration of CBZ decreased the WBC and lymphocytes by 26.3% and 18%, respectively. However, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored the WBC count by 77.3%, 94.5%, and 79.9%, whereas lymphocytes count was restored by 82.2%, 76.8%, and 79.7%, respectively. Furthermore, CBZ exposure increased neutrophil count by 30% and decreased platelet count by 20.2%, respectively, when compared with the control

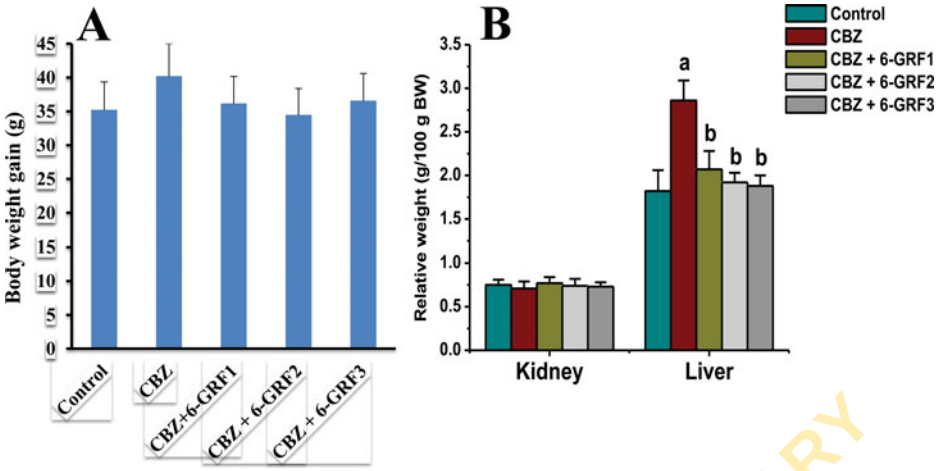

FIGURE 2. Effects of 6-GRF on body weight gain and relative organ weights in CBZ-treated mice. 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg of 6-GRF, respectively. Each bar represents mean ± SD of 10 rats. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CBZ group ($p < .05$).

rats. Coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored the neutrophil count by 107%, 109%, and 109%, whereas platelet count was restored by 54%, 77.1%, and 68.8%, respectively.

Markers of Hepatic and Renal Damage

The functionality of the liver was confirmed by measuring the plasma levels of ALT, AST, ALP, GGT, and bilirubin, whereas renal functionality was confirmed by estimating the levels of urea and creatinine. As depicted in Figure 3, a significant ($p < 0.05$) elevation in all the markers of hepatic and renal damage was observed in the plasma of CBZ-treated rats when compared with the control rats. However, coadministration of CBZ-treated rats with 6-GRF significantly restored the levels of these biomarkers to near normalcy. Administration of CBZ increased plasma ALT, AST, and ALP levels by 134%, 162.8%, and 131.4%, respectively,

TABLE 1. Effects of 6-Gingrol-rich fraction (6-GRF) on the erythrocyte parameters in carbendazim-treated rats

Parameters	Control	CBZ	CBZ + 6-GRF1	CBZ + 6-GRF2	CBZ + 6-GRF3
PCV (%)	49.67 ± 2.08	49.33 ± 1.52	49.53 ± 2.12	50.5 ± 1.71	49.33 ± 1.52
RBC ($10^3/\mu$L)	9.04 ± 0.14	8.11 ± 0.42	9.65 ± 0.38	8.98 ± 0.15	9.13 ± 0.28
Hb (g/dL)	15.80 ± 1.61	15.73 ± 1.37	15.91 ± 1.48	16.30 ± 1.28	15.53 ± 1.47
MCV (fL)	59.52 ± 2.77	61.58 ± 1.89	59.64 ± 2.12	60.23 ± 2.05	60.15 ± 2.16
MCH (pg)	19.33 ± 1.57	19.33 ± 1.33	18.33 ± 1.48	19.67 ± 1.35	19.33 ± 1.48
MCHC (g/dL)	35.75 ± 2.11	31.06 ± 2.08	33.41 ± 2.53	34.60 ± 2.61	33.71 ± 2.15

CBZ, carbendazim; 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg; PCV, packed cell volume; RBC red blood cell; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume. Values are expressed as mean ± Standard Deviation (SD) of 10 rats.

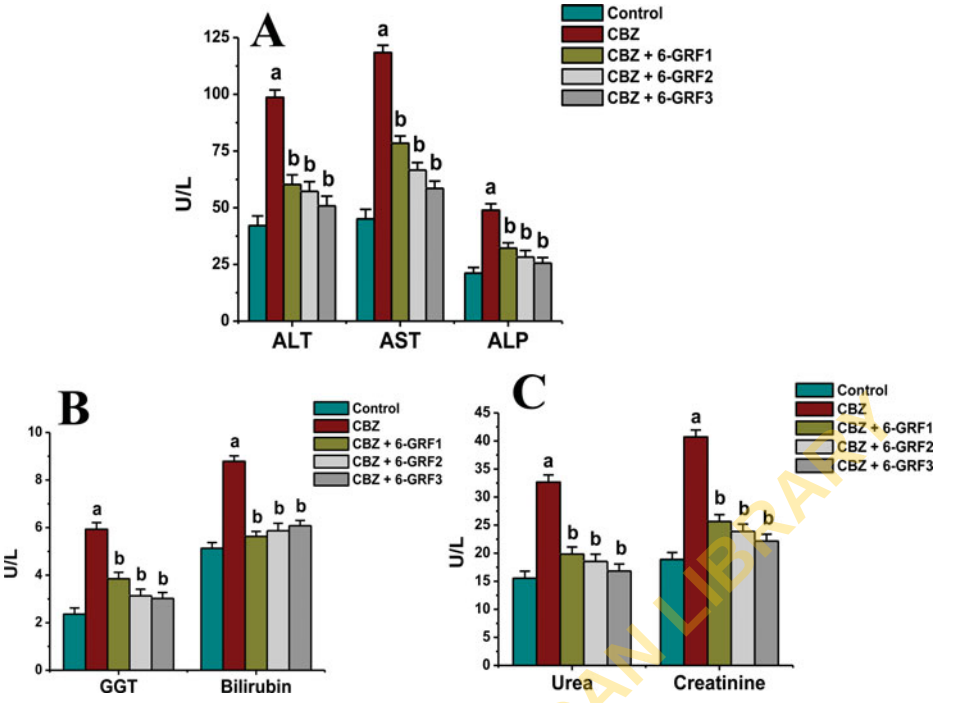

FIGURE 3. Effects of 6-GRF on plasma AST, ALT, and ALP activities in CBZ-exposed rats (A). Plasma GGT activity and bilirubin level in CBZ-exposed rats (B). Plasma creatinine and urea level in CBZ-exposed rats (C). 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg of 6-GRF, respectively. Each bar represents mean ± SD of 10 rats. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CBZ group ($p < .05$).

when compared with the control rats. Conversely, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored ALT level by 73.2%, 80.3%, and 88.1%, and AST level by 64%, 78.8%, and 88.4%, whereas ALP level was restored by 60.2%, 74.4%, and 84%, respectively. CBZ exposure increased plasma GGT and bilirubin levels by 151.3% and 71.5% respectively, when compared with the control rats. Conversely, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored GGT level by 58.3%, 78.4%, and 81.5%, whereas bilirubin level was restored by 86.3%, 79.8%, and 74.1%, respectively.

TABLE 2. Effects of 6-Gingrol-rich fraction (6-GRF) on the differential white blood cell and platelets counts in carbendazim-treated rats

Parameters	Control	CBZ	CBZ + 6-GRF1	CBZ + 6-GRF2	CBZ + 6-GRF3
WBC ($10^3/\mu L$)	14.73 ± 1.33	10.85 ± 1.91^a	13.85 ± 1.77^b	14.52 ± 1.32^b	13.95 ± 2.08^b
Neutrophils (%)	29.82 ± 2.50	38.76 ± 2.16^a	28.17 ± 1.85^b	28.21 ± 1.77^b	28.50 ± 1.38^b
Lymphocytes (%)	69.37 ± 2.82	56.85 ± 2.55^a	67.26 ± 3.01^b	66.46 ± 2.42^b	66.83 ± 1.53^b
Monocytes ($10^3/\mu L$)	5.13 ± 0.58	4.56 ± 0.80	4.87 ± 0.422	5.03 ± 0.58	4.89 ± 0.47
Eosinophil ($10^3/\mu L$)	2.50 ± 0.71	2.28 ± 0.14	2.40 ± 0.64	2.48 ± 0.31	2.38 ± 0.41
Platelets ($10^3/\mu L$)	659.85 ± 14.37	526.53 ± 17.57^a	598.55 ± 16.26^b	629.38 ± 11.31^b	618.26 ± 15.62^b

CBZ, carbendazim; 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg; WBC, white blood cell. Values are expressed as mean ± SD of 10 rats. [a]$p < .05$ versus control; [b]$p < .05$ versus CBZ only.

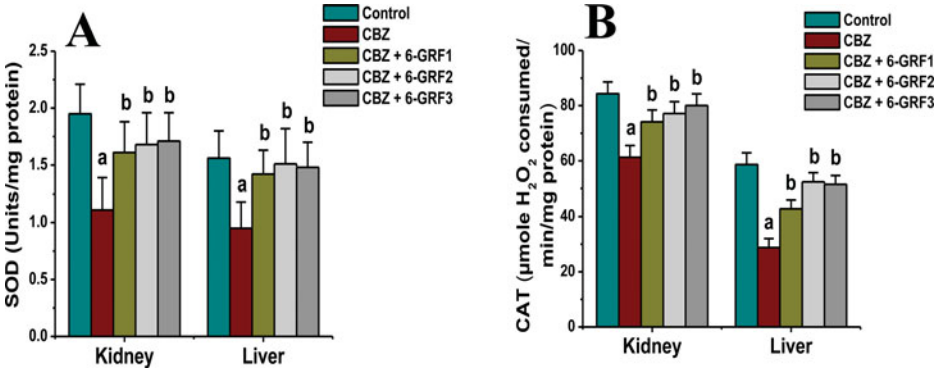

FIGURE 4. Effect of 6-GRF on SOD and CAT activities in livers and kidneys of CBZ-treated rats. 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg of 6-GRF, respectively. Each bar represents mean ± SD of 10 rats. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CBZ group ($p < .05$).

Hepatic and Renal Antioxidant Status

Figures 4–6 depict the influence of CBZ exposure and 6-GRF administration on antioxidant defense systems and lipid peroxidation in the kidney and liver of experimental animals. The activities of SOD, CAT, GPX, GST, and CAT as well as the level of GSH were markedly ($p < .05$) decreased in the kidney and liver of CBZ-treated rats. Coadministration of 6-GRF ameliorated the decrease in the activities of these antioxidant enzymes and GSH level, and restored their normalcy in CBZ-exposed rats. Administration of CBZ decreased SOD activity in the liver and kidney by 43.07% and 39.1%, respectively, when compared with the control rats. However, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored renal SOD activity by 77.1%, 91.8%, and 86.9%, whereas hepatic SOD activity was restored by 59.5%, 67.86%, and 71.4%, respectively. CBZ exposure decreased CAT activity in the liver and kidney by 27.3% and 50.9%, respectively, when compared with

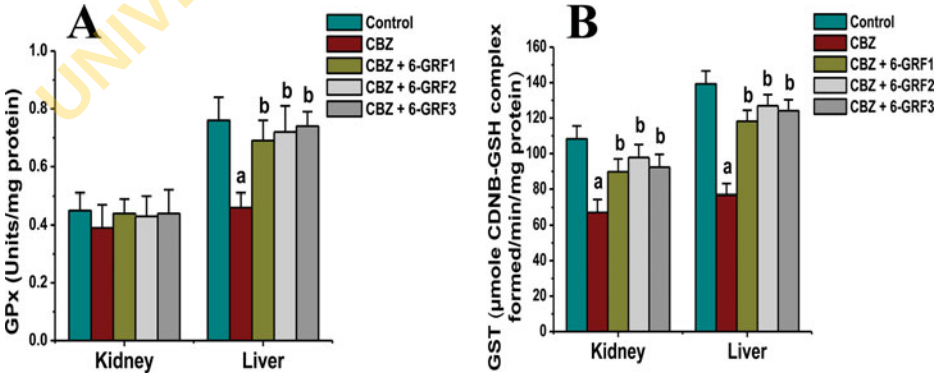

FIGURE 5. Effect of 6-GRF on GPX and GST, activities in livers and kidneys of CBZ-treated rats. 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg of 6-GRF, respectively. Each bar represents mean ± SD of 10 rats. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CBZ group ($p < .05$).

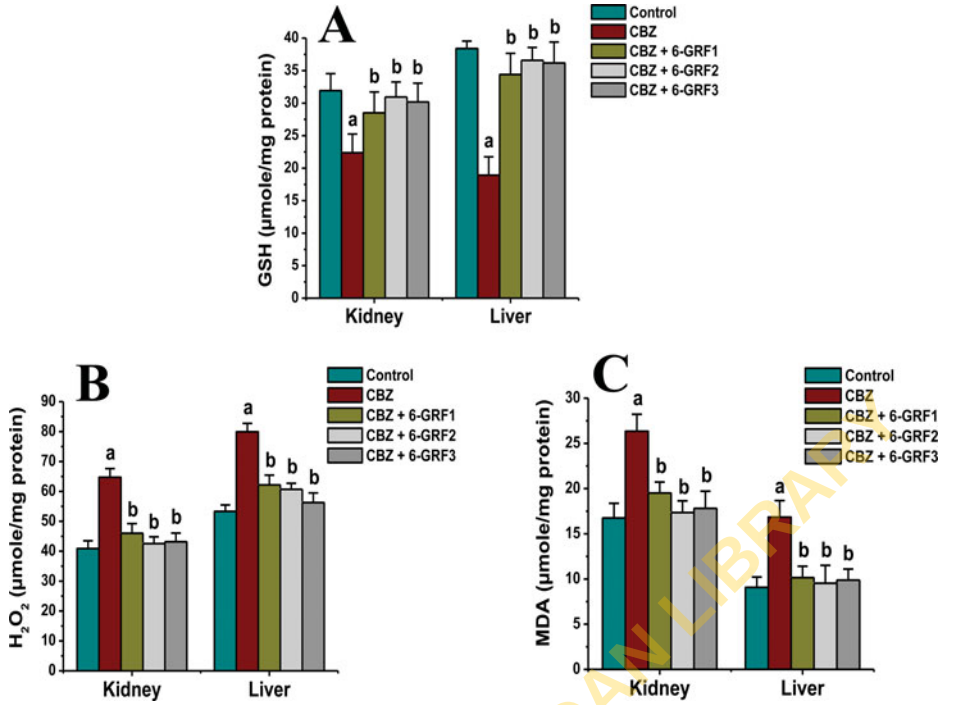

FIGURE 6. Effect of 6-GRF on GSH, H_2O_2, and Lipid Peroxide (LPO) levels in livers and kidneys of CBZ-treated rats. 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg of 6-GRF, respectively. Each bar represents mean ± SD of 10 rats. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CBZ group ($p < .05$).

the control rats. However, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored renal CAT activity by 55.9%, 68.9%, and 81.3%, whereas hepatic CAT activity was restored by 47.0%, 79%, and 76%, respectively.

Moreover, there was a significant elevation in the levels of H_2O_2 and MDA, a biomarker of lipid peroxidation, in the kidney and liver of the CBZ-exposed rats. Coadministration of 6-GRF remarkably decreased the H_2O_2 and MDA levels in the investigated organs when compared with the CBZ-treated rats. CBZ treatment elevated H_2O_2 level in the liver and kidney by 58.6% and 49.9%, respectively, when compared with the control rats. However, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored renal H_2O_2 level by 78.5%, 93.1%, and 90.4%, whereas hepatic H_2O_2 level was restored by 66.8%, 72.3%, and 88.9%, respectively. CBZ treatment increased MDA level in the liver and kidney by 57.3% and 85.6%, respectively. Conversely, coadministration with 6-GRF1, 6-GRF2, and 6-GRF3 restored renal MDA level by 71.1%, 93.7%, and 88.9%, whereas hepatic MDA level was restored by 86.2%, 94.3%, and 89.7%, respectively.

Histopathological Observations

Figures 7 and 8 represent the photomicrographs of the kidneys and livers of experimental rats. The kidney and liver of control rats appeared structurally and functionally normal. The glomeruli and hepatocytes showed a well-preserved morphology. The mesangia and the capillaries appeared normal and the urinary space was

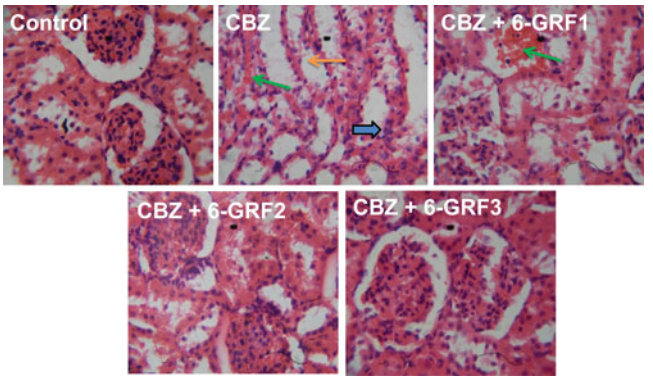

FIGURE 7. Photomicrographs of the kidneys of control and experimental rats. The kidney of control rats appeared structurally and functionally normal. The glomeruli showed a well-preserved morphology. The mesangia and the capillaries appeared normal and the urinary space intact. CBZ-treated kidney showed severe degeneration (yellow arrow), focal area of inflammation with mild disseminated congestion of vessels (blue block arrow), fatty infiltration of the medulla and convoluted tubules. Kidneys of rats cotreated with 6-GRF at 50 mg/kg (CBZ + 6-GRF1) showed normal mesangia and capillaries. The urinary space is intact but contained few red blood cells (green arrow). The kidneys of rats cotreated with 6-GRF at 100 and 200 mg/kg (6-GRF2 and 6-GRF3) appeared structurally and functionally normal. Original magnification: 240×.

intact. However, the kidney of rats treated with CBZ alone showed focal area of inflammation with mild disseminated congestion of vessels. The medulla and convoluted tubules show fatty infiltration with a congested vasa recta. The liver showed focal area of necrosis, slight dilatation of sinusoid with the presence of inflammatory cells. The epithelial cells lining the duct show vesicular nuclei, architectural anarchy, and increased nucleocytoplasmic ratio. Conversely, the kidneys of rats cotreated with 6-GRF at 50 mg/kg (CBZ + 6-GRF1) showed normal mesangia and capillaries. The urinary space is intact but contain few RBCs. The hepatocytes appear normal but the sinusoid has mild infiltration of inflammatory cells. The kidneys and livers of rats cotreated with 6-GRF at 100 and 200 mg/kg (6-GRF2 and 6-GRF3) appeared structurally and functionally normal. The observed tissue damage due to CBZ administration in the rats was independent of corn oil (the vehicle). The modulatory effects of 6-GRF on the frequency of observed lesions in the kidney and liver of CBZ-treated rats are summarized in Table 3.

DISCUSSION

In spite of the ample benefits of using fungicides in agriculture, many studies have also shown the wide spectrum of human health hazards resulting from their applications especially through the produce that reaches consumers (Blasco et al., 2006; Cabras and Angioni, 2000; Hjorth et al., 2011; Jardim and Caldas, 2012; Zubrod et al., 2014). The present study demonstrated that CBZ exposure significantly increased the relative weight of liver without affecting the body weight gain and kidney in the treated rats. This increase indicates hypertrophy of the liver. Our

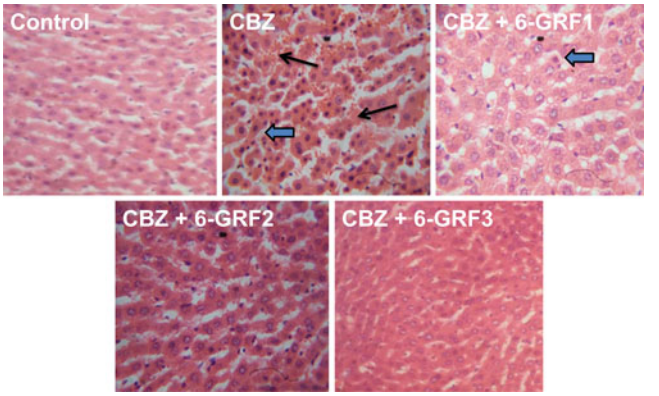

FIGURE 8. Photomicrographs of the livers of control and experimental rats. The hepatocytes of control rats appeared structurally and functionally normal. CBZ-exposed liver showed focal area of necrosis (black arrow) and slight dilatation of sinusoid with the presence of inflammatory cells (blue block arrow). The epithelial cells lining the duct showed vesicular nuclei, architectural anarchy, and increased nucleocytoplasmic ratio. The hepatocytes of rats cotreated with 6-GRF at 50 mg/kg (CBZ + 6-GRF1) appeared normal but the sinusoid had mild infiltration of inflammatory cells. The livers of rats cotreated with 6-GRF at 100 and 200 mg/kg (6-GRF2 and 6-GRF3) showed structurally and functionally hepatocytes. Original magnification: 240×.

result is in agreement with previously reported observations in CBZ-treated rats (WHO, 1974). However, the restoration of the relative liver weight to near normal following treatment with the 6-GRF showed an improvement and prevention of tissue damage during exposure to CBZ.

Hematological parameters are generally assessed to determine the functional health status and the internal environment of an organism (Etim et al., 2013; Owoeye et al., 2014). Exposure to CBZ did not affect the erythrocyte parameters such as RBC count, hemoglobin (Hb) level, PCV, MCV, MCH, and MCHC in the present investigation. This observation is in agreement with previous studies (Selmanoglu et al., 2001). However, the deleterious effects of CBZ on the total WBC, lymphocytes, neutrophils, and platelets counts were evident in this study. The WBC count

TABLE 3. Effects of 6-Gingrol-Rich fraction (6-GRF) on the frequency of observed lesions in the kidney and liver of carbendazim-treated rats

Parameters	Control	CBZ	CBZ + 6-GRF1	CBZ + 6-GRF2	CBZ + 6-GRF3
Kidney					
Congestion of vessels	0	3	1	0	0
Focal area of inflammation	0	2	0	0	0
Fatty infiltration	0	2	0	0	0
Liver					
Inflammatory cells	0	4	1	0	0
Large vacuoles	0	2	0	0	0
Focal area of necrosis	0	3	0	0	0
Hepatic degeneration	0	3	0	0	0

CBZ, carbendazim; 6-GRF1, 6-GRF2, and 6-GRF3 denote 50, 100, and 200 mg/kg. $n = 10$. Values indicate the number of animals with observed lesions in their tissues.

is a stable and well-standardized biomarker of systemic inflammation, because the cells are involved in the regulation of immunological function in many organisms (Margolis et al., 2005). Each type of white blood cell protects the body from infection. Thus, the observed decrease in these defense cells in CBZ-exposed rats indicates a widespread suppression of the immune response and high risk of infection in the treated rats. Neutrophils are normally found in the blood stream at the onset of infections or exposure to environmental contaminants (Kaminsky et al., 2002). The increased neutrophils' count in these experimental rats may partly be a compensatory response to the CBZ-induced functional impairment, whereas the marked depletion in the platelets counts indicates that CBZ could lead to impaired blood clotting. The restoration of the altered hematological parameters in rats coadministered with 6-GRF obviously revealed the effectiveness of 6-GRF in inhibiting the hematotoxicity resulting from CBZ exposure.

Elevated levels of plasma ALT, AST, ALP, and GGT are sensitive indicators of hepatocellular damage and dysfunction (Adedara et al., 2010; Lin et al., 2003; Kaplan, 1993). In the present study, these indicators of hepatic damage were higher in CBZ-treated rats than control. The chemical interaction between CBZ and the liver cell membranes could result in structural damage and leakage of these marker enzymes into blood circulation in the CBZ-treated rats. Elevated level of total bilirubin is also a characteristic of hepatic injury. The increase in the total bilirubin level in CBZ-exposed rats in the present study indicates hyperbilirubinemia due to the liver's inability to conjugate and secrete bilirubin into bile. Moreover, the increase in the levels of plasma creatinine and urea is an indication of renal dysfunction in CBZ-exposed rats. High plasma creatinine indicates impairment in the renal functions, mostly for glomerular filtration rate, whereas elevated plasma urea may suggest reduced reabsorption at the renal epithelium in the CBZ-exposed rats (Adedara et al., 2012). However, coadministration with 6-GRF significantly reversed the CBZ-mediated increase in the plasma levels of both hepatic and renal functional indices. The restoration of these biomarkers revealed a chemoprotective ability of 6-GRF against hepatorenal damage resulting from CBZ exposure in the experimental rats.

Oxidative stress, as a result of increased intracellular generation of reactive oxygen species, induces cytotoxicity and oxidative degradation of tissues. However, antioxidant defense systems comprised of enzymatic and nonenzymatic antioxidants are known to scavenge free radicals and prevent oxidative damage (Adedara and Farombi, 2010). In the present investigation, the CBZ-exposed rats exhibited a significant elevation in H_2O_2 and lipid peroxidation concomitantly with marked decrease in the GSH level and activities of antioxidant enzymes namely SOD, CAT, GPX, and GST. The observations indicate a state of oxidative stress. The diminution of antioxidant defenses could result in the accumulation of H_2O_2 molecules and consequently increased tissue lipid peroxidation as evidenced by the elevated hepatic and renal MDA level in CBZ-exposed rats. CBZ has previously been reported to induce oxidative stress in Leydig cells and the testes of rats (Adedara et al., 2013; Rajeswary et al., 2007; Sakr and Shalaby, 2014). Conversely, the antioxidant status was restored in the rats cotreated with 6-GRF. The reestablishment of the antioxidant status in the liver and kidney by 6-GRF could be attributed to its ability to scavenge free radicals, consequently diminishing the inhibition of the

antioxidant enzymes or causing their synthesis and preventing oxidative stress in CBZ-treated rats. The present observation corroborated the antioxidant property of 6-GRF previously reported (Chakraborty et al., 2012; Dugasani et al., 2010; El-Bakly et al., 2012).

The histopathological observations of the kidney and liver confirmed the biochemical results that CBZ-induced oxidative damage in the experimental rats. The present investigation revealed that the kidneys of CBZ-treated rats had focal areas of inflammation, congestion of vessels, and fatty infiltration, whereas the liver showed focal areas of necrosis, presence of inflammatory cells, and architectural anarchy with increased nucleocytoplasmic ratio in the epithelial cells lining the duct. The present histopathological observations are in accordance with previous reports (Akbarsha et al., 2000; Selmanoglu,et al., 2001). However, coadministration of 6-GRF at 100 and 200 mg/kg significantly ameliorated the CBZ-induced hepatic and renal oxidative injuries in the treated rats, thus indicating that 6-GRF protected these tissues from damage by CBZ via an antioxidative mechanism.

The protective effect of 6-Gingerol against CBZ-induced hepatorenal toxicity observed in the present investigation is associated with the structure of this natural antioxidant. In mechanistic terms, previous investigation on the structure-activity relationship of 6-Gingerol revealed that the presence of hydroxyl groups is accountable for hydrogen atom donation activity essential for the reduction of peroxyl radicals before their deleterious interaction with the cell membranes and other cell components (Dugasani et al., 2010). Moreover, 6-Gingerol has been reported to scavenge Fenton-generated hydroxyl radicals and superoxide radicals *in vitro* (Dugasani et al., 2010). We, therefore, hypothesize that 6-Gingerol, by way of scavenging deleterious free radicals and Reactive Oxygen Species (ROS), maintains the renal and hepatic antioxidant capacity and is able to combat CBZ-induced hepatorenal toxicity. The overall assessment of biochemical and histological data in the present study showed that the administration of 6-GRF produced dose-dependent ameliorative effects with 200 mg/kg (6-GRF3) being most effective, followed by 100 mg/kg (6-GRF2), and then 50 mg/kg (6-GRF1) on CBZ-induced toxicity in rats.

CONCLUSION

Taken together, the findings from this study clearly indicated that induction of oxidative stress by CBZ led to impairment of hepatic and renal functions in rats. The hematotoxic effect of CBZ was largely on the WBC parameters and the platelets. Coadministration of 6-GRF effectively restored the hematological parameters and the structures and functions of investigated tissues by a mechanism involving inhibition of lipid peroxidation and augmentation of the antioxidant defense systems. Thus, dietary supplementation of 6-Gingerol could exert protective effects against hematotoxicity and hepatorenal damage resulting from CBZ exposure.

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