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ARTICLE

Ameliorative Effect of Gallic Acid in Doxorubicin-Induced Hepatotoxicity in Wistar Rats Through Antioxidant Defense System

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ABSTRACT

Hepatotoxicity has been found to be one of the main side effects associated with doxorubicin (Dox) administration in cancer therapy. The aim of the present study was to examine the ameliorative effect of gallic acid (GA) in Dox-induced hepatotoxicity. Sixty male Wistar rats of 10 rats per group were used in this study and were randomly divided into 6 experimental groups (A–F). Rats in Group A served as the control group and received distilled water orally for 7 days; Group B was given Dox at 15 mg/kg body weight intraperitoneally (IP) on Day 8. Group C was given GA at 60 mg/kg body weight orally for 7 days + Dox at 15 mg/kg IP on Day 8. Group D was given GA at 120 mg/kg body weight orally for 7 days + Dox at 15 mg/kg IP on day 8. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight orally for 7 days, respectively. Dox administration led to a significant reduction in hepatic reduced glutathione and nonprotein thiol (NPT) together with significant increase in hepatic malondialdehyde, hydrogen peroxide generation, superoxide dismutase, and catalase activity; hepatic glutathione peroxidase and glutathione-S-transferase activity were significantly inhibited in Dox-treated rats. The serum alanine aminotransferase (ALT), alkaline phosphatase, and total bilirubin concentrations were significantly elevated following Dox administration. Pretreatment with GA ameliorated Dox-induced hepatotoxicity and oxidative stress. The results suggest that GA may offer protection against hepatic damage in Dox cancer chemotherapy.

KEYWORDS

antioxidant; doxorubicin; gallic acid; hepatotoxicity; oxidative stress

Introduction

Doxorubicin (Dox) is an anthracycline glycoside antibiotic that is effective against a number of cancers and hematological malignancies (Nudelman et al., 2005). It is broad spectrum in action and has been widely used over the past several decades to treat patients

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with various cancers, including hepatocellular carcinoma, because of its ability to kill transformed/neoplastic cells (Choi et al., 2008). The mechanism of Dox antitumor activity involves alterations of deoxyribonucleic acid (DNA) and production of free radicals (Forrest et al., 2012). This has limited the use of this drug in chemotherapy because of the diverse toxicities, such as cardiac, hepatic, hematological, and testicular toxicities (Yilmaz et al., 2006; Zaletok et al., 2015). Nicotinamide adenine dinucleotide phosphate– (NADPH-) dependent cellular reductase has been shown to convert Dox to semiquinone free radicals that can generate reactive oxygen species (ROS) including superoxide, hydroxyl radicals, and hydrogen peroxide (Yagmurca et al., 2007). These free radicals are critical to Dox-mediated cytotoxicity, including cardiotoxicity (Wang et al., 2015) and hepatotoxicity (Dai et al., 2015; Nagai, Oda, & Konishi, 2015).

Gallic acid (GA) has also been shown to possess antiallergic, antimutagenic, anti-inflammatory, and anticarcinogenic ability via its antioxidant property (Lee et al., 2014; Sarkaki et al., 2015; Shi et al., 2016). Free radical scavenging activity of GA has been proposed as a probable mechanism for reduction of oxidative stress (Nikbakht et al., 2015; Pandurangan et al., 2015). From our laboratory, the protective effect of GA on the toxicity of the anticancer agent cyclophosphamide on the central nervous system, reproductive system, and liver has been reported (Oyagbemi, Omobowale, Saba, Adedara, et al., 2016; Oyagbemi, Omobowale, Saba, Olowu, et al., 2016; Oyagbemi, Omobowale, Asenuga, et al., 2016). Hence, this study was designed to study the hepatoprotective effect of GA prior to Dox cancer chemotherapy.

Materials and methods

Chemicals

Thiobarbituric acid (TBA), ammonium ferrous sulfate, potassium iodide, sodium potassium tartrate, copper sulfate, glacial acetic acid, trichloroacetic acid, potassium dichromate, hydrogen peroxide (H_2O_2), hydrochloric acid, sulfuric acid, xylenol orange, sodium hydroxide, ethanol, sodium azide, Ellman's reagent (DTNB), O-dianisidine, reduced glutathione (GSH), 2-dichloro-4-nitrobenzene (CDNB), and sorbitol were purchased from Sigma (St Louis, MO, USA). All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

Experimental design

Sixty adult male rats weighing between 120 and 180 g obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were randomly divided into 6 groups (A–F) of 10 animals per group. The animals were kept in wire mesh cages under controlled light cycle (12h light/12h dark); they were fed with commercial rat chow ad libitum and liberally supplied with water.

Rats in Group A served as the control group and received distilled water orally for 7 days; Group B was given Dox at 15 mg/kg body weight IP on Day 8. Group C was given GA at 60 mg/kg body weight orally for 7 days + Dox at 15 mg/kg IP on Day 8. Group D was given GA at 120 mg/kg body weight orally for 7 days + Dox at 15 mg/kg IP on Day 8. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight orally for 7 days, respectively. The choice for Dox administration was chosen from the previous study (El-Moselhy & El-Sheikh, 2014). The Dox was administered at a single intraperitoneal injection. Rats were sacrificed 24 hours after the last administration.

Care of animals

All the experimental animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Science and published by the National Institutes of Health (Garber et al., 2011). The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments according to the Public Health Service (Garber et al., 2011).

Blood collection and serum preparation

Fresh whole blood (5 mL) was collected from each rat through the retro-orbital venous plexus into sterile plain tubes and left for about 30 minutes to clot. The clotted blood was thereafter centrifuged at 4,000 g for 10 minutes. Serum was harvested into sample bottles and stored at −20°C till the time of analysis.

Hepatic tissue preparation for biochemical assays

The rats were sacrificed by cervical dislocation 24 hours after the last administration. The liver was removed and weighed and a portion of it was rinsed in 1.15% potassium chloride (KCl) and homogenized in potassium phosphate buffer (0.1 M, pH 7.4) and centrifuged at 12, 000 g for 15 minutes to obtain the postmitochondria fraction (PMF)/cytosolic fractions. The PMF of the liver was obtained and subsequently stored at -20°C until the time of use.

Biochemical assays

Protein determination

Protein concentrations were determined as described by Gornal, Bardawill, and David (1949); 1 mL of diluted serum was added to 3 mL of the biuret reagent. The reaction mixture was incubated at room temperature for 30 minutes. The mixture was thereafter read with spectrophotometer at 540 nm using distilled water as blank. The final value for total protein was extrapolated from the total protein standard curve.

Determination of hepatic glutathione peroxidase and Glutathione-S-transferase (GST) activity

The hepatic glutathione peroxidase (GPx) activity was measured according to Buetler, Duron, and Kelly (1963). The reaction mixtures contain 0.5 mL of potassium phosphate buffer (pH 7.4), 0.1 mL of sodium azide, 0.2 mL of GSH solution, 0.1 mL of H_2O_2, 0.5 mL of PMF, and 0.6 mL of distilled water. The mixture was incubated in the water bath at 37°C for 5 minutes, and 0.5 mL of trichloroacetic acid (TCA) was added and centrifuged at 4,000 g for 5 minutes; 1 mL of the supernatant was taken and added to 2 mL of K_2PHO_4 (dipotassium hydrogen phosphate) and 1 mL of Ellman's reagent. The absorbance was read at 412 nm. Glutathione-S-transferase (GST) was estimated by the method of Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene as substrate.

Determination of hepatic superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972) with modification from our laboratory (Oyagbemi et al., 2015; Omobowale et al.,

2014); 100 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. This preparation prevents auto-oxidation of epinephrine and is stable for 4 weeks. Then 0.01 mL of hepatic PMF was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) followed by the addition of 0.3 mL of 0.3 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds; 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

Determination of hepatic catalase activity

Catalase (CAT) activity was determined according to the method of Sinha (1972). Briefly, a 1 mL portion from the reaction mixture (2 ml of H_2O_2 solution, 2.5 ml of 0.01 M potassium phosphate buffer [pH 7.0], and 1 mL of properly diluted PMF) was blown into 1 mL dichromate/acetic acid solution by a gentle swirl at room temperature at 60-second intervals for 3 times. The mixture was incubated in the water bath at 100°C for 10 minutes. The absorbance was read at 570 nm. One unit of CAT activity represents the amount of enzyme required to decompose 1 µmol of H_2O_2/minute.

Determination of hepatic reduced glutathione

The content of hepatic reduced glutathione (GSH) was estimated by the method of Jollow et al. (1994). Briefly, 0.5 mL of 4% sulfosalicylic acid (precipitating agent) was added to 0.5 mL of PMF and centrifuged at 4,000 g for 5 minutes. To 0.5 mL of the resulting supernatant, 4.5 mL of Ellman's reagent (0.04 g of DTNB in 100 ml of 0.1 M phosphate buffer, pH 7.4) was added. The absorbance was read at 412 nm.

Determination of hepatic nonprotein thiol levels

Hepatic NPSH levels were determined by the method of Ellman (1959). A 500 µL aliquot of hepatic PMF was mixed with 10% TCA (V/V). After centrifugation, the protein pellet was discarded and free-sulfhydryl (SH) groups were determined in a clear supernatant. A 100 µL aliquot of supernatant was added to 850 µL of 1 M potassium phosphate buffer (pH 7.4) and to 50 µL of DTNB (10 mM). Absorbance of colorimetric reaction was measured at 412 nm.

Determination of hepatic total thiol content

The hepatic total thiol content was determined using the method of Ellman (1959). The reaction mixture contained 170 µL potassium phosphate buffer (0.1 M, pH 7.4), 20 µL of sample, and 10 µL of DTNB (10 mM). After incubation, for 30 min at room temperature, the absorbance was measured at 412 nm and the sample total thiol levels were estimated using GSH as standard.

Determination of hepatic thiobarbituric acid reactive substance

Thiobarbituric acid reactive substance (TBARS) was quantified as malondialdehyde (MDA) in the hepatic PMF. The MDA was determined according to the method of Varshney and Kale (1990). To 1.6 mL of Tris-KCl, 0.5 mL of 30% TCA, 0.4 mL of sample, and 0.5 mL of 0.75% thiobarbituric acid (TBA) prepared in 0.2 M HCl were added. The reaction mixture was incubated in the water bath at 80°C for 45 minutes, cooled on ice and centrifuged at 4,000 rpm for 15 minutes. The absorbance was measured at 532 nm. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}Cm^{-1}$.

Measurement of hepatic hydrogen peroxide generation

Hydrogen peroxide (H_2O_2) generation was determined according to the method of Wolff (1994). To 2.5 mL of 0.1 M potassium phosphate buffer (pH 7.4), 0.250 mL of ammonium ferrous sulfate (AFS), 0.1 mL of sorbitol, 0.1 mL of xylenol orange (XO), 0.025 mL of H_2SO_4, and 0.050 mL of hepatic PMF were added. The mixture was mixed thoroughly by vortexing, and a light pink color of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 minutes. The absorbance was measured at 560 nm. The hydrogen peroxide (H_2O_2) generated was extrapolated from the hydrogen peroxide standard curve.

Measurement of hepatic nitric oxide contents

Hepatic nitric oxide (NO) was measured as described by Olaleye et al. (2007) by indirectly measuring the nitrite concentration. After incubation at room temperature for 20 minutes, the absorbance at 540 nm was measured by spectrophotometer. The concentration of nitrite in the sample was determined from a sodium nitrite ($NaNO_2$) standard curve and was expressed as μmol nitrite/mg protein.

Determination of serum alanine aminotransferase, alkaline phosphatase, and total bilirubin

The activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin were determined using the Randox Kit (Randox Laboratories Limited, UK). The manufacturer's instructions were followed in the assay protocol.

Histopathology

Small pieces of liver tissues were collected in 10% buffered formalin (pH 7.4) for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5–6 µm in thickness were made and stained with hematoxylin and eosin for histopathological examination (Drury, Wallington, & Cancerson, 1976).

Statistical analysis

All values are expressed as mean ± standard deviation (SD). One-way ANOVA with Dunnett's posttest was also performed using GraphPad Prism version 4.00. The level of statistical significance was considered as $p < .05$.

Results

The activities of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) together with total bilirubin level increased significantly ($p < .05$) in Dox-only treated rats compared to the control (Table 1). The hepatoprotective effect of GA was demonstrated with significant ($p < .05$) reduction in the serum ALT, ALP, and total bilirubin of rats pretreated with GA (60 and 120 mg/kg) (Table 1).

The hepatic reduced glutathione (GSH) content was significantly reduced in rats administered Dox only. Further, rats pretreated with GA (60 and 120 mg/kg) showed significant ($p < .05$) improvement in GSH content compared to rats administered Dox only (Figure 1). The improvement in GSH content was dose dependent with 120 mg/kg of GA showing higher

Table 1. Effects of doxorubicin on markers of hepatic damage.

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
ALT (U/L)	72.09 ± 0.86	73.47 ± 0.08[a]	72.59 ± 0.21[b]	72.60 ± 0.14[b]	72.05 ± 0.08	72.26 ± 0.07
ALP (U/L)	5.80 ± 0.55	10.70 ± 0.29[a]	5.38 ± 0.14[b]	5.24 ± 0.24[b]	5.73 ± 0.29	5.84 ± 032
Total bilirubin (mg/dL)	10.64 ± 0.65	23.74 ± 1.41[a]	6.94 ± 0.65[a,b]	9.25 ± 0.93[b]	8.79 ± 0.65[a]	9.25 ± 1.31

Data are presented as mean ± standard deviation ($n = 10$). Rats in Group A served as the control group. Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered with GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally.
[a]Significant difference at $p < .05$ when groups B, C, D, E, and F are compared with group A.
[b]Significant difference at $p < .05$ when groups C and D are compared with group B.

antioxidant capacity (Figure 1). The hepatic malondialdehyde (MDA) also increased significantly in Dox-only treated rats compared to the control and rats pretreated with GA (60 and 120 mg/kg) as shown in Figure 2. In the same experiment, pretreatment with GA (60 and 120 mg/kg) dose-dependently reduced hepatic MDA content (Figure 2). On the other hand, the hepatic nitric oxide (NO) content was also significantly decreased in Dox-only treated rats as opposed to the aforementioned markers of oxidative stress when compared to the control (Figure 3). However, pretreatment with GA (60 and 120 mg/kg) significantly reduced hepatic NO content compared to Dox-only treated rats (Figure 3). Similarly, hepatic hydrogen peroxide (H_2O_2) generated was significantly increased in Dox-only treated rats compared to the control (Figure 4), and this increase in marker of oxidative stress was significantly ($p < .05$) reduced in rats pretreated with GA (60 and 120 mg/kg), as indicated in Figure 4.

The activity of hepatic glutathione peroxidase (GPx) was significantly reduced in Dox-only treated rats compared to the control (Figure 5). However, pretreatment with GA (60 and 120 mg/kg) dose-dependently increased hepatic GPx activity (Figure 5). The hepatic catalase (CAT) increased significantly in Dox-only treated rats and rats pretreated with GA (60 and 120 mg/kg) when compared to the control (Figure 5). In this study, Dox administration led to a significant ($p < .05$) decrease in hepatic nonprotein thiol (NPT) compared to the control and other treatment groups (Figure 6). However, pretreatment with GA (60 and 120 mg/kg) dose-dependently increased hepatic NPT. By contrast, the administration of Dox

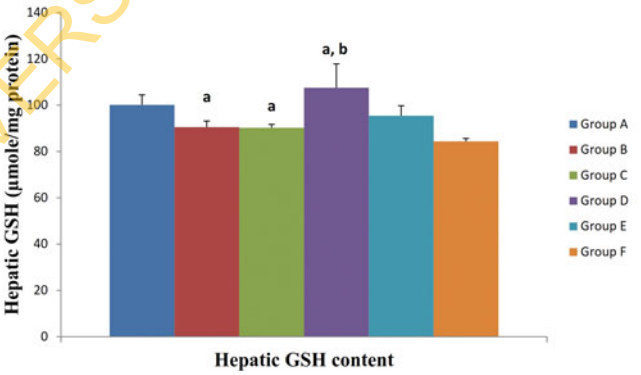

Figure 1. The effect of doxorubicin intoxication on hepatic reduced glutathione (GSH). Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

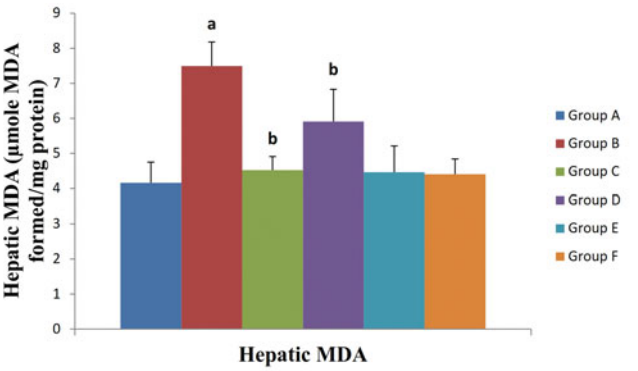

Figure 2. The effect of doxorubicin intoxication on hepatic malondialdehyde (MDA). Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

significantly increased total thiol (TT) when compared to the control and rats pretreated with GA (Figure 6). Our results showed that Dox administration significantly increased hepatic superoxide dismutase (SOD) activity in Dox-only treated rats and rats pretreated with GA (60 and 120 mg/kg) when compared to the control (Figure 7). By contrast, the activity of GST was reduced significantly by Dox administration compared to the control and other treatment groups (Figure 7).

The liver weight also showed significant ($p < .05$) increase in Dox-only treated rats and rats pretreated with 60 mg/kg of GA compared to the control (Figure 8). However, rats pretreated with 120 mg/kg of GA showed significant reduction in the liver weight when compared to the Doxonly treated rats (Figure 8). The histology section of rats administered Dox -only showed congestion of vessels, mild infiltration of zone 2 by inflammatory cells, and moderate microvesicular steatosis, whereas rats pretreated with 60 mg/kg gallic acid (GA) orally for 7 days prior to administration of Dox showed no visible lesions except for areas of

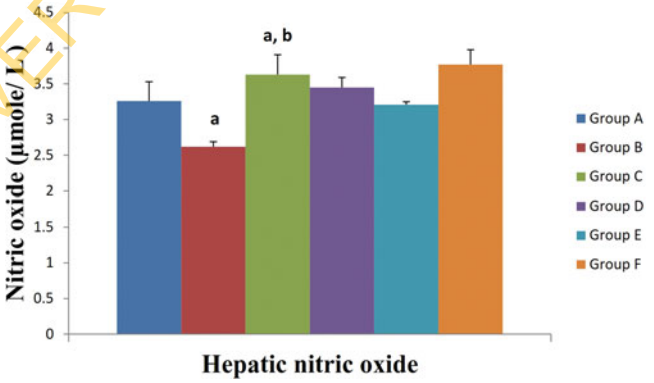

Figure 3. The effect of doxorubicin intoxication on hepatic nitric oxide (NO). Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given GA at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

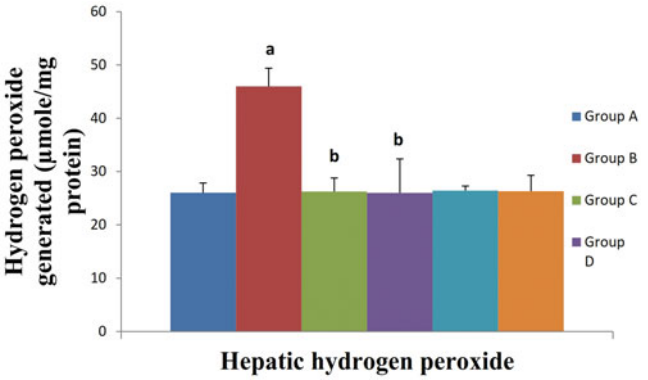

Figure 4. The effect of doxorubicin intoxication on hepatic hydrogen peroxide (H_20_2) generated. Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

slight congestion; a similar result was obtained in rats pretreated with 120 mg/kg gallic acid (GA) orally for 7 days prior to administration of Dox 15 mg/kg. Rats pretreated with 60 and 120 mg/kg gallic acid (GA) orally alone for 7 days showed no visible lesions except for areas of slight congestion (Figure 9).

Discussion and conclusion

In this study, Dox administration led to a significant increase in serum ALT, ALP, and total bilirubin. The increases in serum levels of ALT, ALP, and total bilirubin have been reported as

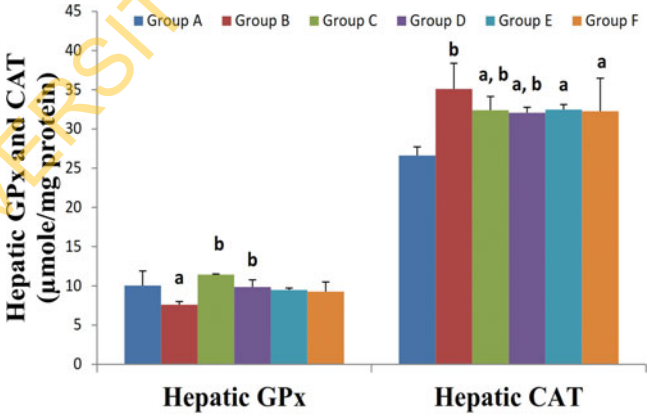

Figure 5. The effect of doxorubicin intoxication on hepatic glutathione peroxidase (GPx) and catalase (CAT) activity. Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

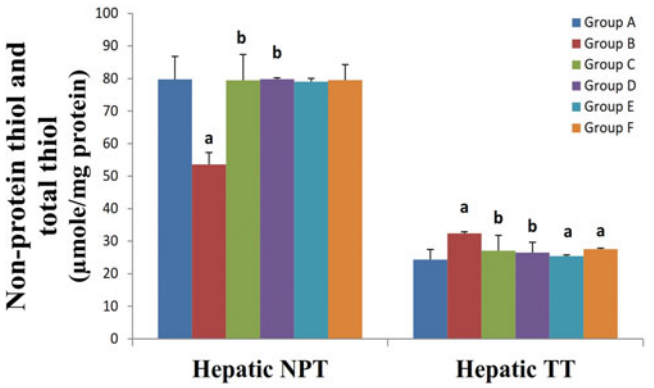

Figure 6. The effect of doxorubicin intoxication on hepatic nonprotein thiol (NPT) and total thiol (TT). Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

markers of liver damage and hepatocyte death due to membrane breakdown of the liver cell and ultimate leakage of these markers from the intracellular to extracellular milieu (Wang et al., 2015; Kobylinska et al., 2015; Nagai et al., 2016). According to our result, pretreatment with GA ameliorated and mitigated Dox-induced hepatotoxicity. The toxicity associated with Dox might be due to the toxic metabolite following metabolism of Dox after its administration (Niu et al., 2015). Furthermore, medicinal foods that contain GA might be of potential benefit to cancer patients prior chemotherapy with Dox.

The administration of Dox precipitated significant reduction in hepatic nitric oxide (NO), reduced glutathione, and nonsulfhydryl content together with significant increase in hepatic hydrogen peroxide (H_2O_2) generated, and malondialdehyde content. Therefore, we propose

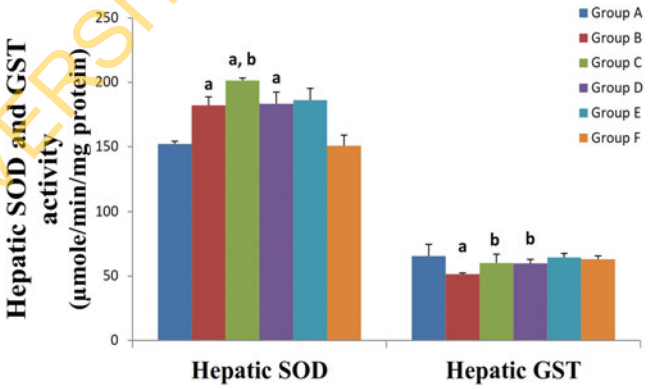

Figure 7. The effect of doxorubicin intoxication on hepatic superoxide dismutase (SOD) and glutathione-S-transferase (GST) activity. Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group B.

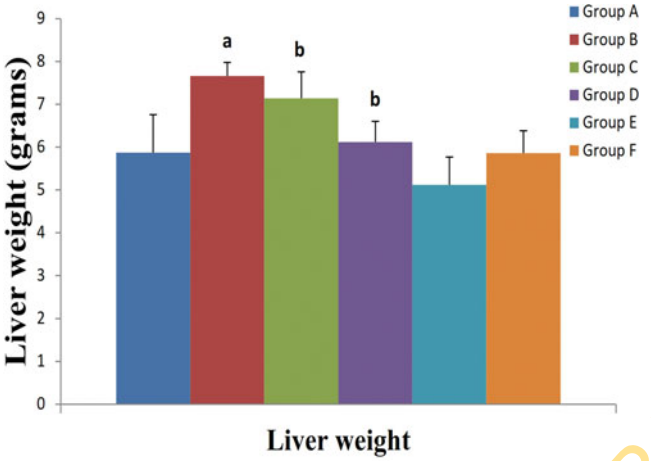

Figure 8. The effect of doxorubicin intoxication on liver weight. Rats in Group A served as the control; Group B was given doxorubicin (Dox) at 15 mg/kg body weight; Group C was given gallic acid (GA) at 60 mg/kg body weight + Dox at 15 mg/kg; Group D was given GA at 120 mg/kg body weight + Dox at 15 mg/kg. Rats in Groups E and F were administered GA alone at 60 and 120 mg/kg body weight, respectively. Dox was administered intraperitoneally while GA was given orally. a indicates $p < .05$ when Groups B, C, D, E, and F are compared with Group A. b indicates $p < .05$ when Groups C and D are compared with Group A.

from this study that Dox administration enhanced both hepatic oxidative and nitrosative stress. The observed reduction in the NO content following Dox might be related to the overproduction of superoxide radical anion that can possibly combine with NO, thereby facilitating its reduction. The combination of NO and superoxide radical anion forms peroxynitrite, which is a cytotoxic molecule that can damage proteins, DNA, and ribonucleic acid (RNA). Peroxynitrite has also been reported to participate in nitrosative stress (Lee et al., 2016). Hence, we speculate that the generation of nitrosative stress might be associated with the formation of peroxynitrite. The involvement of Dox in oxidative stress and generation of reactive oxygen species (ROS) has been widely accepted as the mechanism of action of Dox-induced hepatotoxicity (Wu et al., 2015; Liu et al., 2015; Mokni et al., 2015; Szwed et al., 2016). The ability of GA as a potent antioxidant was strongly demonstrated by reducing markers of oxidative stress and improving the hepatic GSH content. Oxidative stress is considered when production of ROS/free radicals exceeds and overwhelms that of the antioxidant defense system.

However, activities of the antioxidant defense system were altered in a different manner. The hepatic glutathione-S-transferase (GST) and glutathione peroxidase (GPx) were significantly inhibited following Dox administration. By contrast, the hepatic superoxide dismutase (SOD) and catalase (CAT) activities increased significantly. The SOD participates in the first line of defense during oxidative stress by converting superoxide anion radical (O_2-) to H_2O_2 while GPx and CAT quench H_2O_2 to water and oxygen (O_2), respectively. Furthermore, GST detoxifies toxic electrophiles including Dox metabolites with the help of GSH as a cofactor to more soluble and less toxic metabolites that can easily be excreted by the kidney (Chen et al., 2015; Beyerle et al., 2015). However, the reduction in hepatic GSH by a high dose GA (120 mg/kg) suggests that a high dose of GA might be pro-oxidant. Hence, care must be taken in the use of phytochemicals as antioxidants in disease prevention. The observed reduction in the activities of GST and GPx might suggest production/generation of ROS/free radicals by Dox, thereby enhancing the accumulation of toxic metabolites of Dox, which further exacerbated the hepatotoxicity of Dox. On the contrary, the increase in the activity of SOD and

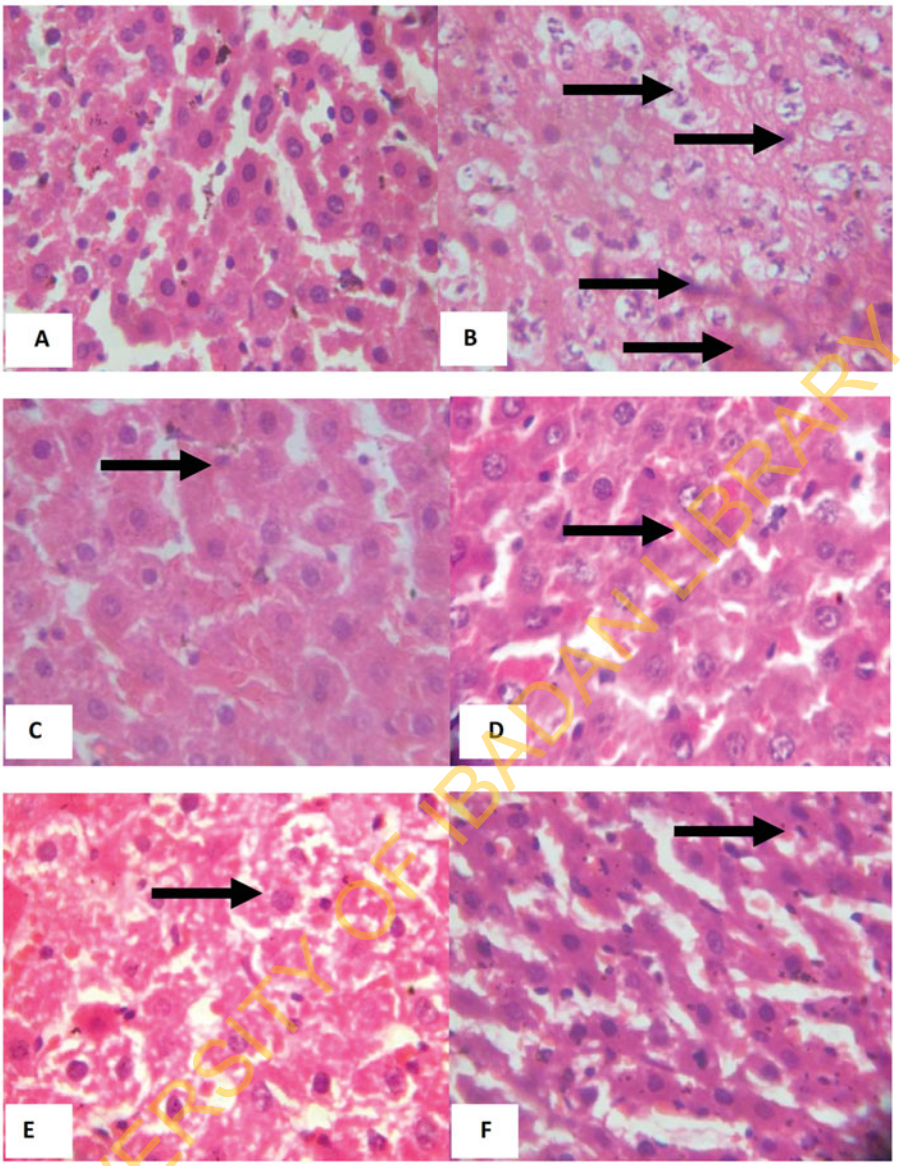

Figure 9. Histology of the liver following doxorubicin (Dox) administration. Group A shows slight areas of congestion; rat liver intoxicated with doxorubicin at 15 mg/kg body weight intraperitoneally (IP). Group B shows congestion of vessels, mild infiltration of zone 2 by inflammatory cells, and moderate microvesicular steatosis (black arrow). Group C pretreated with 60 mg/kg gallic acid (GA) orally for 7 days prior to administration of Dox 15 mg/kg. Group D pretreated with 120 mg/kg GA orally for 7 days prior to administration of Dox 15 mg/kg Group E received 60 mg/kg GA orally alone for 7 days. Group F received 120 mg/kg GA orally alone for 7 days. There are no visible lesions in the photomicrograph of the liver section in rats in Groups C, D, E, and F, but there are areas of congestion (black arrows). Representative hematoxylin-eosin- (H&E-) stained liver sections (X 400 objectives).

CAT could be said to be an adaptive response of hepatic tissue to Dox toxicity. The mechanism through which Dox induces antioxidant enzyme activity might be via up-regulating SOD and CAT messenger ribonucleic acid (mRNA) and activation of the Nrf2-ARE pathway. Hence, the hepatoprotective effect and the antioxidant activities of GA were clearly demonstrated by reducing the hepatic markers of oxidative stress and improving the antioxidant

defense system in a dose-dependent manner. In addition, excessive production of NO was observed in rats administered Dox only, which was indicative of nitrosative stress (Wanyong et al., 2015). The hepatic NO may combine with (O_2-) to produce peroxynitrite (ONOO-), a cytotoxic molecule (Wen et al., 2015). GA administration prior to doxorubicin chemotherapy may be potentially beneficial to cancer patients by modulating, ameliorating, or reversing toxicity associated with Dox treatment. The ameliorative effect of GA in the present study could be ascribed to its antioxidant and anti-inflammatory properties. The results suggest that GA may offer protection against hepatic damage in Dox cancer chemotherapy. However, the major limitation in this study was lack of funding.

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Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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