

Original Article**Gallic Acid Ameliorates Bisphenol A-Induced Toxicity in Wistar Rats***Eunice Olufunke Ola-Davies^{*1}, Samuel Gbadebo Olukole², Damilare Olaniyi Lanipekun²**Received: 23.03.2018**Accepted: 05.05.2018***ABSTRACT**

Background: Bisphenol A (BPA) has received attention in environmental and toxicological research due to its widespread effects in biological systems. While several anti-oxidants have been used in ameliorating BPA-induced toxicities in experimental animals, there is the scarcity of research information on the use of Gallic acid (GA) in protecting against BPA-induced toxicity. This study investigated the ameliorative effect of Gallic acid in BPA-induced toxicities of the adult male Wistar rats.

Methods: Thirty two adult male Wistar rats were randomly assigned into four groups of eight animals each as follows: Group 1 (Control rats): 0.2 ml of corn oil; Group 2 (GA-treated rats): 20 mg/kg/day GA (dissolved in distilled water); Group 3 (BPA-treated rats): 10 mg/kg/day BPA suspended in 0.2 ml corn oil; Group 4 (BPA+GA-treated rats): BPA (10 mg/kg/day) with a concomitant GA (20 mg/kg/day). All treatments were orally administered for 14 days.

Results: BPA significantly increased ($P<0.05$) in the values of liver function enzymes (ALP, AST, ALT, GGT), total globulin, conjugated globulin, triglycerides, total cholesterol, low-density lipoprotein, creatinine, and urea as well as sodium ions. Concomitant treatment with GA ameliorated these elevated values. Moreover, BPA-induced histopathological alterations in the liver and kidney while GA ameliorated them.

Conclusion: BPA caused structural and cellular perturbations of the blood, liver, and kidney of rats while concomitant treatment with GA ameliorates the condition. Hence, GA has hepatoprotective and nephroprotective actions against BPA-induced toxicity in Wistar rats.

Keywords: Bisphenol A, Gallic acid, Kidney, Liver, Toxicity.

IJT 2018 (4): 11-18**INTRODUCTION**

Bisphenol A (BPA), an endocrine disrupting chemical (EDC) has received attention in environmental and toxicological research due to its widespread effects in biological systems.

BPA is a component of epoxy resins, widely used in the production of plastic containers for food and beverages, adhesives, paints, dental sealants, drinking water pipe linings and household paper products [1]. Being exogenous agents, EDCs are able to interfere with the synthesis, metabolism, and action of endogenous hormones in animals and humans.

Most studies on BPA have focused on its endocrine disrupting ability and its consequence adverse effect on the development of the reproductive organs and fertility at large [2, 3]. However, there have been reports on the effect of BPA on the digestive and excretory systems especially, the liver and kidney [4, 5]. The adverse

effect of BPA on the liver and kidney was demonstrated to include reactive oxygen species (ROS) generation with oxidative DNA in the liver, the formation of multinucleated giant cells in rat liver hepatocytes, the alteration of liver and kidney biochemical profiles as well as degeneration of renal tubules in kidney of rat and mice [4, 6].

Gallic acid {(GA),3,4, 5-trihydroxybenzoicacid)} is one of recognized tea polyphenols widely used in the traditional medicine due to its anti-allergic, anti-mutagenic, anti-inflammatory, anti-oxidant and anti-cancer activities [7]. GA can be found in several natural products including lemon, red wine, strawberries, pineapples, bananas, gallnuts and tea leaves [7, 8]. Gallic acid, like all tea polyphenols, is able to sequester metal ions and to scavenge ROS thereby preventing oxidative stress in tissues since ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues [8].

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A number of antioxidants, including melatonin, indole-3-carbinol, Vitamins C and E have been used in ameliorating BPA-induced toxicities in experimental animals [9-11]. There is the scarcity of research information on the use of Gallic acid in protecting against BPA-induced toxicity. This study was designed to investigate the ameliorative effect of Gallic acid in BPA-induced toxicities of the liver and kidney in adult male Wistar rats.

MATERIALS AND METHODS

Chemicals

BPA and GA used in the study were purchased from Sigma-Aldrich Co. (St Louis, Missouri, USA). Every other reagent used was of standard grades.

Experimental Animals

Thirty-two adult male Wistar rats kept in the Animal House, Faculty of Veterinary Medicine, University of Ibadan were used.

All procedures were carried out according to the guidelines for the care and use of experimental animals (National Institute of Health (NIH), USA). The study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee.

Commercial rat feed pellets and water was given *ad libitum*. The rats were stabilized for two weeks before the commencement of the treatment protocol. The rats used in the study were randomly assigned into four groups of eight animals each as follows:

- Group 1 (Control rats): 0.2 ml of corn oil orally administered.
- Group 2 (GA-treated rats): 20 mg/kg body weight per day GA (dissolved in distilled water) administered orally for 14 d [12].
- Group 3 (BPA-treated rats): 10 mg/kg body weight per day BPA suspended in 0.2 ml corn oil, orally administered for 14 d [13].
- Group 4 (BPA+GA-treated rats): Orally administered BPA (10 mg/kg body weight per day) with a concomitant GA (20 mg/kg body weight per day) orally administered.

Blood Sampling

The rats were fasted overnight, weighed, anesthetized using diethyl ether and blood samples collected 24 h after the last treatment prior to sacrifice. Blood sampling was carried out [14]. Briefly, blood samples were collected from the orbital sinus of the rats into clean lithium heparinized tubes. Blood samples were also collected for biochemical analysis, centrifuged at

3000 rpm for ten minutes to isolate the serum. Na^+ , K^+ , Cl^- , HCO_3^- were determined by use of automated analyzers [15]. Commercially available kits were used according to the respective manufacturer's protocol for the measurement of serum liver enzyme activity. Serum alkaline phosphatase (ALP) activity was determined by a kit from BioSystems SA., Spain. Serum aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alanine transaminase (ALT), acid phosphatase and prostatic acid phosphatase activities, Urea, Creatinine, Total Bilirubin (TB), Conjugated Bilirubin (CB), Total Protein (TP), *high density lipoprotein (HDL)*, *low density lipoprotein (LDL)*, were measured using RANDOX[®] laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmere, United Kingdom. Serum Cholesterol and Triglyceride levels were determined by Ecoline CHOD-PAP and Ecoline 25 GPO-PAP assay kits (1.14856.0001, Merck KGaA, Darmstadt, Germany), respectively.

Histological Study

Liver and kidney samples were collected in 10% formalin for histopathological analysis. Tissues were processed and embedded in paraffin wax and sections were made of about 4-6 μm . After staining with hematoxylin and eosin, slides were examined under the microscope (Olympus, Japan) for histopathological changes and photographed.

Statistical Analysis

Data generated from the treatments were analyzed using mean-standard deviation and was subjected to one-way ANOVA with Dunnett's multiple comparisons test (GraphPad Prism 6).

RESULTS

There were significant increases ($P < 0.05$) in the values of ALP, AST, ALT, GGT, TB and CB in the BPA-treated rats (Fig. 1A-F). However, concomitant treatment with GA ameliorated these increases. Moreover, the BPA-treated rats had significant increases in UREA and CREAT values compared to the control group (Fig. 2A-B). The BPA+GA-treated rats, however, showed significant decreases in these values compared to the BPA-treated rats. The results of the lipid profile assay are given in Fig. 3A-D. There were significant increases in the values of TG, TC, and LDL in the BPA-treated group compared to the control. Moreover, the BPA+GA group showed significant decrease in these parameters compared to the BPA-treated group. However, BPA caused a significant

decrease in HDL value compared to the control while GA ameliorated the condition. GLB and TP showed no significant difference ($P>0.05$) among the groups (Fig. 4 A-C). However, there was a significant increase ($P<0.05$) in the value of ALB between the BPA-treated rats and those of the control while the BPA+GA group showed a significant decrease in ALB value compared to the BPA group. The results of the electrolyte test for the rats used in the present study were given in Table 1. There was a significant increase ($P<0.05$) in sodium ions in the BPA-treated rats compared to those of the control. The BPA+GA group, however, ameliorated the condition. With respect to bicarbonate ions, there was a significant decrease ($P<0.05$) between the BPA-treated rats and those of the control with the BPA+GA attenuating the reduction. However, there were no significant differences ($P>0.05$) in potassium and chloride ions across the groups. The frequency of occurrence of structural alterations of the liver and kidney of the rats used in the present study is given in Table 2. The liver of the control rats had normal hepatic

plates radiating from the central plate being surrounded by sinusoids (Fig. 5 A). The BPA-treated rats showed disarrangement of hepatic plates with congested central vein, dilated sinusoids, pyknotic nuclei, and cellular infiltrations in the portal area as well as proliferative and congested bile ducts (Fig. 5 B-D). The liver plates of the GA treated showed no such lesions while the BPA + GA rats showed a restoration of the hepatic plates with lesser lesions compared to the BPA-treated rats (Fig. 5 F&E). The kidney histopathological findings followed the same pattern observed with the liver. While the control and GA groups showed normal renal corpuscles with the tubules and connecting ducts (Fig. 6 A& E), the BPA group showed a number of lesions including segmented glomerular tuft, loss of glomerular epithelium and widened urinary space, widened Loop of Henle, exfoliated cells within the connecting ducts, congested cortical blood vessels and cytoplasmic vacuolations (Fig. 6 B-D). The BPA + GA group however, showed a restored renal tissue with minimal lesions (Fig. 6 F).

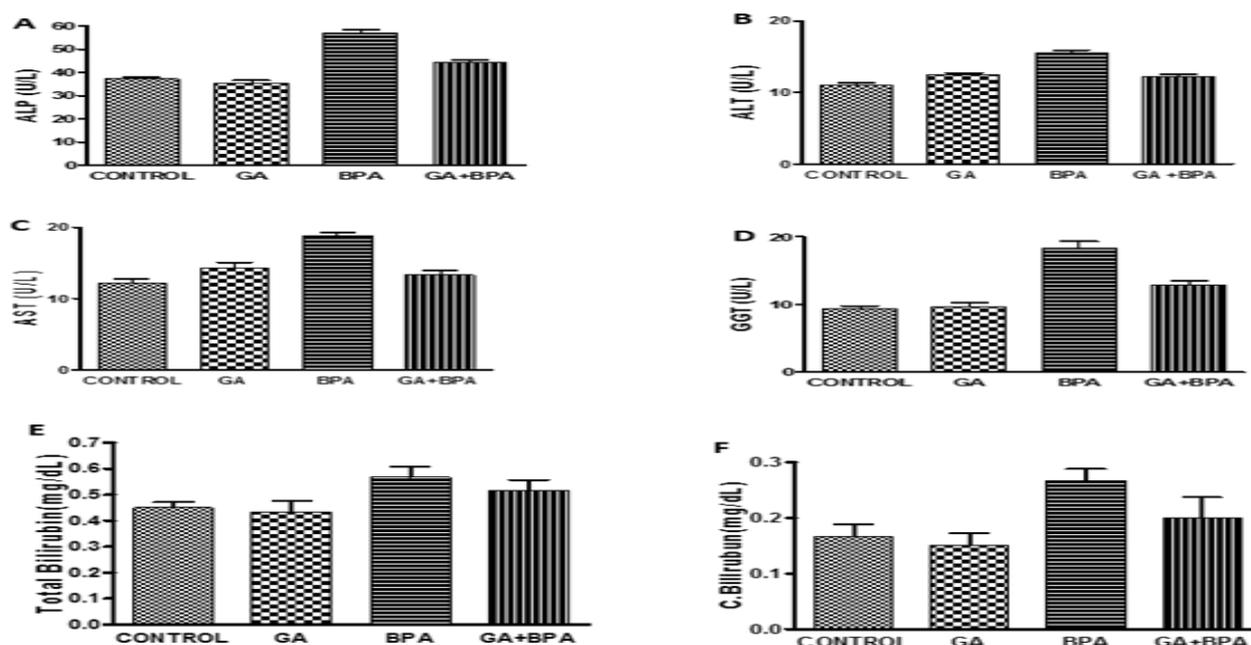


Figure 1. Bar chart of liver function parameters in rats showing A (ALP), B (ALT), C (AST), D (GGT), E (TB) and F (CB).

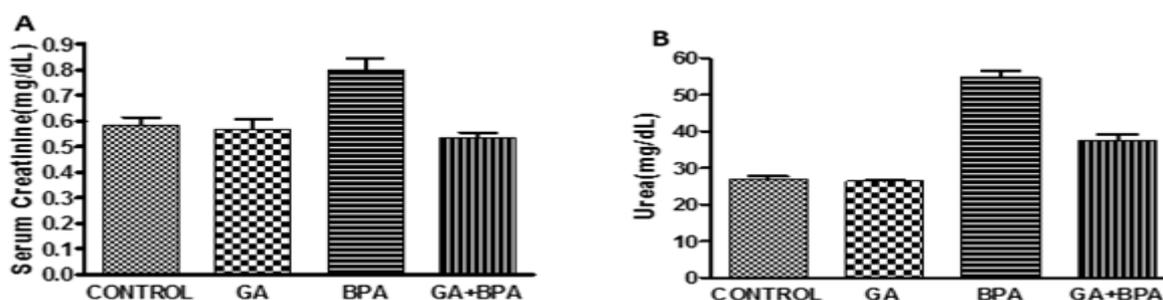


Figure 2. Bar chart of kidney function parameters in rats showing serum creatinine (A) and Urea (B).

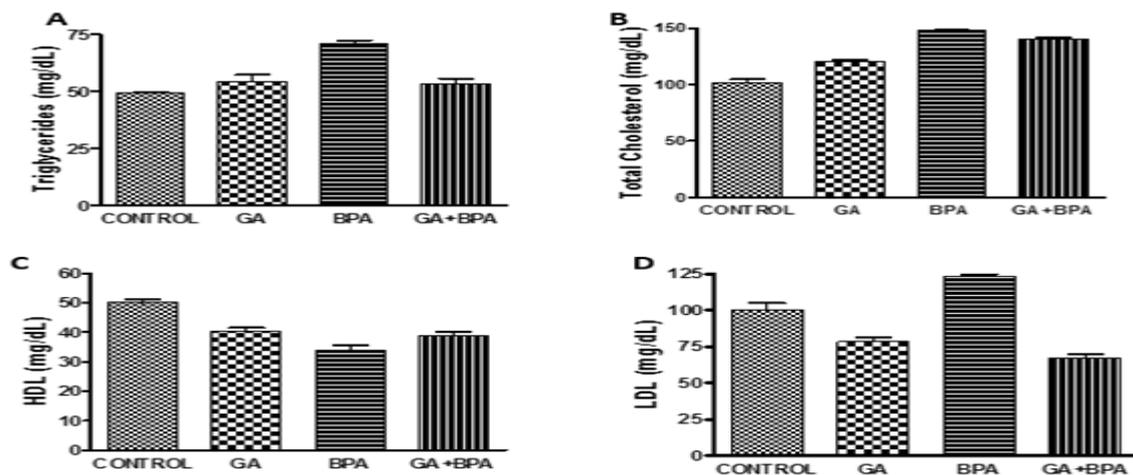


Figure 3. Bar chart of lipid function parameters: Triglycerides (A), Total cholesterol (B), High-density lipoprotein, HDL (C) and Low-density lipoprotein, LDL (D).

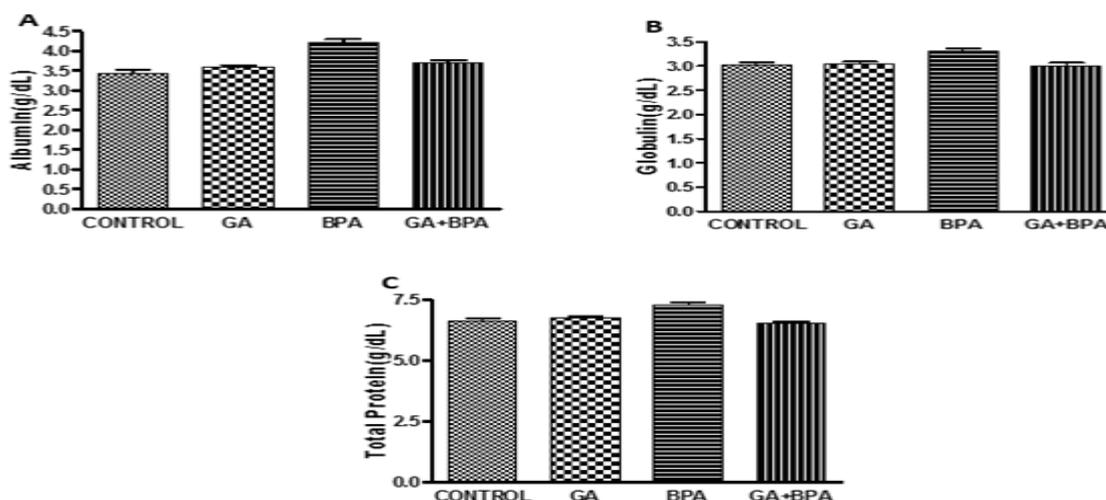


Figure 4. Bar chart of serum proteins in rats: A (Albumin), B (Globulin) and C (Total protein).

Table 1. Mean and Standard deviation (SD) values of Electrolyte in rats (n=8).

Electrolyte	CONTROL	GA	BPA	GA+BPA
Na ⁺	135±2.4 ^a	133±0.89 ^a	140±1.4 ^b	139±2.4 ^b
K ⁺	3.9±0.24	3.9±0.14	4.0±0.05	3.8±0.14
Cl ⁻	107±5.2	107±2.6	108±2.6	106±2.6
HCO ₃ ⁻	23±0.89 ^a	21±0.52 ^b	20±1.40 ^b	24±1.40 ^a

Table 2. Frequency of BPA-induced lesions of the liver and kidney in rats.

Liver Lesions	CONTROL	GA	BPA	GA+BPA
Hepatic necrosis and peri-portal congestion	0/8	1/8	7/8	3/8
Cellular infiltration of lymphocytes in the portal area	1/8	1/8	8/8	4/8
Disarrangement of hepatic plates with Swollen	0/8	0/8	8/8	3/8
Pyknotic nucleus	0/8	0/8	7/8	4/8
Proliferative bile ducts	1/8	2/8	8/8	3/8
Cytoplasmic vacuolization	0/8	1/8	8/8	4/8
Kidney Lesions	CONTROL	GA	BPA	GA+BPA
Atrophic renal corpuscles with segmented Glomerular tuft	0/8	0/8	8/8	3/8
Widened urinary space	0/8	0/8	7/8	3/8
Degeneration of tubular and glomerular epithelium	0/8	0/8	8/8	4/8
Loss of brush borders in tubules	0/8	1/8	8/8	3/8
Exfoliated cells and cellular debris in tubules	0/8	1/8	8/8	4/8
Cytoplasmic vacuolization	0/8	1/8	7/8	4/8

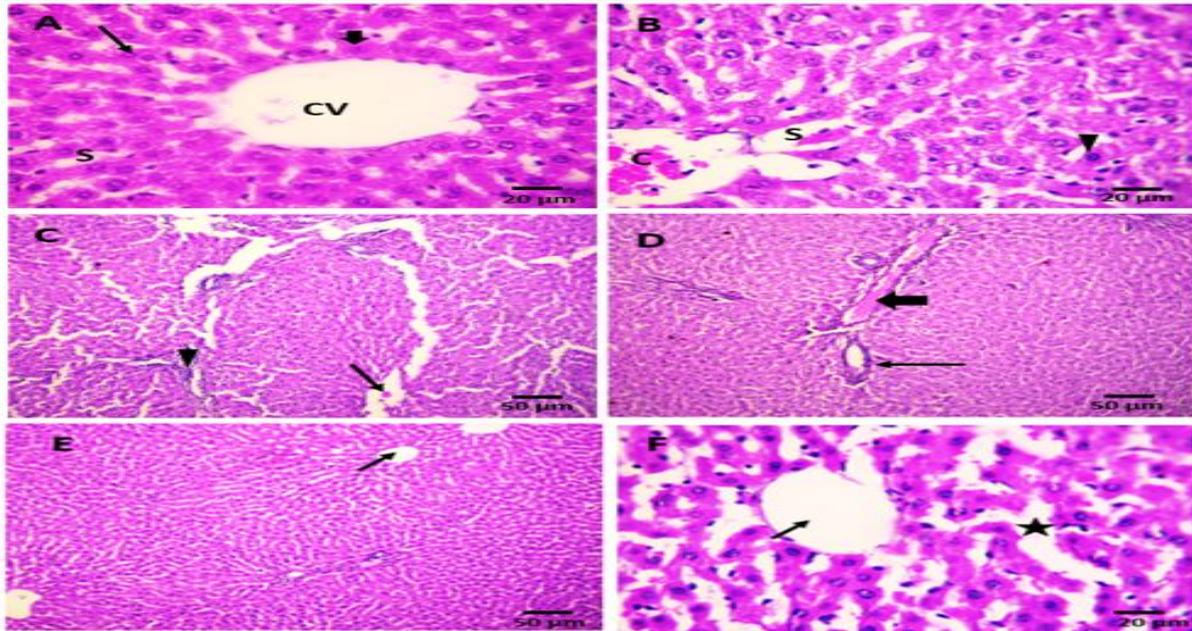


Figure 5. Photomicrographs of the liver of rats. A. Control rats showing normal central veins (CV), sinusoids (S), mono-nucleated (block arrow) and bi-nucleated (arrow) hepatocytes. B. BPA-treated rats showing dilated sinusoids (S), congested central vein (C) and pyknotic nucleus (arrowhead). C. BPA-treated rats showing disarrangement of hepatic plates with congested central vein (arrowhead) and cellular infiltrations in the portal area (arrow). D. BPA-treated rats showing proliferative bile ducts (arrow) and congested bile duct (black arrow). E. GA-treated rats showing well-arranged hepatic plates and normal central vein (arrow). F. BPA + GA-treated rats showing restored hepatic plates with unaffected lobules and widened sinusoid (Star).

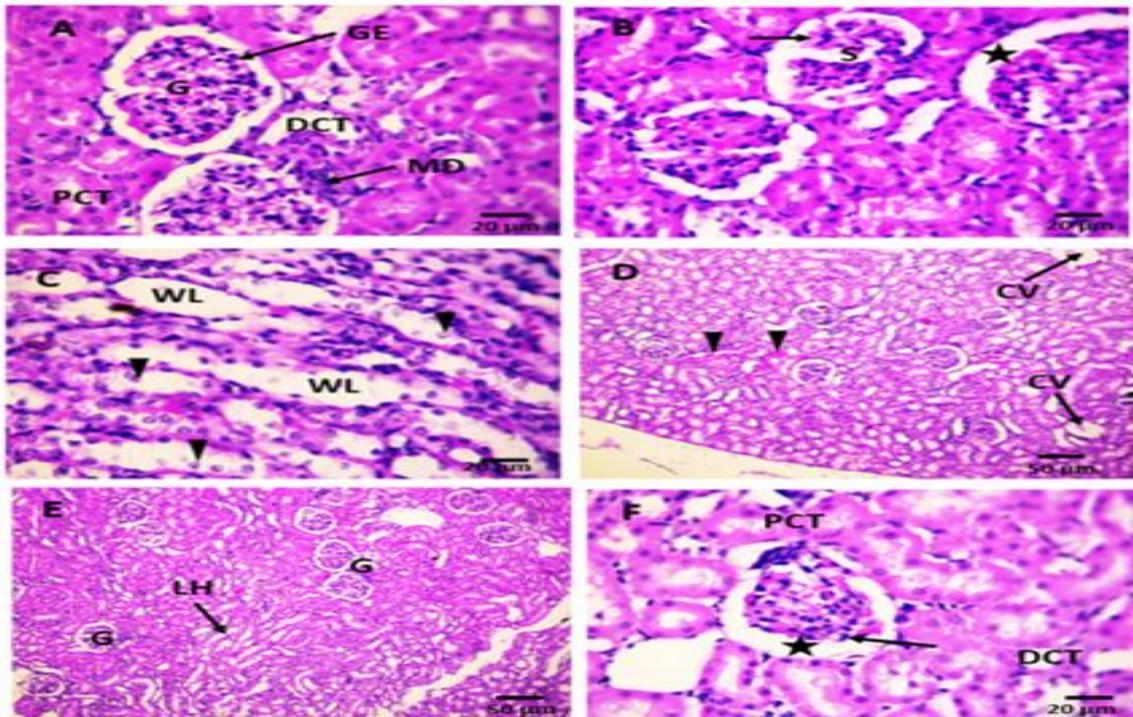


Figure 6. Photomicrographs of the kidney of rats. A. Control rats showing normal glomerulus (G), proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and macula densa (MD). B. BPA-treated rats showing segmented glomerular tuft (S), loss of glomerular epithelium and widened urinary space (star). C. BPA-treated rats showing widened Loop of Henle (WL) and exfoliated cells within the connecting ducts (arrowhead). D. BPA-treated rats showing congested cortical blood vessels (arrowheads) and cytoplasmic vacuolations (CV). E. GA-treated rats showing normal renal corpuscles with normal glomerulus (G) and normal Loop of Henle (LH). F. BPA + GA-treated rats showing restored renal corpuscles with a reduction in renal space (star) compared to those of BPA-treated rats, restored glomerular epithelium (arrow) and restored proximal convoluted tubule (PCT) as well as distal convoluted tubule (DCT).

DISCUSSION

The ubiquitous nature of BPA, as well as its widespread use in the manufacture of plastics and other consumer products, raises concerns over the extent of potential BPA exposure to humans and its perturbations on reproductive and other vital organs. Liver function assays are diagnostic tools in the assessment of the functional status of the liver. The BPA-induced elevated values of serum hepatic marker enzymes observed in the present study shows that the liver is a target tissue of EDCs and is indicative of cellular leakage as a result of damage to the hepatic tissue in the rats. Cellular leakage and loss of the functional integrity of the membrane architecture of the liver have been identified as the direct consequences of hepatic cellular damage [16]. Thus, hepatic injury induced by BPA causes the subsequent release of liver marker enzymes into the blood.

Our findings from the histopathology of the liver of the BPA-treated rats confirmed the loss of functional integrity of the membrane architecture. Elevated levels of AST, ALP, ALT, and GGT have been identified as important markers in the detection of hepatic damage by several authors [16-18]. Hence, the membrane integrity maintenance activity of ALT and ALP will be affected while the mitochondrial maintenance role of AST, as well as the hepatic injury prevention action of GGT, will be adversely affected in the BPA-treated rats. BPA-induced elevated serum hepatic marker enzymes have been reported by several authors [19-21]. The ameliorative effect of GA on these serum hepatic marker enzymes shows its hepatoprotective activities and confirms previous report on its ameliorative actions [22]. The BPA-induced elevated total and conjugated bilirubin levels observed in the study may be indicative of hemolytic anaemia in the rats with a concomitant alteration in the conjugative ability of the liver. The toxic effects of BPA on the liver further as observed in elevated serum hepatic markers correlate very well with cellular infiltrations, hepatic necrosis and peri-portal congestion observed in the BPA-treated rats. Moreover, the BPA-induced disruption of hepatic plates, cytoplasmic vacuolization, nuclear degeneration as well as inflammation of the portal area observed in the study are consistent with BPA-induced hepatic lesions earlier reported in rats [19]. The ameliorative effect of GA on the histopathology of the liver is indicative of the anti-inflammatory activity of GA and is in agreement with previous studies [23, 24].

The elevated levels of urea and creatinine observed in the BPA-treated rats are suggestive of the nephrotoxic activity of BPA. Hence, there was the accumulation of BPA metabolites as well as a reduced ability of the kidney to excrete these toxic substances from the rats. This correlates positively with previous reports on the effect of BPA on serum kidney markers [19, 25-27] and is confirmed by the renal histopathological observations in the BPA-treated rats. The atrophic renal corpuscles with segmented glomerular tuft complicated with widened urinary space in the BPA-treated rats are able to potentiate dysfunctional filtration in the Bowman's capsule of the rats. In addition, the exfoliated cells and cellular debris observed in the renal tubules of the BPA-treated rats are suggestive of a decrease in the re-absorptive activities of the proximal and distal convoluted tubules of the kidney. BPA-induced lesions of the kidney such as disruption in the Bowman's capsule architecture (hypertrophy, shrinkage, degeneration and rupture), degeneration and dissociation of renal tubules, pyknotic nucleus, and degeneration of renal tubular epithelium as well as loss of apical brush borders in renal tubules have been described in experimental animals [6, 19, 21]. Interestingly, the concomitant treatment with GA ameliorated the alterations in serum markers and tissue of the kidney. This is indicative of the nephron-protective ability of GA against BPA-induced toxicity. This is in conformity with previous reports on the role of GA in nephrotoxicity due to EDCs [24].

In the present study, the BPA-induced significant increases in the values of triglycerides, total protein, and low-density lipoprotein were not observed for high-density lipoprotein. Significant increases in total cholesterol, triglycerides, low-density lipoprotein as well as high-density lipoprotein in rats exposed to BPA have been reported at acute levels [28]. With the exception of the decrease in high-density lipoprotein, our findings are in agreement with [29] on the effect of BPA-induced alterations in lipid function in rats. This difference is likely due to dosage and duration of BPA exposure. For example, while the present study utilized 10 mg/kg body weight BPA daily for 14 d, Oguazu and Ezeonu used lower doses not more than 250 µg BPA/kg body weight/day once to determine effect of acute exposure of BPA in rats. Nevertheless, the hypercholesterolemia observed with the BPA-treated rats in the present study has also been reported in BPA-exposed rats [27]. Moreover, significant decrease in triglycerides has

been reported in rats exposed to BPA doses as high as fifteen times the dose used in this study with prolonged exposure [27]. Similarly, GA attenuated the elevated levels of triglycerides, total protein and low-density lipoprotein indicating protective ability in BPA-induced lipid function alterations.

The BPA-treated rats in the present study all showed increases in serum proteins. However, only albumin showed a significant increase compared to the control group. This observation is not in agreement with the findings of Abdel-Wahab who reported a decrease in serum proteins in rats exposed to BPA of same dose used in the study but for a longer period of exposure [30]. Hence, the effect of BPA on serum proteins in rats can be said to be based on dosage as well as the period of exposure. This increase in albumin is indicative of accumulation of BPA metabolites with an impaired ability of the liver to excrete them. However, GA ameliorated the BPA-induced increase in serum protein.

With respect to electrolytes investigated in the present study, the significant increase in sodium ion observed in the BPA treated rats is suggestive of hypernatremia due to loss of body fluid resulting from the inability of the kidneys to excrete adequate sodium from the tubular fluid [31]. Hypernatremia has been reported to result in rats due to loss of body fluids containing less sodium than plasma alongside reduced intake of fluid or when there is excessive sodium intake with limited liquid intake [31]. However, GA ameliorated the BPA-induced hypernatremia, indicating its protective ability on the renal tubules. The significant decrease in bicarbonate ions observed in the BPA-treated rats is suggestive of reduced blood pH. Reduction in blood pH can be as a result of increased excretion of bicarbonate ions by the kidney [31]. Similarly, GA was able to prevent loss of bicarbonate ions in the rats.

CONCLUSION

Observations from serum biochemistry and histopathology showed that BPA caused structural and cellular perturbations of the liver and kidney of rats while concomitant treatment with GA ameliorates the condition. Hence, GA has hepatoprotective and nephroprotective actions against BPA-induced toxicity in Wistar rats.

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