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Chronic exposure of adult male Wistar rats to bisphenol A causes testicular oxidative stress: Role of gallic acid

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Objectives. Bisphenol A (BPA) has been reported that among other male reproductive dysfunctions, it can cause marked estrogenic effects including alteration in serum hormones as well as testicular lesions in exposed animals. This work sought to study the role of gallic acid (GA), a known antioxidant, on the BPA-induced testicular oxidative stress in adult male Wistar rats using serum hormone analysis, histopathology, and biochemical assays.

Methods. Adult male rats were divided into four groups (n=10) including control (0.2 ml of corn oil), GA (20 mg/kg/day), BPA (10 mg/kg/day), BPA+GA (BPA, 10 mg/kg/day + GA, 20 mg/kg/day). All medications were given by oral gavage for 45 consecutive days. The body and testicular weights were measured. Blood and organ samples were collected for the serum hormonal assay: testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL), and tissue biochemistry analysis: superoxide dismutase (SOD), reduced glutathione (GSH), glutathione-S-transferase (GST), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), respectively.

Results. The BPA-treated rats showed significant reduction in the gonadosomatic index. BPA also caused significant decrease in the levels of the serum testosterone and prolactin. Furthermore, BPA induced testicular oxidative stress by decreasing the activities of antioxidant enzymes and increasing reactive oxygen species. However, co-treatment with GA protected against these alterations.

Conclusion. Findings from the present study confirmed the previously reported data and show that the ability of GA, as a potent antioxidant, may protect against BPA-induced alterations in the male reproductive function. Hence, GA protects against testicular oxidative stress in adult male Wistar rats following chronic exposure to BPA.

Key words: bisphenol A, gallic acid, oxidative stress, testis, serum hormone

Endocrine disrupting chemicals (EDCs) are exogenous substances capable of altering the biological activities by their interactions with hormone synthesis, transportation, metabolism, and elimination in human and animal populations (Ahbab et al. 2017). There is a plethora of evidence that a variety of EDCs including phthalates, bisphenol A (BPA), and by-products of alkyl phenol ethoxylate surfactants are being released into landfills and municipal wastewater through sewage into water bodies, thereby causing great health concerns (Yuan et al. 2015).

Despite the fact that BPA has been first synthesized by Dianin in 1891, its estrogenic activity was not discovered until 1936 (Dodds and Lawson 1936). However, in the 1950s, due to BPA's ability to resist high temperature, shattering, and electricity, this led to its industrial use as a monomer to produce epoxy resins and polycarbonate plastics (Tian et al. 2017).

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BPA is readily applied in the impaction of flexibility, durability, and longevity to a variety of household and industrial products. Hence, BPA is used in the production of refillable drinking containers, plastic utensils, dental sealants, the linings of metal cans, electronics, medical devices, infant feeding bottles, toys, pharmaceuticals, compact disks, waxes, and food packaging materials (Mendonca et al. 2014). The not observable adverse effect level (NOAEL) dose for BPA in rats is 50 mg/kg/day b.w., while BPA (50 μg/kg/day b.w.) is the standard daily tolerable dose for humans (Doerge et al. 2011). However, studies in rodents have demonstrated that exposure to BPA doses lower than the NOAEL for 14 days caused a significant reduction in testosterone levels and an elevation in the luteinizing hormone (LH) level in the serum of adult rats (Sun et al. 2006; Kazemi et al. 2016).

The polyhydroxyphenolic compound, gallic acid, GA (3,4,5- trihydroxybenzoic acid), has been detected in vegetables and fruits including banana, pineapple, gallnut, grapes, apple, sumac, oak bark, lemons, and as a content of a number of beverages derived from plants including teas and fruit juices (Galati and O'Brien 2004; Madlener et al. 2007). Studies have demonstrated the antiallergic, antimutagenic, anti-inflammatory, and anticarcinogenic activities of gallic acid via its antioxidant property (Rather et al. 2013; Shi et al. 2016). Gallic acid extracted from the rose flowers had been demonstrated to possess certain anti-oxidative properties (Li et al. 2005).

GA has been shown to sequester metal ions and scavenge ROS, thereby preventing oxidative damage of cellular macromolecules in tissues (Canbek et al. 2011). The mitigating effects of lipoic acid as well as melatonin on BPA-induced testicular oxidative toxicity have been reported in rodents (El-Beshbishy et al. 2012; Othman et al. 2014). However, very little is known about the role of GA in testicular oxidative stress due to chronic BPA exposure in adult rats. This study was aimed to investigate the role of GA in testicular oxidative stress induced by chronic exposure of adult rats to oral low dose of BPA.

Materials and methods

Chemicals. BPA and GA used in this study were sourced from Sigma-Aldrich Co. (St Louis, Missouri, USA). Every other reagent used in this investigation was of standard grade.

Experimental animals. All procedures were carried out according to the National Institutes of Health's protocol on handling of laboratory animals (Garber et al. 2011). Forty male albino rats obtained from the Experimental Animal Unit, Veterinary Medicine, University of Ibadan, Nigeria, were used for the study. The rats were kept in plastic cages under controlled environmental conditions, being kept on commercial rat pellets and clean water provided *ad libitum*. The rats used in the study were divided into four groups (n=10): Group 1 (Control): corn oil, 0.2 ml was administered; Group 2 (GA alone): rats received GA (20 mg/kg b.w./day); Group 3 (BPA alone): rats received BPA (10 mg/kg b.w./day); Group 4 (BPA+GA): rats received same doses of BPA and GA as in groups 2 and 3 above. All medications were by gavage and lasted for 45 days to cater for a complete spermatogenic cycle in the rodent (Othman et al. 2014). The mode of exposure and administration of BPA and GA utilized in this study were according to the reports of Othman et al. (2014) and Rather et al. (2013), respectively.

Necropsy. Twenty-four h after the final BPA and GA administration, body weights of the animals were taken, blood was sampled and centrifuged at 3000 rpm for 20 min, at 4 °C to isolate the serum and later stored at –20 °C until used. Then the rats were euthanized under mild diethyl ether anesthesia. The testes were retrieved and weighed. The left testis was stored at –20 °C until used for biochemical assays, while the right one was fixed in buffered neutral formalin for histopathological analysis.

ELISA serum hormonal assays. Earlier stored frozen serum samples were allowed to thaw at room temperature and vortexed. Then, they were centrifuged at 1000 rpm for 5 min. Using the commercial kits (Beckman Coulter, Fullerton, CA USA), the serum hormones were quantified in triplicates to prevent errors due to inter-assay. Using ELISA reader (Rayto microplate RT-2100C) at wavelengths of 450 and 630 nm, optical densities of the samples were measured after which the concentrations of testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) were determined (Kazemi et al. 2016).

Biochemical assays. 10% testicular tissues homogenate (w/v) was made in ice-cold 0.1 M Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4 with the use of a homogenizing vessel made of ice-chilled glass. The centrifugation of the homogenate was performed as described by Abd-Elrazek and Ahmed-Farid (2018) and the supernatant used for the subsequent biochemical assays. The determination of the superoxide dismutase (SOD) level was performed according to the protocol of Misra and Fridovich (1972). The reduced glutathione (GSH) level was

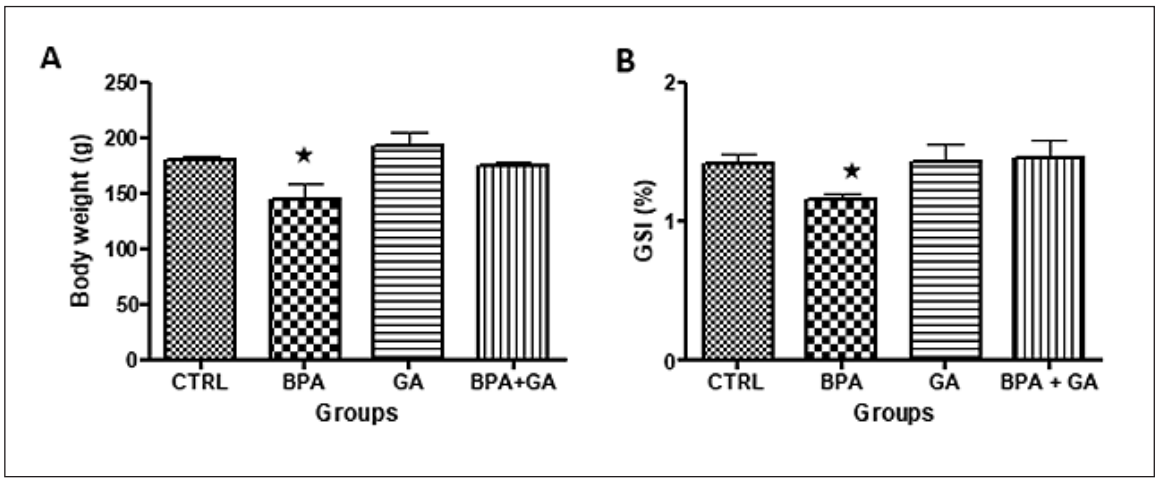

Figure 1. Effect of gallic acid (GA) on bisphenol-A (BPA)-induced changes in body weight **(A)** and gonadosomatic index (GSI) **(B)** in rats. *p<0.05.

assessed using the method of Jollow et al. (1974). The estimation of glutathione peroxidase (GPx) was carried out based on the method of Beutler et al. (1963) and glutathione-S-transferase (GST) activity using the protocol of Habig et al. (1974). The estimation of nitric oxide (NO) was carried out using the protocol of Olaleye et al. (2007) and the activity of myeloperoxidase (MPO) by the method of Xia and Zweier (1997). Testicular hydrogen peroxide (H_2O_2) level was carried out using the method of Wolff (1994) and malondialdehyde (MDA) content by the method of Varshney and Kale (1990).

Histopathological analysis. Samples from the testis fixed in buffered neutral formalin were processed for routine histology. Analysis of slides for testicular lesions was done with the use of Olympus BX63 light microscope attached to a DP72 camera.

Statistical analysis. Data obtained were expressed as means and standard deviation. Mean values across groups were compared using One-Way ANOVA. Statistical significance among parameters was considered at p<0.05 and all data analysis and presentation in graphical formats were carried out with the aid of GraphPad Prism 5 software (La Jolla, California, USA).

Results

Changes in the body weight and gonadosomatic index (GSI). Treatment with BPA significantly reduced the body weight of the rats compared to control and GA groups. However, the co-treatment of BPA with GA protected against the BPA-induced reduction in weight (Figure 1A). Similarly, BPA induced a significant decrease in GSI in the rats compared to all other groups (Figure 1B). Also, BPA+GA protected against the BPA-induced decrease in GSI of rats (Figure 1B).

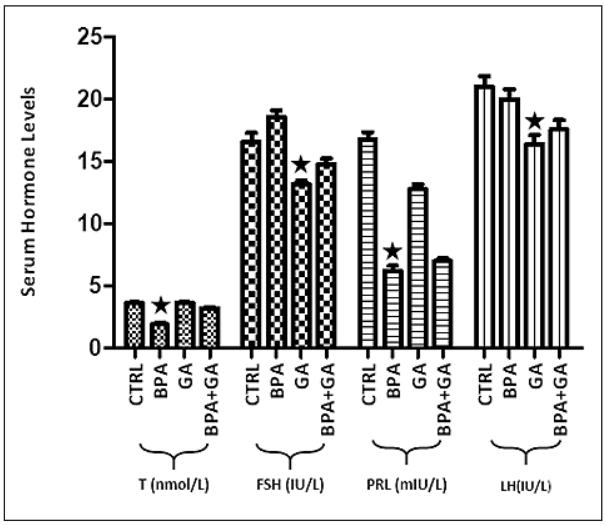

Figure 2. Effect of gallic acid (GA) on bisphenol A (BPA)-induced changes in serum hormone levels in rats. Bars with asterisks shows significant differences (p<0.05).

Changes in the serum hormone levels. BPA administration caused significant reduction in the serum levels of T and PRL compared to the control, but resulted in non-significant increases in the serum levels of FSH and LH (Figure 2). However, co-treatment with GA protected against the observed alterations in the serum levels of T, PRL, FSH and LH.

Changes in the biochemical parameters. BPA caused significant reductions in the activities of SOD, GP_X, GST, and GSH compared to the control (Figures 3A–D), while it caused significant elevations in the levels of NO, MPO, MDA, and H_2O_2 when compared with the control (Figures 3E–H). However, co-treatment with GA offered protection against these alterations in activities of antioxidant enzymes as well as levels of markers of oxidative stress (Figures 3A–H).

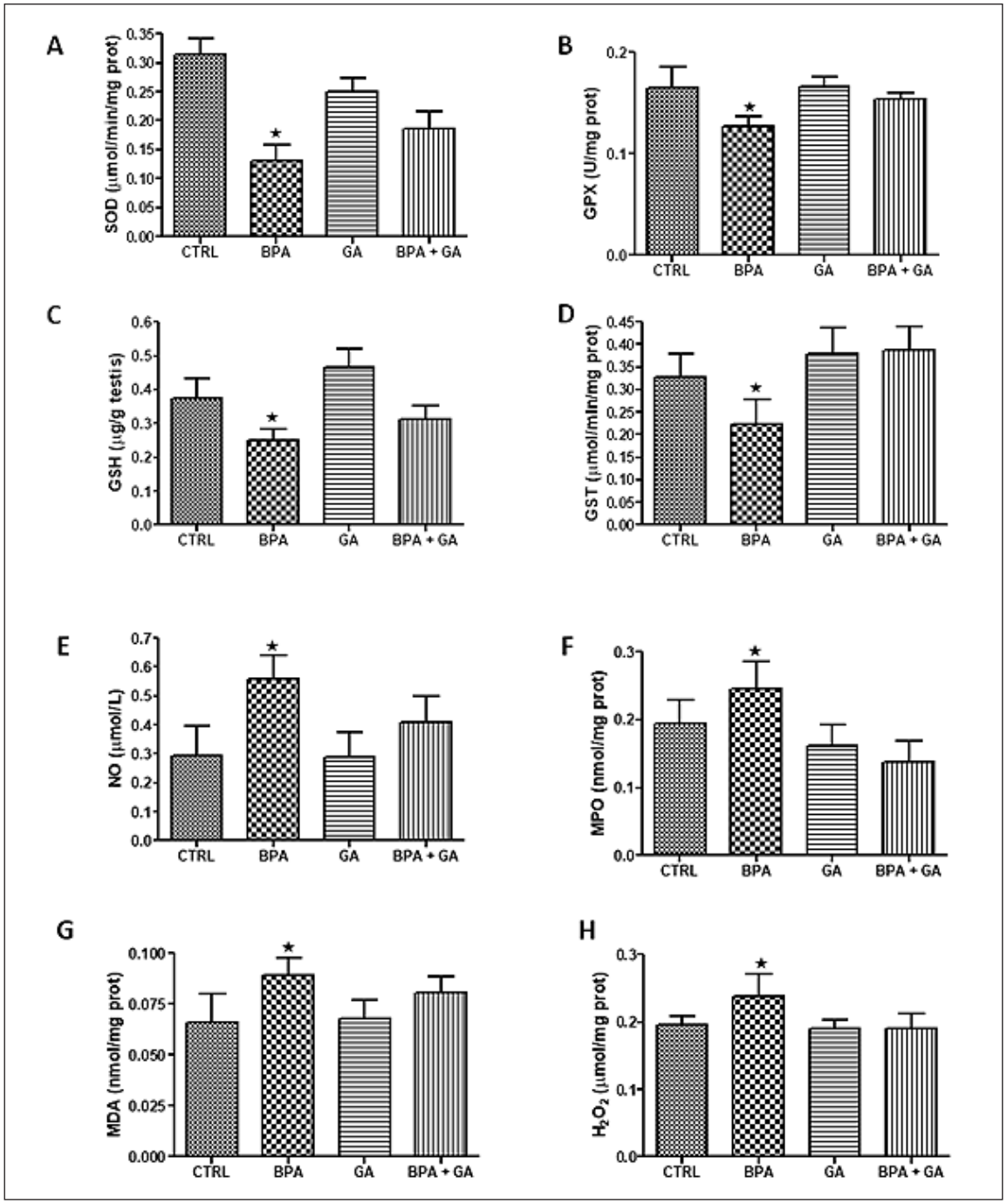

Figure 3. Role of gallic acid (GA) on bisphenol A (BPA)-induced testicular oxidative stress in adult rats. **(A–D)** Effect of GA on BPA-induced changes in antioxidant enzymes. **(E–H)** Effect of GA on BPA-induced changes in reactive oxygen species. Bars with asterisks shows significant differences ($p<0.05$).

Changes in the histopathology. Histopathological examination of the testis in control as well as GA-treated rats showed structural regularity in the seminiferous tubules, mediastinum, and interstitium (Figures 4A,C). BPA induced marked lesions including vacuolations of spermatogenic cells, presence of immature sperm cells within the lumen of seminiferous tubules, the disruption of intercellular junctions between spermatogenic and Sertoli cells, and the sloughing of spermatogenic cells (Figures 4B,D). Other BPA-induced testicular lesions included the congestion of the testicular interstitium and mediastinum, especially of the rete testis and the erosion of the interstitium (Figures 5A,B). However, rats that received BPA+GA co-treatment had better testicular architecture, including normal pattern of spermatogenic cells, compact interstitium, and mild congestion (Figures 5C,D).

Discussion

The present study showed that chronic BPA treatment of adult male rats at a concentration lower than the not observable adverse effect level (NOAEL) is capable of inducing reproductive alterations that could lead to infertility, while the co-treatment with GA ameliorates these perturbations. In the present study, the BPA-induced significant decreases in body weight and GSI are in consonance with the reports of a number of studies (El-Beshbishy et al. 2012; Yuan et al. 2015). The observed ability of GA to ameliorate BPA-induced decreases in body weight and GSI shows that it is protective against EDC-induced toxicity. This is in accord with the reports of Lu et al. (2006) and Tung et al. (2009) on the role of GA against EDCs-induced reduction in body as well as organ weights.

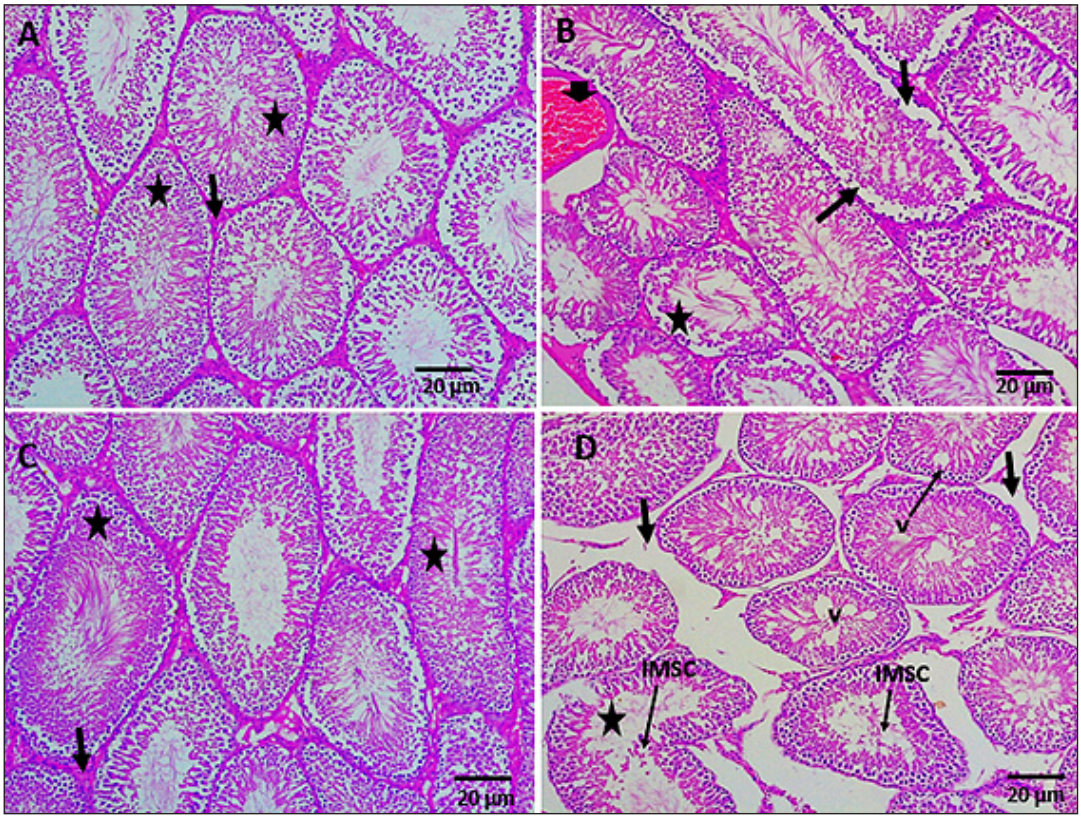

Figure 4. Representative histological sections of the testis of rats (H&E). (**A**) Control group showing normal pattern of spermatogenic cells (asterisk) and well-formed testicular interstitium (arrow). **(B)** BPA-treated group showing disruption of intercellular junctions between spermatogenic and Sertoli cells (asterisk); sloughing of spermatogenic cells (arrow) and severe congestion of testicular interstitium (arrow head). **(C)** GA-treated group showing normal pattern of spermatogenic cells (asterisk) and well-formed testicular interstitium (arrow). **(D)** BPA-treated group showing degeneration of spermatogenic cells (asterisk); erosion of testicular interstitial cells (arrow); vacuolations (v) and diminution of seminiferous tubules with presence of immature spermatogenic cells (IMSC) within the lumen.

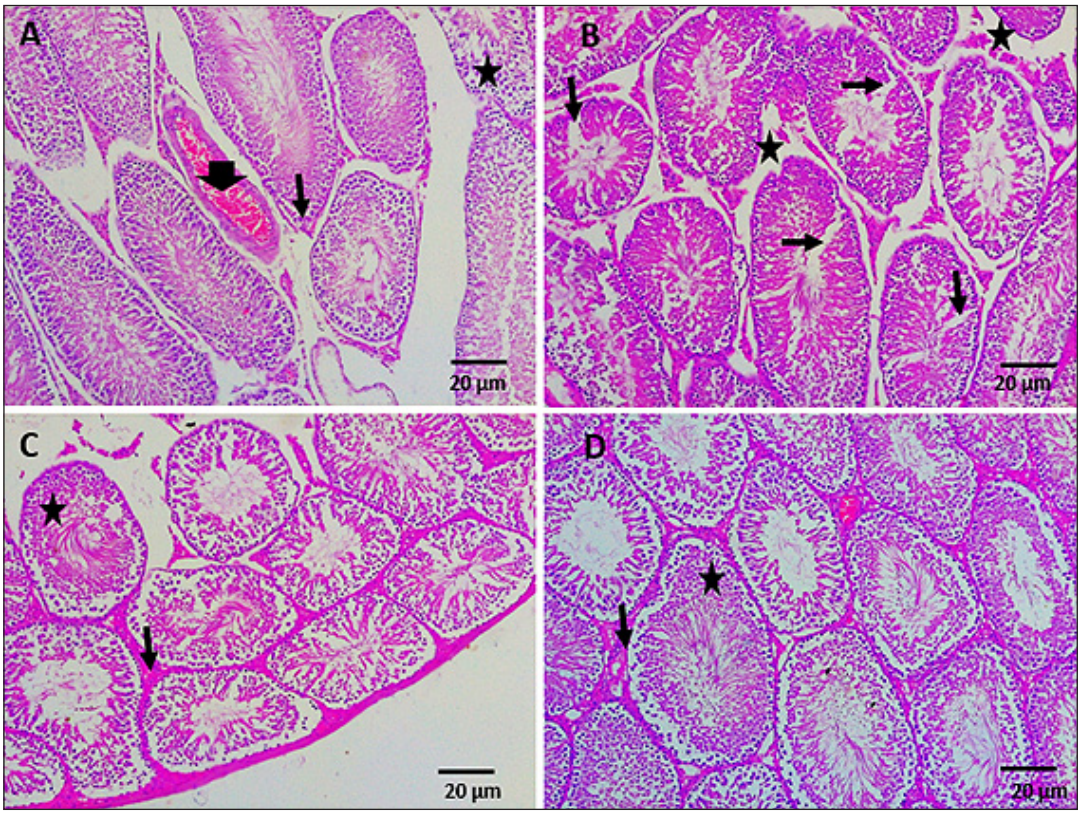

Figure 5. Representative histological sections of the testis of rats (H&E). **(A)** BPA-treated group showing disruption of intercellular junctions between spermatogenic and Sertoli cells (asterisk); erosion of testicular interstitium (arrow) and severe congestion of rete testis (arrow head). **(B)** BPA-treated group showing disruption of intercellular junctions between spermatogenic and Sertoli cells (arrow); erosion of testicular interstitium (asterisk). **(C)** BPA + GA group showing improved pattern of intercellular junctions between spermatogenic and Sertoli cells (asterisk) and normal testicular interstitium (arrow). **(D)** BPA + GA group showing improved pattern of intercellular junctions between spermatogenic and Sertoli cells (asterisk); normal testicular interstitium (arrow) and reduced mild congestion of testicular interstitium (arrow head).

The changes in serum hormone levels observed in this study agrees with our findings on spermatozoa parameters and GSI. These changes are consistent with previous reports on the BPA-induced serum hormone levels (Kazemi et al. 2016; Ahbab et al. 2017; Tian et al. 2017). The present study shows that BPA significantly reduced the serum levels of T and PRL. Decrease in serum T has also been reported following the exposure of adult male rats to environmentally relevant doses of BPA (Dhanabalan et al. 2013). The BPA-induced significant decrease in serum PRL that paralleled a significant difference in serum T has also been reported in rats following exposure to one or a combination of EDCs (Doerge et al. 2011; Othman et al. 2014). It could be inferred that BPA induced modulations in the steroidogenic functions of Leydig cells thereby inhibiting T production as well as causing an increase in secretion of LH by the pituitary gland. The significant elevation in FSH levels as a result of BPA treatment as against the observed significant decreases in T and PRL in this study are in consonance with the reports of Sun et al. (2006). This clearly shows that BPA exhibited anti-androgenic actions in the treated rat. However, Kazemi et al. (2016) have observed that treatment of adult male rats with low BPA doses (5, 25 and 25 µg/kg) for a consecutive 35-day did not show any significant difference in serum T, FSH, and LH although there were significant changes in male reproductive functions.

In this study, BPA-exposed rats exhibited significant reduction in the activities of antioxidant enzymes as well as significant elevations in the levels of reactive oxygen species (ROS) and myeloperoxidase activity. Similar observations have been documented in adult rats treated with oral doses of BPA for sub-acute (Anjum et al. 2011; El-Beshbishy et al. 2012) and chronic cases (Chitra et al. 2003). In the

testis of the BPA-treated rats, it could be inferred that SOD converted the superoxide anion radicals into H_2O_2, which thereafter accumulated in the testes due its reduced elimination. Elevated testicular MDA levels have been suggested to be responsible for the pathologic lipid peroxidation of spermatozoa membrane and reduction of sperm motility (Hsieh et al. 2006). The BPA-induced reduction in the activities of the antioxidant enzymes and increase in the MPO in the testis of the rats are suggestive of an improved platform for inflammation and testicular dysfunction via the degeneration of spermatogenic cells due to excessive generation of ROS by peroxidation of the membranes of the seminiferous tubules. This condition of reduced antioxidant enzymes concurrently with increased generation of ROS would in turn adversely affect the mitotic and meiotic activities of spermatogonia and spermatocytes as well as the maturation of spermatids thereby reducing the quality and quantity of spermatozoa within the adluminal compartment of the seminiferous tubules. In this study, GA ameliorated the BPA-induced decrease in the activities of antioxidant enzymes together with the elevated levels of ROS. This is in confirmation of the antioxidant properties of GA reported earlier (Rather et al. 2013; Shi et al. 2016). Our findings show the ability of GA as a potent antioxidant capable of ameliorating BPA-induced oxidative stress.

Testicular lesions induced by BPA seen in the present study are similar to the previous reports in BPA-induced testicular toxicity (Anjum et al. 2011; El-Beshbishy et al. 2012; Othman et al. 2014). The lesions positively correlate with our findings on BPA-induced serum hormone changes as well as those of biochemical parameters. Othman et al. (2014) have reported testicular lesions including vacuolization, interstitial hemorrhage, sloughing, and reduction of spermatogenic cells following chronic exposure of rats to BPA. The sloughing off of interstitial cells of Leydig observed in the BPA-exposed rat accounts for the reduced levels of T in the rat since Leydig cells are directly involved in secretion of T.

In conclusion, the present study showed that exposure of adult male rats to BPA (10 mg/kg) for 45 consecutive days is capable of inducing marked male reproductive alterations, including those of testicular weights, serum hormone levels, antioxidant enzymes, ROS as well as the induction of testicular lesions. However, our findings also showed that GA is capable of ameliorating BPA-induced perturbations of the male reproductive functions. Hence, GA protects against BPA-induced testicular oxidative toxicity in the adult male Wistar rats.

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