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Protective role of melatonin on Bisphenol A-induced Adrenal Gland dysfunction in pubertal Wistar rats exposed *in-utero*

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ABSTRACT

Most studies on the effect of Bisphenol A (BPA) on the endocrine organs are either not environmentally relevant due to high doses of BPA exposure or involve direct exposure to adult rats. To bridge this knowledge gap, this present study was designed to investigate the protective effects of melatonin (MLT) on adrenal gland dysfunction in pubertal male Wistar rats exposed to environmentally relevant doses of BPA *in utero*. Pregnant Wistar rats were randomly assigned into five groups (n=5): Group 1 (control) received 0.2 ml 1% dimethyl sulfoxide (DMSO)/99% canola oil as the vehicle; Group 2 received BPA at 50 µg/kg/day; Group 3 received BPA at 150 µg/kg/day; Group 4 received BPA at 50 µg/kg/day with concurrent MLT 1 mg/kg/day; and Group 5 received BPA at 150 µg/kg/day with concurrent MLT 1 mg/kg/day. All treatments were administered by gavage from gestational day (GD) 10-21. Thereafter, two male offspring randomly from each litter per group (n=10) were weighed, and blood samples were collected for serum hormonal assay before they were euthanized on post-natal day (PND) 42. Biomarkers for oxidative stress (MDA, H_2O_2) and antioxidants (GPx and GST) were estimated. The levels of corticosterone and ACTH were determined using ELISA kits. Histopathology and histomorphometry of the gland were carried out using standard methods. BPA exposure induced marked dose-dependent adrenal alterations, including changes in body and organ weights, alterations in serum hormone levels, decreased activities of antioxidant enzymes while increased ROS and MDA as well as the induction of adrenal lesions. However, melatonin co-administered with BPA protected against BPA-induced alterations of adrenal function. Hence, melatonin protects against BPA-induced toxicity of the adrenal gland of pubertal male rats exposed *in utero*.

Keywords: Bisphenol A; Melatonin, Adrenal gland; Serum hormones; Oxidative stress.

Short title: BPA-induced adrenal dysfunction

Introduction

Bisphenol A (BPA) is used to manufacture epoxy resins and polycarbonate plastics present in everyday-used items, including refillable drinking containers, plastic utensils, dental sealants, the linings of metal cans, electronics, medical devices, infant feeding bottles and toys, pharmaceuticals, compact

disks, waxes, food and beverage packaging, adhesives, building materials, as well as paper receipts (Ma *et al.*, 2017; Olukole *et al.*, 2019; Gorini *et al*., 2020; Farrugia *et al*., 2021). BPA is an endocrine-disrupting chemical (EDC). EDCs have been at the centre of several environmental and biomedical investigations because they possess the ability to interfere with the endocrine system via (but not limited to) estrogenic, androgenic, anti-androgenic and anti-thyroid mechanisms (Schiller *et al*., 2013; Shi *et al*., 2015; Gorini *et al*., 2020).

Research on bisphenol A (BPA) has been on the increase in the last two decades due to its incrimination in worsening environmental health and decline in male reproductive ability among other endocrine-disrupting activities of BPA (Al-Hiyasat *et al*., 2002; Richter *et al*., 2007; Salian *et al*., 2009; Olukole *et al*., 2019). There are more than 5000 safety-related studies published on the effects of BPA exposure on humans and animals (Alonso-Magdalena *et al*., 2015; Medwid *et al*., 2016; Tian *et al*., 2017). There is enough evidence to demonstrate that EDCs may alter biological processes by either binding to or blocking hormone receptors, thereby initiating or obstructing hormonal response (Hotchkiss *et al*., 2008; Flint *et al*., 2012). EDCs mimic biological axes, including the hypothalamic–pituitary–gonadal axis being able to disrupt spermatogenesis, steroidogenesis, and the functions of the male reproductive system (Singh *et al*., 2017).

BPA's release into the environment has been a major source of exposure to humans and animals including wildlife. The release of BPA can occur during the chemical manufacture, transport, and processing of plastic products. Documentations have shown that post-consumer releases of BPA are mainly via effluent discharge from municipal wastewater treatment plants, leaching from landfills, combustion of domestic waste, as well as from natural breakdown of plastics in the environment (Crain *et al*., 2007; Kang *et al*., 2007). Hence, wildlife are exposed to BPA in their natural habitats, including polluted air, contaminated soil and water bodies. The short half-life of BPA notwithstanding, its continuous release into the environment makes it ubiquitous thereby acting through several physiological receptors, including estrogen receptors α and β (ERα and ERβ), membrane-bound ERs, androgen receptor, peroxisome proliferator-activated receptor gamma and thyroid hormone receptor, represents a significant potential risk for humans and wildlife (Flint *et al*., 2012; Peretz *et al*., 2014; Gorini *et al*., 2020).

Melatonin (N-acetyl-5-methoxytryptamine, MLT) is a neuro-hormone derived from tryptophan, an amino acid and is mainly released from the pineal gland (Olukole *et al*., 2020). This MLT possesses antioxidants with prophylactic properties against oxidative stress (Reiter *et al*., 2000). The antioxidant properties of melatonin have been demonstrated in several experimental and clinical conditions with a wide safety margin upon its administration (El-Missiry, 2000; Reiter *et al*., 2004). Previous reports have demonstrated the prevention and or reduction of BPA-induced toxicity using antioxidants such as Lipoic acid, N-acetylcysteine and Vitamin C (Korkmaz *et al*., 2010; Jain *et al*., 2011; El-Beshbishy *et al*., 2012). However, Anjum *et al*. (2011) demonstrated the ameliorative role of melatonin in BPA-induced biochemical toxicity in testicular mitochondria of mice. Olukole *et al*. (2018, 2020) demonstrated the ameliorative role of melatonin in BPA-induced perturbations of the prostate gland of adult rats.

Toxicological investigations have demonstrated that the adrenal gland is one of the most susceptible endocrine organs associated with EDC-induced lesions

(Olukole *et al*., 2019). The adrenal gland is the first target of EDC toxicity due to the critical role of glucocorticoids in the maintenance of body homeostasis in man and animals (Rosol *et al*. 2001; Medwid *et al*. 2016). Despite being the first target of EDCs, particularly BPA, most studies on BPA have focused mainly on reproductive, cardiovascular, immunological, neuronal, and metabolic functions (Rochester 2013). Olukole *et al*. (2019) investigated the effects of BPA exposure in adult rats, while Medwid *et al.* (2016, 2018) demonstrated the effects of prenatal BPA exposure on the adrenal gland of mice. Zhou *et al*. (2015) investigated the effects of perinatal exposure to low-dose BPA on adrenal axis regulation and behaviours of rat offspring. However, there still exists a dearth of information on the protective actions of melatonin on adrenal gland dysfunction in the F1 pubertal male Wistar rats following maternal exposure to environmentally relevant doses of BPA. To bridge this knowledge gap, this present study was designed to investigate the protective effects of melatonin on adrenal gland dysfunction in the F1 pubertal male Wistar rats exposed to environmentally relevant doses of BPA *in utero*.

Materials and Methods

Experimental Animals and Protocols

Bisphenol A and Melatonin were purchased from Sigma-Aldrich Co. (St Louis, Missouri, USA). All reagents used for this study were of standard grade. Procedures used in this study were carried out according to the criteria contained in the guide for the care and use of Laboratory animals published by the National Institutes of Health (Garber *et al*., 2011). They were also approved by the University of Ibadan Animal Care and Use Research Ethics Committee. Sexually mature female Wistar rats (average weight, 180 ±10 g) obtained from the Animal House, Faculty of Veterinary Medicine, University of Ibadan. The rats were stabilized for two weeks before the commencement of the treatment protocol. All rats received free access to commercial pelletized rat feed and glass bottles containing BPA-free drinking water. Feed and water were provided *ad libitum*, and animals were kept on a 12-h light, 12-h dark cycle.

The rats were mated overnight with mature male Wistar rats of proven fertility by placing 2 females in a cage with 1 male. This was followed by the observance of the peri-anal regions of the rats for the presence or absence of vaginal plug. The presence of a vaginal plug on any female rat was taken as a sign of mating and was designated as day 1 of gestation (GD1). On GD9, pregnant rats were weighed and randomly assigned into five (5) groups of three dams each (n=3) as follows:

- Rats in group 1 (control) received oral (by gavage) 0.2 ml 1% dimethyl sulfoxide (DMSO)/99% canola oil as a vehicle (Sigma–Aldrich Co., St. Louis, MO, USA).
- Rats in group 2 received oral (by gavage) 50µg/kg BW/day bisphenol A only (Sigma–Aldrich Co., St. Louis, MO, USA), dissolved in DMSO and solubilized in canola oil.
- Rats in group 3 were treated orally by gavage with bisphenol A, dissolved in DMSO and solubilized in canola oil at 150µg/kg BW/day.
- Rats in group 4 were treated with 50µg/kg BW/day bisphenol A with concomitant melatonin at 1mg/kg BW/day (Sigma–Aldrich, 98% pure, dissolved in 0.5% ethanol in normal saline) both administered orally.
- Rats in group 5 received oral 150µg/kg BW/day of bisphenol A with concomitant 1mg/kg BW/day melatonin via the same route.

The daily tolerable intake (TDI) of BPA established by the US Environmental

Protection Agency (EPA) is 50 µg /kg/d (NTP, 1982; Doerge *et al.,* 2011). Hence, the BPA 50 µg and 150 µg doses used in the study are the TDI and three-fold TDI for BPA. All treatments lasted from GD10-GD21 and all offspring remained with their dams until postnatal day (PND 21). The duration and route of administration for BPA and melatonin used in this study were as described by Bernado *et al.* (2015), Tiao *et al*. (2014), respectively and Olukole *et al*. (2020). At weaning, two male offspring were selected at random from each of the three dams per group making a total of six males for each group (n=6). The male rats were kept on commercial pelletized rat feed and glass bottles containing BPA-free drinking water till PND 42.

Necropsy

Rats were weighed at PND 42 and blood samples were collected into plain sample bottles, centrifuged at 3000 rpm for 20 minutes, at 4° C to isolate the serum and later stored at -20° C till the time of use. The animals were then euthanized. The adrenal glands of rats were harvested and weighed. Samples were collected from the right adrenal glands for routine histology and microstereology, while the left adrenal glands were used for biochemical assays (Ola-Davies and Olukole, 2018). The adrenal index (%) of rats was calculated as the weight of adrenal glands divided by body weight multiplied by 100 (Olukole *et al*., 2019).

ELISA Serum Hormonal Assays

Frozen serum samples earlier stored were allowed to thaw at room temperature and vortexed. They were then centrifuged at 1000 rpm for 5 min. Corticosterone and adrenocorticotropic hormone (ACTH) levels (ng/mL) were determined using ELISA kits (MP Biomedicals, Ohio, USA) as specified by the manufacturer (Wei *et al*., 2011). To eliminate inter-assay variations, all samples were quantified in triplicates in one assay with the intra-assay coefficient of variation being < 5% (Medwid *et al*., 2016; Olukole *et al*., 2019).

Histopathology and micro stereology

Samples of the right adrenal gland of rats were fixed in buffered neutral formalin and embedded in paraffin blocks. Sections 2–4-µm thick were stained with Hematoxylin and Eosin (H&E) and used for histopathological and microstereological analyses. The slides were analysed and microscopic fields were digitized using a light microscope (Olympus BX63 with a DP72 camera) and sections were observed for lesions of the adrenal gland. Microstereological analyses of sections were performed with the aid of GIMP 2 Software. Five histological fields per section of adrenal gland stained with H&E from six different rats randomly chosen per group, totalling 30 fields per parameter [capsule thickness, depths of cortex (zona glomerulosa, zona fasciculata, and zona reticularis), and medulla].

Biochemical assays

Adrenal tissues of rats were homogenised to make 10% homogenate (w/v) in ice-cold 0.1 m Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4. Homogenisation was carried out using an ice-chilled glass-homogenising vessel in a homogeniser fitted with a Teflon pestle (Glas-Col, USA). Thereafter, the homogenate was then centrifuged in a refrigerated centrifuge at 2,000 × *g* at 4°C for 15 min to remove nuclei and debris (Abd-Elrazek and Ahmed-Farid, 2018). The supernatant obtained from the homogenate was used as a sample for the following assays by Agilent HP 1100 series HPLC apparatus (USA).

Determination of adrenal-reduced glutathione (GSH) level

Reduced glutathione (GSH) was estimated by the method of Jollow *et al.* (1974). Briefly, 0.5 mL of 4% sulfosalicylic acid (precipitating agent) was added to 0.5 mL of sample and centrifuged at 4, 000 rpm 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 0.1M phosphate buffer, pH 7.4 was added. The absorbance was read at 412 nm against distilled water as blank.

Determination of adrenal Glutathione peroxidase (GPx) Activity

Glutathione peroxidase activity was measured according to Beutler *et al.* (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 sample and 0.6 mL of distilled water. The mixture was incubated in the water bath at 37°C for 5 min and 0.5 mL of TCA was added and centrifuged at 4,000 rpm for 5 min. A volume of 1 mL of the supernatant was taken and added 2 mL of K_2PHO_4 and 1 mL of Ellman's reagent. The absorbance was read at 412 nm using distilled water as blank.

Determination of adrenal hydrogen peroxide (H_2O_2) generation

This was done as described by Wolff (1994). To 50µL of PMFs of tissues, add 100 µL of 0.1M potassium phosphate buffer (pH 7.4), 50 µL of ammonium ferrous sulphate, 20 µL of sorbitol, 20 µL of xylenol orange (XO) and 10 µL of H_2SO_4. The mixture was mixed thoroughly by vortexing and a light pink colour of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 minutes. The mixtures were read at an absorbance of 560 nm. The H_2O_2 generated was extrapolated from the H_2O_2 standard curve.

Determination of adrenal malondialdehyde (MDA)

The Malondialdehyde (MDA) content as an index of lipid peroxidation was quantified in the PMFs of the tissues according to the method Varshney and Kale, (1990). 400 µL of Tris-KCl, 125 µL of 30% TCA, 100 µL of sample and 125 µL of 0.75% TBA in 0.2 M HCl was immediately added. The reaction mixture was incubated in the water bath at 80°C for 45 minutes, cooled on ice and centrifuged at 3000 rpm for 15 minutes. 200 µL of supernatant was taken. The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of 1.56×10^5/M/cm.

Statistical analysis

Quantitative data were recorded as mean and standard deviation. A comparison of the mean was performed using one-way ANOVA. Statistical significance among parameters was considered at $p<0.05$. Graphical presentation of data was performed using GraphPad Prism 5 software (GraphPad Software, Inc. La Jolla, California, USA). A contingency table followed by Fisher's test was used to analyze the incidence of adrenal lesions.

Results

Changes in body weight and Adrenal Index (AI)

Maternal exposure to BPA resulted in a significant decrease in body weight of pubertal rats ($P<0.05$) between the control and the 150 µg/kg BPA-treated group (Figure 1 A). Nevertheless, there was no significant difference ($P>0.05$) between the control and the 50 µg/kg BPA-treated group. However,

melatonin significantly increased the BPA-induced reduction in body weight of rats in the BPA 250 µg/kg group.

Similar observations were recorded for both doses of BPA with respect to changes in AI. (Figure 1 B). However, these differences were dose-dependent as there was a significant difference in AI between both BPA doses. Melatonin co-administered with BPA significantly reduced the BPA-induced elevation in AI of pubertal rats.

Changes in hormone concentration

Treatment with BPA resulted in significant dose-dependent increases in corticosterone and ACTH compared to the control rats (Figure 2 A & B) with the greatest effect being on the BPA 150 µg/kg body weight. Concerning corticosterone and ACTH, there was a significant difference between the low and high BPA dose groups (Figure 2 A & B). Co-treatment with melatonin protected against increases in corticosterone and ACTH in the rats both for the low and high BPA dose groups.

Changes in biochemical parameters

Treatment with BPA resulted in a dose-dependent reduction in the activities of GSH and GPx compared to the control with BPA 150µg/kg exerting a greater effect on the activities of oxidative enzymes. However, the co-treatment with melatonin protected against the observed alterations (Figure 3 A & B). Both the 50 and 150 µg/kg BPA groups induced significant increases in the levels of MDA and H_2O_2 compared to the control (Figure 3 C & D). Similarly, concomitant treatment with melatonin protected against the BPA-induced increases in the levels of MDA and H_2O_2.

Histopathology and micro stereology

The control group showed normal architecture of the adrenal gland including an enclosing capsule of dense irregular connective tissue containing smooth muscles (Fig. 4 A). The adrenal cortex comprised the zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR). The ZG comprised polyhedral cells arranged in irregular cords while the ZF comprised radially arranged cords of cuboidal cells. The ZR is comprised of an irregular network of anastomosing cells that are surrounded by sinusoids. The low BPA-treated group showed mild cortical vascular congestion, while the 150 µg/kg dose caused ballooned vacuoles, and congested and pyknotic nuclei in the medulla region ZG (Fig. 5c).

However, the BPA, co-administered with melatonin improved the glandular architecture (Figures 4 & 5). Microstereological analysis of the adrenal gland across the groups revealed that BPA induced dose-significant changes in the cortex and medulla of the gland (Table 1). Both BPA doses resulted in cortical depth while increasing the depth of the medulla. However, the BPA, co-administered with melatonin ameliorated these alterations.

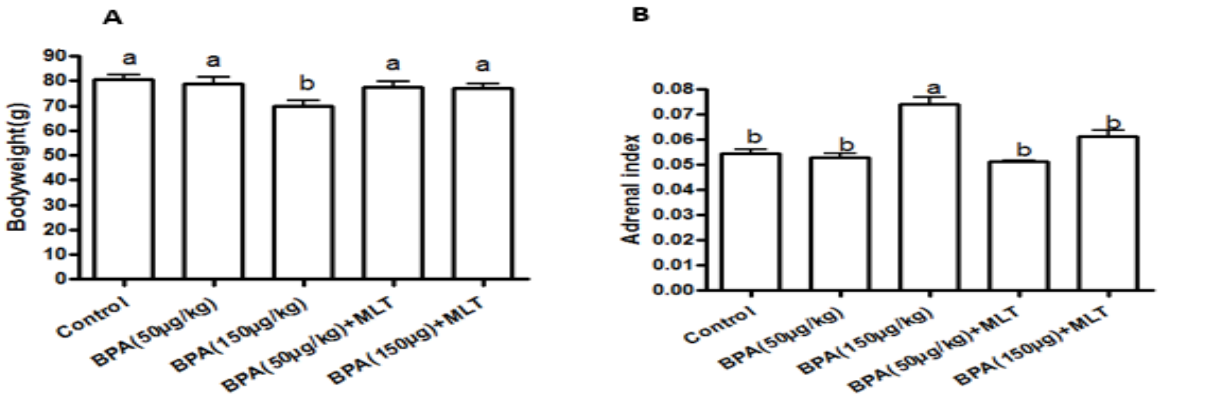

Figure 1. Effect of Melatonin on BPA-induced changes in body weight (A) and adrenal index (B) in pubertal male Wistar rats.

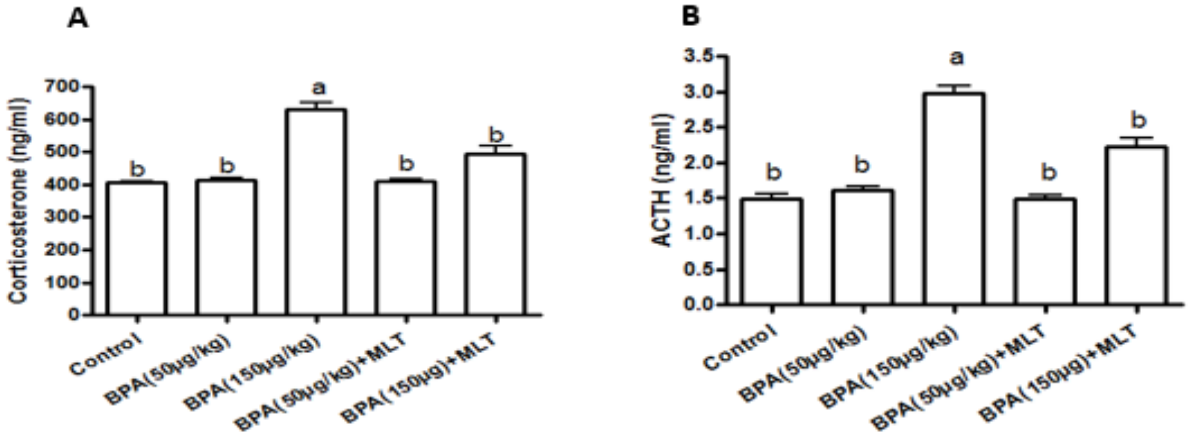

Figure 2. Effect of Melatonin on BPA-induced changes in corticosterone (A) and ACTH (B) levels in pubertal male Wistar rats.

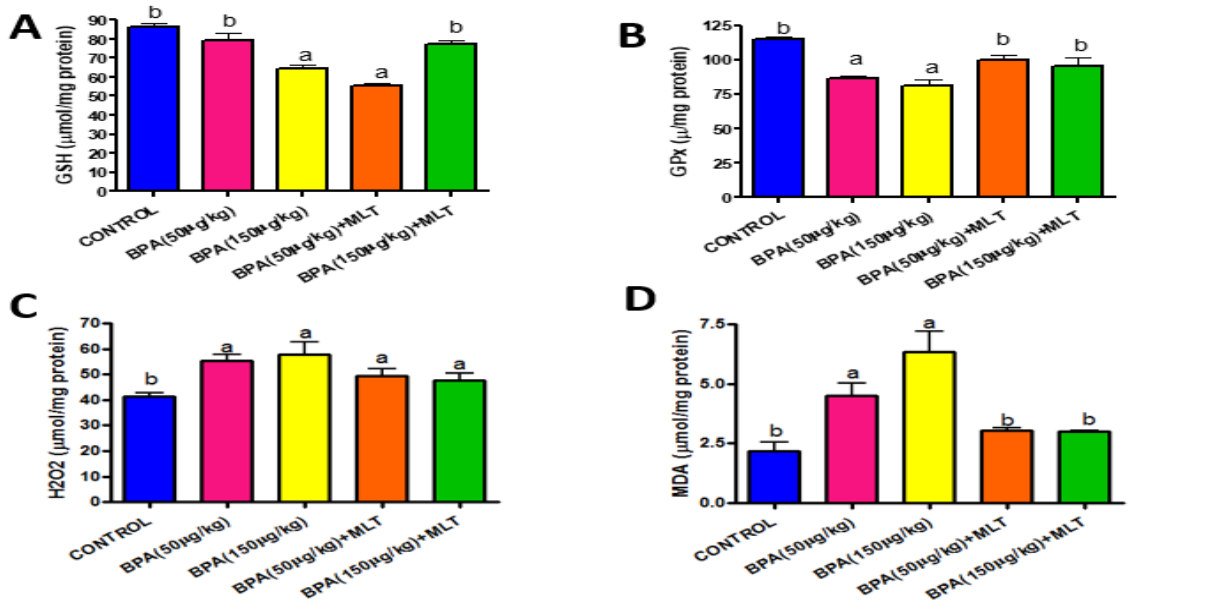

Figure 3. Effect of melatonin on BPA-induced changes in antioxidant enzymes activities in pubertal male Wistar rats.

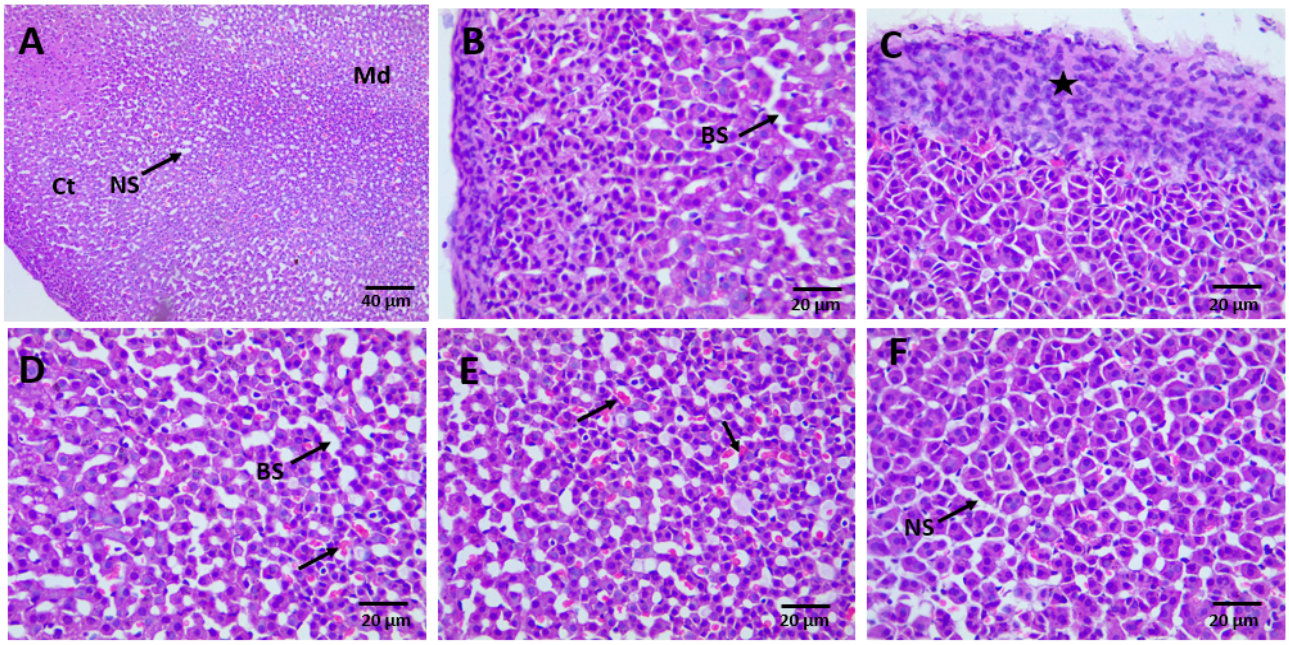

Figure 4. Adrenal gland of rats. A: Control, showing normal cortex (Ct), normal sinusoid (NS), and normal medulla (Md). B: BPA (50 µg/kg) showing ballooned sinusoid (BS). C: BPA (150 µg/kg) showing capsular hyperplasia (asterisk). D: BPA (150 µg/kg) showing ballooned sinusoid (BS) and congested sinusoid (arrow). E: BPA (50 µg/kg + MLT) showing congested sinusoid (arrow). F: BPA (150 µg/kg + MLT) showing normal sinusoid (NS).

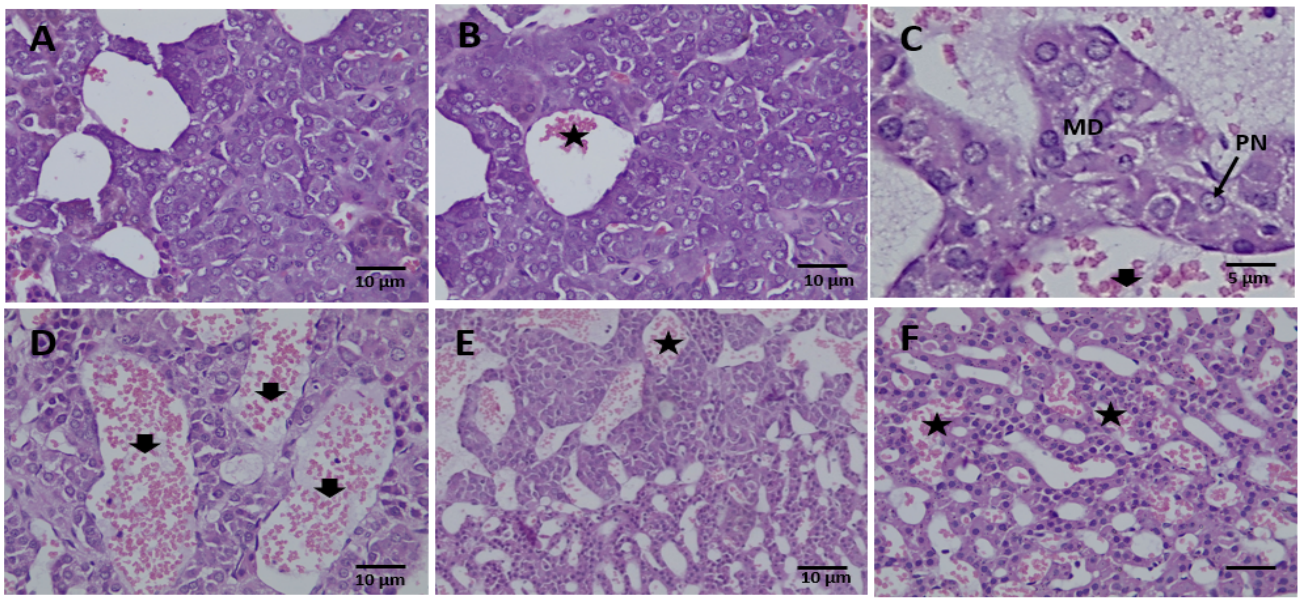

Figure 5. Adrenal gland of rats. A: Control, showing normal medulla, B: BPA (50 µg/kg) showing mild medullary vascular congestion (asterisk). C: BPA (150 µg/kg) showing severe medullary congestion (arrow head), PN- pyknotic nuclei, MD- medullary degeneration D: BPA (150 µg/kg) showing severe medullary congestion (arrow head). E: BPA (50 µg/kg + MLT) showing mild medullary vascular congestion (asterisk). F: BPA (150 µg/kg + MLT) showing mild medullary vascular congestion (asterisk)

Table 1. Effect of melatonin on BPA-induced changes in histomorphometric parameters of adrenal gland pubertal male Wistar rats.

Parameter	Control	BPA (50µg/kg)	BPA (150 µg/kg)	BPA (50µg/kg) + MLT	BPA (150 µg/kg) + MLT
Capsule thickness (µm)	13.6 ± 2.4	13.7 ± 1.8	13.4 ±2.6	12.9 ± 1.9	13.5 ±2.7
ZG depth (µm)	52 ± 11.2	50 ± 10.7	35 ± 8.2	48 ± 11.3	47 ± 10.3
ZF depth (µm)	230 ± 25.7	216 ± 23.1	180 ± 32.2	214 ± 29.3	218 ± 19.3
ZR depth (µm)	102 ± 15.3	113 ± 32.7	114 ± 25.9	118 ± 20.1	106 ± 17.4
Depth of cortex (µm)	384 ± 18.4	379 ±25.3	329 ± 21.8	380 ± 18.3	371 ± 15.6
Depth of medulla (µm)	242 ± 17.2	284 ± 12.7	295 ± 23.9	252 ± 25.6	257 ± 19.6

Discussion

Findings from this study have shown that exposure of pregnant rats from gestation day ten to twenty-one (GD10-21) to environmentally relevant doses of bisphenol A is capable of inducing dose-dependent

changes in adrenal function in the pubertal F1 rats, while co-administration with melatonin is capable of protecting against BPA-induced adrenal toxicity. The doses of BPA used in the study are environmentally relevant because 50 µg/kg/day BPA has been reported as the standard daily tolerable dose for humans while 50 mg/kg/day BPA has been reported as no observable adverse effect level (NOAEL) for BPA in rats (Doerge *et al.,* 2011). However, studies in rodents have demonstrated that *in utero* exposure of rats to BPA dose as low as 2.4 µg/kg/day from GD12 to PND 21 is capable of disrupting certain endocrine functions including reproductive dysfunctions (Bernardo *et al*., 2015; Kazemi *et al.,* 2016).

In the present study, treatment of rats with BPA resulted in significant dose-dependent decreases in body weight as well as AI. These findings suggest that *in utero* exposure of rats to BPA (50 µg/kg and 150 µg/kg) is capable of causing adverse effects on general metabolism as well as organ function in rats. Some authors have documented similar reports on the effect of BPA exposure on organ and body weights in rodents (Akingbemi *et al.,* 2004; Yuan *et al.,* 2015; Kazemi *et al.,* 2016). However, Chitra *et al*. (2003) reported that exposure of adult male rats to BPA (10 mg/kg) for 14 days did not result in any significant difference in body weight. This disparity in effect on body weight could be traced to the different modes of exposure as *in utero* exposure of rats to BPA has been shown to present with more adverse effects compared to exposure in the adult stage of life. The observed significant difference in body weight and AI of BPA-treated groups and those co-treated with melatonin shows that melatonin has a protective role against endocrine-disrupting chemical-induced toxicity.

The observed changes in serum hormone levels in the present study correlate positively with findings by Olukole *et al*. (2019) who reported that exposure of adult rats to 10 mg/kg of BPA resulted in a significant increase in serum levels of corticosterone and ACTH. The BPA-induced increase in adrenal index observed in the present study is further corroborated by the significant increase in the corticosterone and ACTH levels of the rats. Elevated serum corticosterone and ACTH levels are suggestive of adrenal disorders. As observed in the present study, elevated levels of ACTH correlate positively with increased hyperplasia of the adrenal gland. Similar positive correlations between serum levels of ACTH and adrenal tissue inflammation have been documented by many authors as the effect of exposure of rats to endocrine-disrupting chemicals (Olukole *et al*., 2019). Interestingly, melatonin co-administered with BPA protected against the observed BPA-induced increases in corticosterone and ACTH.

Treatment with BPA resulted in significant dose-dependent decreases in the activities of antioxidant enzymes GSH and GPx while causing significant increases in the levels of reactive oxygen species (ROS)/ free radicals (MDA and H_2O_2.). A similar decrease in the activities of antioxidant enzymes with concomitant elevated levels of reactive oxygen species (ROS)/ free radicals has been reported by Olukole *et al.* (2019) in adult rats exposed to BPA. This shows that exposure of rats to BPA either *in utero* or adult stage is capable of inducing alterations in metabolism following the report of El-Beshbishy *et al.* (2012). Also, exposure of mice to a standardized dose of BPA (10 mg/kg body weight) orally for 14 days has been reported to result in decreased activities of marker mitochondrial enzymes such as succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, monoamine oxidase and NADH dehydrogenase (Anjum *et al.,* 2011). Similarly, El-Beshbishy *et al.* (2012) also reported exposure of adult male rats to BPA

(10 mg/kg body weight) orally for 14 days which resulted in a decrease in male reproductive function including testes weight, total testicular protein content, testicular enzymes such as acid phosphatase, alkaline phosphatase and lactate dehydrogenase and decline in activities of marker mitochondrial enzymes Increased MDA levels have been suggested to be responsible for pathologic lipid peroxidation of spermatozoa membrane, which eventually causes prostatic cytotoxicity (Hsieh *et al.,* 2006). In the present study, melatonin served to protect the adrenal gland against a BPA-induced decrease in the activities of antioxidant enzymes as well as elevated levels of ROS/ free radicals. This is in confirmation of the antioxidant properties of melatonin as earlier reported (Bonnefont-Rousselot and Collin, 2010; Othman *et al.,* 2014; Olukole *et al*., 2018).

The adrenal lesions induced by BPA in the present study are similar to those earlier reported in adult rats exposed to 10 mg/kg BPA (Olukole *et al*., 2019). It can be inferred from the study that elevated levels of corticosterone and ACTH resulted in adrenal hyperplasia since the gland was undergoing oxidative stress. Observations from the present study have shown that melatonin co-administered with BPA is capable of protecting against vascular congestion, and hyperplasia of the adrenal gland.

In conclusion, the present study has shown that *in utero* exposure of male rats to environmentally relevant doses of BPA (50 µg/kg and 150 µg/kg) from GD 10-21 are capable of inducing marked dose-dependent adrenal alterations, including changes in body and organ weights, alterations in serum hormone levels, decrease in the activities of antioxidant enzymes while increasing H_2O_2 and MDA as well as the induction of adrenal lesions. However, findings from the study have also shown that melatonin co-administered with BPA is capable of protecting against BPA-induced adrenal dysfunction.

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