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Research Article

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In utero exposure to bisphenol A alters prostatic responses in adult male rats: Protective effects of melatonin

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Abstract Exposure to bisphenol A (BPA), an endocrine disrupting chemical (EDC), has been shown to result in a number of reproductive dysfunction. Melatonin (MLT) is a potent antioxidant known to protect against EDC-induced toxicity. We aimed at investigating the protective effects of MLT on prostate gland dysfunction in the F1 adult male Wistar rats exposed to BPA *in utero*.

Rats, confirmed pregnant were divided into five groups (n=5): Control: 0.2 ml canola oil; BPA 25 µg/kg/day; BPA 250 µg/kg/day; BPA 25 µg/kg/day + MLT 1 mg/kg/day and BPA 250 µg/kg/day + MLT 1 mg/kg/day. Blood sample was collected for serum hormonal and biochemical assays. Histopathology of the prostate gland was carried using standard methods. Prostatic index was significantly increased in BPA-treated rats compared to control ($p<0.05$). BPA induced prostatic oxidative stress and caused significant decreases in the levels of serum T and LH but resulted in significant increases in the levels of PSA, PAC and TAC. Prostatic lesions observed in the BPA groups rats included hyperplasia (functional, reactive and atypical). These were attenuated in the rats co-treated with MLT. BPA induced marked prostatic alterations, while melatonin co-administration protected against these alterations.

Keywords Bisphenol A; Melatonin; Endocrine disrupting chemical; Prostate gland; Pregnant rats

Introduction

Bisphenol A (BPA), an endocrine disrupting chemical (EDC), has received research attention as a result of its incrimination in a number of environmental health and decline in endocrine and reproductive ability in exposed human and experimental animal models [1]. BPA, is a plasticizer shown to be a major content of many household items in including infant feeding bottles and toys, medical devices, dental sealants, food and beverage packaging materials, drinking containers, plastic utensils, pharmaceuticals, adhesives, plastic building materials as well as paper coatings [2].Human exposure routes to BPA are mainly ingestion of contaminated food, drinks or breast milk, inhalation BPA contaminated air through environmental breakdown of plastics and transdermal absorption cash register receipts [3].

EDCs have been demonstrated to be capable of interfering with somatic and gonadal development through a number of mechanisms including anti-thyroid, estrogenic, androgenic and anti-androgenic processes by either blocking or in

some cases, binding to hormone receptors[2, 4].BPA-induced endocrine and male reproductive dysfunctions involving the adrenal gland, testis and accessory sex glands have been reported in adult exposed rodents[4, 5].Studies have shown that BPA exposure results in the formation of reactive oxygen species (ROS) as well as hydrogen peroxide leading to the oxidation of DNA. The progressive accumulation of ROS leads to an increase in lipid peroxidation and subsequently membrane damage in tissues [2, 4].

The tryptophan-derived neuro-hormone, melatonin (MLT), released mainly from the pineal gland has been demonstrated as a potent antioxidant against EDCs-induced toxicities in several clinical and experimental exposures [5, 6]. Melatonin has a wide range of safety margin in rodents following its experimental exposure [7]. MLT has been reported to exhibit both direct and indirect antioxidant roles in tissues. The enhancement of the activities of superoxide dismutase and glutathione peroxidase is an indirect antioxidant role of MLT while lipid peroxidation and DNA degradation has been shown to be its direct antioxidant roles [7].

Apart from MLT, other antioxidants have been used experimentally to protect or prevent BPA toxicity. These include gallic acid, Lipoic acid, N-acetylcysteine and Vitamin C [8-11]. We had earlier demonstrated the role of MLT on the prostate gland of adult rats directly exposed to moderately high dose of BPA [4]. However, the role of MLT in *in utero* exposure to BPA on the prostate gland in F1 generationrats has not been reported. This study therefore investigated the protective effects of MLT on prostate gland dysfunction in the F1 adult male Wistar rats exposed to low doses of BPA *in utero*.

Materials and Methods

Experimental Design

Adult female Wistar rats (187±0.8 g) were mated overnight with fertility-proven adult male Wistar rats. All rats were fed standard pellet and provided with drinking water. Mating was confirmed by the presence of vaginal plug on female rats and was designated as day 1 of gestation (GD1). Pregnant rats were weighed and randomly assigned into five (5) groups (n=5) on GD9: control, BPA (25µg/kg BW/day), BPA (250µg/kg BW/day), BPA (25µg/kg BW/day), + MLT (1mg/kg BW/day) and BPA (250µg/kg BW/day) + MLT (1mg/kg BW/day). Both BPA and MLT were orally administered. Dosage and duration of BPA and MLT were as earlier reported [12, 13]. Rats were treated from GD10-GD21 and offspring were allowed to remain with their dams until postnatal day 21 (PND21) when male rats (n=2) were randomly selected from each of the 5 dams/group (n=10).

Necropsy

On PND 120, rats were weighed, blood samples collected and centrifuged to obtain serum samplesstored at -20° C till use. Rats were euthanized, prostate gland retrieved and weighed with a portion of it was stored at -20° C for biochemical assays while the other part fixed in buffered neutral formalin for routine histology. Prostatic index was estimated as the weight of the prostate gland divided body weight multiplied by 100.

Hormonal Assays

Using commercial kits, quantification of serum hormones was carried out in triplicates while the levels of prostate specific antigen (PSA), prostate antigen concentration (PAC), total antigen concentration (TAC) were estimated [14].

Biochemical Assays

Homogenisation of prostate was carried in ice-cold 0.1 m Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4 followed by centrifugation at 2,000 × *g* at 4°C for 15 min to remove nuclei and debris [15]. Supernatant obtained from the homogenate was used as a sample for the following assays by Agilent HP 1100 series HPLC apparatus (USA).Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels as well as the activities of reduced glutathione (GSH), glutathione peroxidase (GPx), gutathione-S-transferase (GST) and superoxide dismutase (SOD) were evaluated spectrophotometrically [10].

Histopathology and Microstereology

For histology, prostatic tissues were processed as earlier reported [4]. Slides were analysed using light microscope (Olympus BX63 with a DP72 camera) with sections observed for lesions. Microstereology of prostatic sections

(epithelial height, luminal diameter of gland and width of stroma compartment) was carried out using standard techniques [14].

Statistical Analysis

Using the GraphPad Prism 5 software, means and standard deviation of prostatic data were captured and comparison performed using one-way ANOVA. Significance was reported at $p<0.05$.

Results

Changes in bodyweight and Prostatic Index (PI)

Maternal exposure to BPA resulted in a significant decrease in body weight ($P<0.05$) of the 250 µg/kg BPA-treated rats compared to control unlike the 25 µg/kg BPA-treated rats (Fig. 1). Melatonin significantly attenuated the BPA-induced reduction in body weight of rats. Both doses of BPA used in the study induced significant increases in prostatic index (PI) compared to the control (Fig. 2). However, these increases were dose dependent. Melatonin co-administered with BPA significantly reduced the BPA-induced elevation in PI of rats.

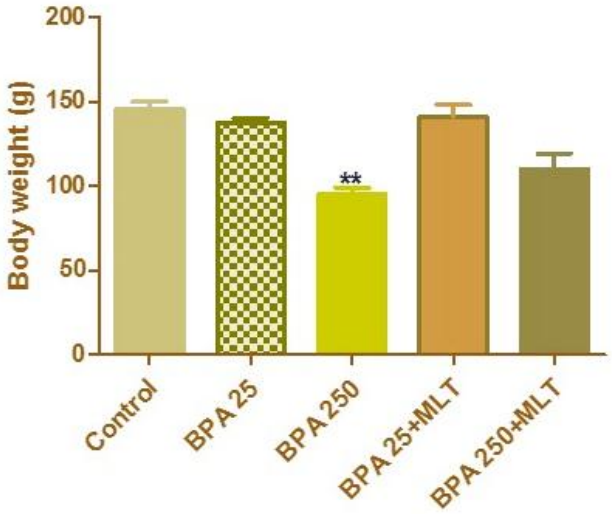

Figure 1: Effect of melatonin on BPA-induced changes in body weight in adult male rats

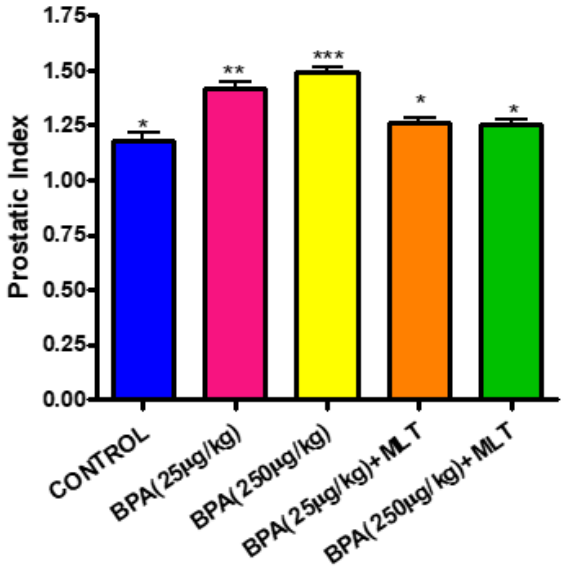

Figure 2: Effect of melatonin on BPA-induced changes in prostatic index (PI) of adult male rats

Changes in hormones concentration

Treatment with BPA resulted in significant dose dependent increases in PSA, PAC and TAC compared to the control rats (Figure 3 A-C) with the greatest effect being on the BPA 250 µg/kg body weight. With respect to PSA and TAC, there were no significant differences between the low and high BPA dose groups (Figure 3 A & C). However, there was a significant difference between the low and high BPA dose groups in terms of PAC level in the rats (Figure 3 B). Co-treatment with melatonin protected against increases in PSA, PAC and TAC in the rats both for the low and high BPA dose groups.

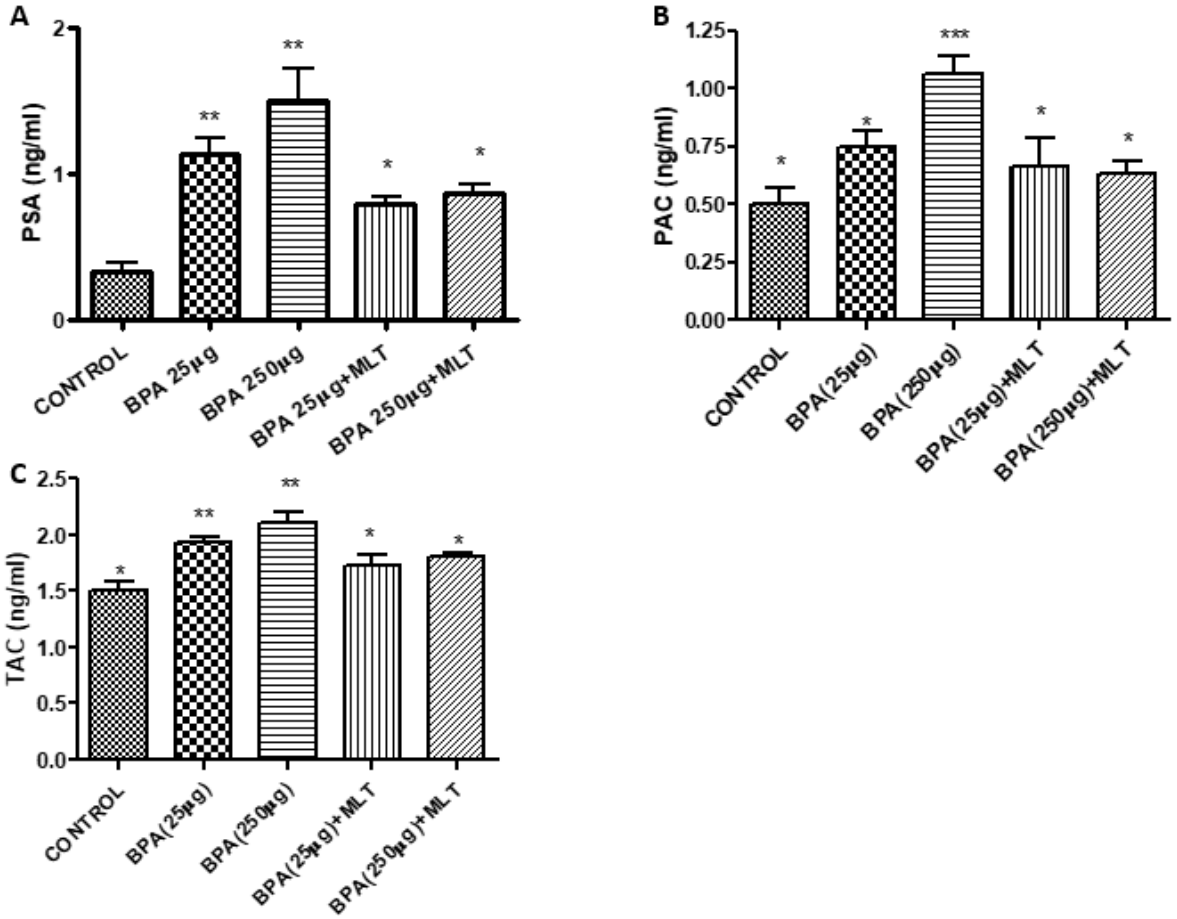

Figure 3: Effect of melatonin on BPA-induced changes in serum hormone levels in adult male rats

Changes in biochemical assays

Treatment with BPA decreased the activities of SOD, GSH, GPx and GST in a dose-dependent manner with BPA 250 µg/kg exerting the greater effect on the activities of oxidative enzymes. However, the BPA combined with melatonin protected against the observed alterations in the activities of SOD, GSH, GPx and GST (Figure 4 A-D).BPA induced significant increases in the levels of MDA and H_2O_2(Figure 5 A-B), while its co-treatment with melatonin attenuated these increases.

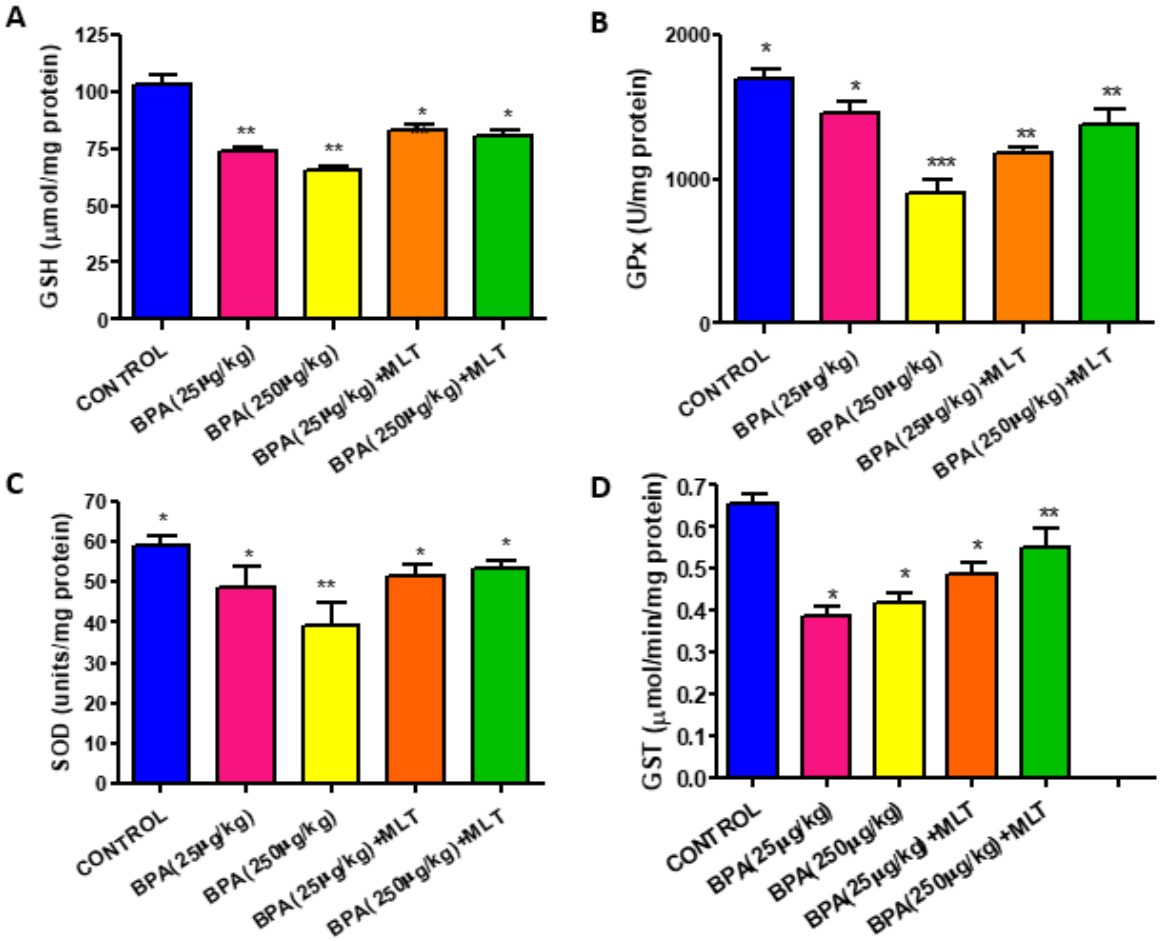

Figure 4: Effect of melatonin on BPA-induced changes in antioxidant enzymes activities in adult Wistar rats

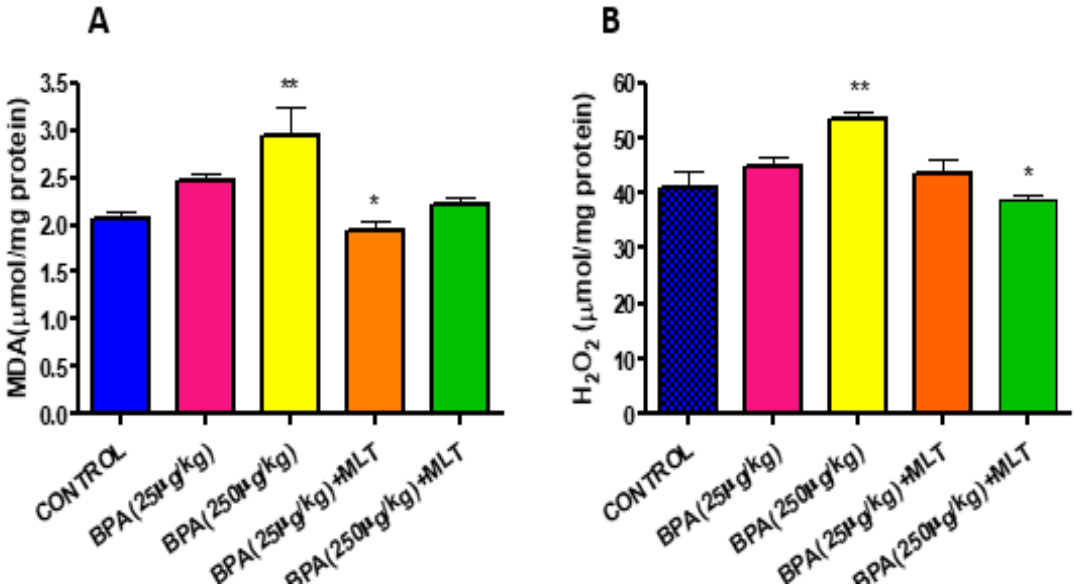

Figure 5: Effect of melatonin on BPA-induced changes in the markers of oxidative stress in adult Wistar rats

Effects on histopathology and histomorphometricparamaters of the prostate gland

The control rats had normal prostate parenchyma and stroma composed of simple columnar epithelium and blood vessels within the stromal elements (Figure 6 A). Prostatic lesions induced by BPA included congested blood vessels as well as functional hyperplasia (increased in-folding of the glandular epithelium), tubular shrinkage, reactive hyperplasia(cellular infiltration); atypical hyperplasia (cribriform pattern) as well as epithelial cell stratification (Figure 6 B-D; 7 A-B). However, BPA+ MLT-treated rats had improved prostatic structure with reduced lesions (Figures 6 E-F; 7 C-D; Table 1).

Treatment with BPA resulted in dose-dependent increases in the epithelial height of the duct of the prostate gland compared to the control while resulting in significant decrease in the luminal diameter of the gland (Figure 8 A-B). Similarly, treatment with BPA resulted in dose-dependent increases in the stromal compartment of the gland (Figure 8 C). However, the concomitant melatonin administration protected against these alterations.

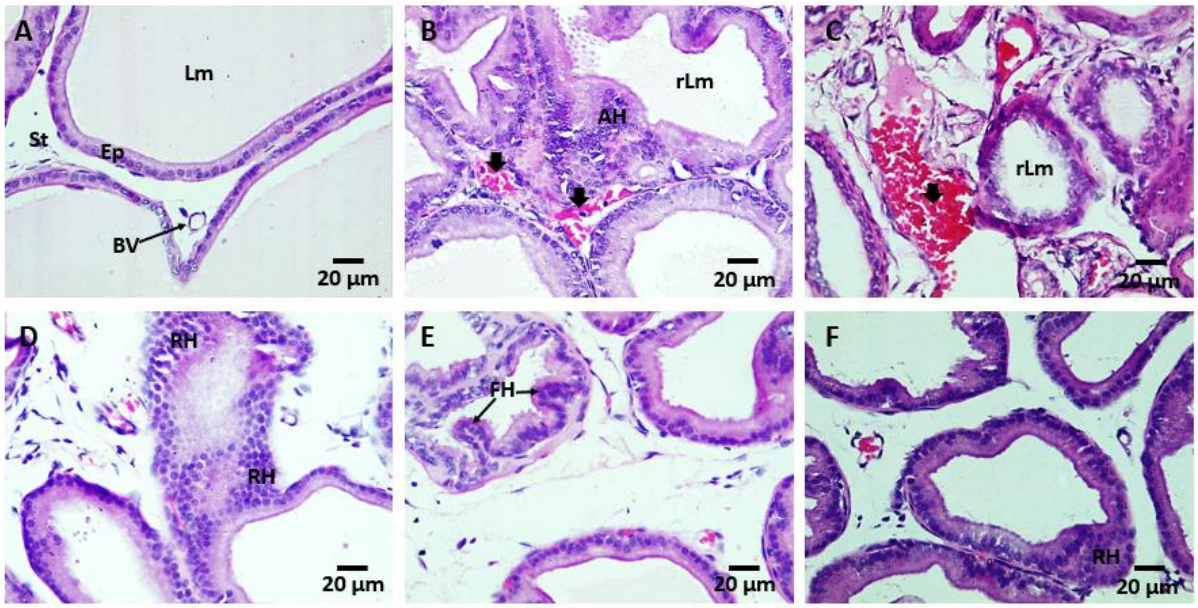

Figure 6: Histological sections showing the effect of melatonin on BPA-induced changes in the prostate gland of adult rats (H&E)

A: Control group showing normal architecture of prostate gland. Ep: epithelium; St: stroma; BV: blood vessel; Lm: lumen of prostatic acini.

B: BPA 25 µg-treated group showing atypical hyperplasia (AH), vascular congestion (arrow heads) and reduced lumen (rLm).

C: BPA 250 µg-treated group showing severe vascular congestion (arrow head) and reduced lumen (rLm).

D: BPA 250 µg-treated group showing reactive hyperplasia (RH) of the prostate gland.

E: BPA 25 µg-treated group with concomitant MLT administration showing improved prostate architecture with functional hyperplasia (FH)

F: BPA 250 µg-treated group with concomitant MLT administration showing improved prostate architecture with reactive hyperplasia (RH)

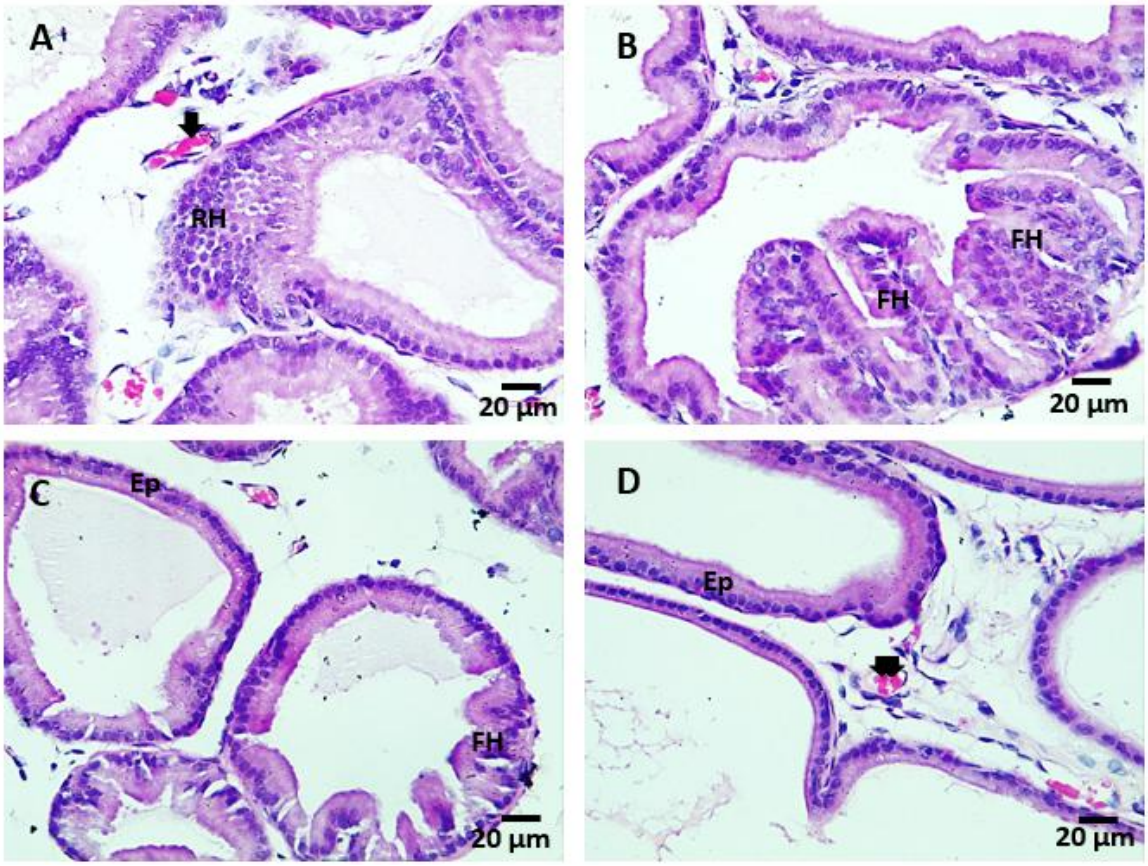

Figure 7: Histological sections showing the effect of melatonin on BPA-induced changes in the prostate gland of adult rats (H&E)

A: BPA 25 µg-treated group showing reactive hyperplasia (RH) and vascular congestion (arrow heads).
B: BPA 250 µg-treated group showing widespread functional hyperplasia (FH) with numerous infolding of prostatic epithelium.
C: BPA 25 µg-treated group with concomitant MLT administration showing mild functional hyperplasia (FH).
D: BPA 250 µg-treated group with concomitant MLT administration showing mild vascular congestion (arrow heads).

Table 1: Incidence of morphological alterations in the prostate gland of rats

Parameters of prostate gland	**Control (n=10)**	**BPA (25 µg/kg) (n=10)**	**BPA (250 µg/kg) (n=10)**	**BPA (25 µg/kg) + MLT (n=10)**	**BPA (250 µg/kg) + MLT (n=10)**
Functional hyperplasia (%)	0	40	60	20	30
Reactive hyperplasia (%)	0	50	70	30	30
Atypical hyperplasia (%)	10	30	50	20	20
Inflammation (%)	10	60	80	20	20
Atrophic tubules (%)	10	60	60	30	30
Vascular congestion (%)	10	60	80	40	30
Decreased glandular diameter (%)	0	40	60	20	10

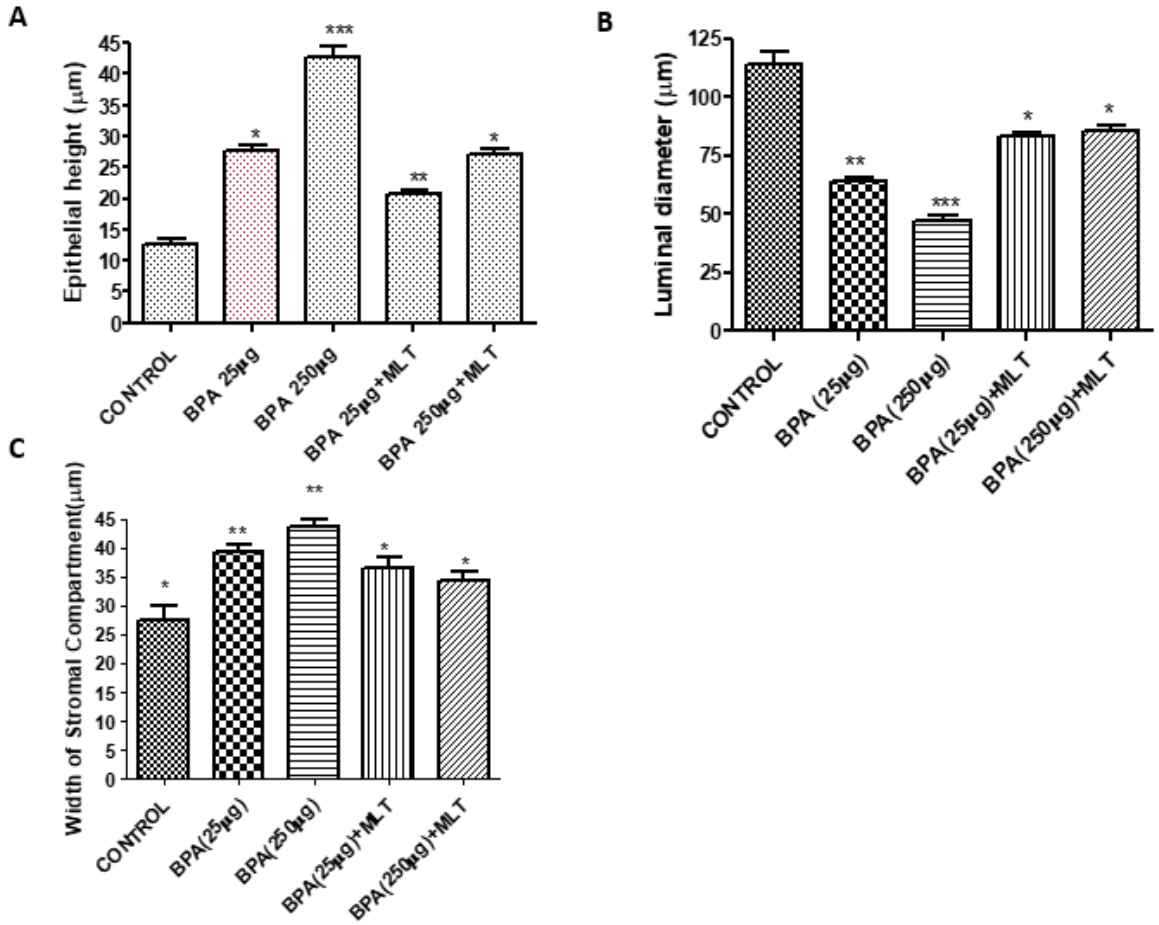

Figure 8: Effect on melatonin on BPA-induced changes in the histomorphometric parameters of the prostate gland of adult Wistar rats

Discussion

Treatment of pregnant rats from gestation day ten to twenty-one (GD10-21) with BPA (25 and 250 µg/kg) induced dose-dependent alterations in prostate function in the adult F1 generation, while its co-administration with MLT attenuated these alterations. The doses of BPA used in the study are environmentally relevant since 50 µg/kg/day and 50 mg/kg/day are the established standard daily tolerable dose in humans and the no observable adverse effect level (NOAEL) in rats, respectively [16].

The observed BPA-induced dose-dependent decreases in body weight as well as PI suggest that maternal exposure of rats to low doses of BPA is capable causing adverse effects on general metabolism as well as organ function in rats. Authors have documented similar reports on the effect of *in utero* exposure to BPA on organ and body weights in rodents [12, 16, 17]. However, exposure of adult rats to low doses of BPA resulted in no significant difference in organ and body weights [8, 18, 19].

In utero exposure of rats to BPA has been associated with more adverse effects compared to exposure in the adult stage of life [12, 16]. The BPA-induced significant decrease in levels of PSA has earlier been observed in adult rats [4].

Elevated serum PSA levels, a vital clinical finding in diagnosis of prostate disorders, has been related to benign prostate hyperplasia [20].The concurrent increased levels of serum PSA with increased hyperplasia of the prostate gland observed in this study is consistent with previous reports [4, 12]. Also, increased prostatic index coinciding with significant elevation in the serum levels of PSA, PAC and TAC of the rats is indicative of prostatic alteration.

Interestingly, melatonin co-administered with BPA protected against BPA-induced increases in PSA, PAC and TAC.

The BPA-induced significant decreases in the activities of antioxidant enzymes and elevation in the levels of reactive oxygen species (ROS)have been reported by other authors [11, 19]. Increase in the levels of prostatic ROS with a concomitant decrease in antioxidant enzymes function is expected to result in the lipid peroxidation of the membranes of the prostate gland. Increased ROS levels has been linked to lipid peroxidation of spermatozoa membrane as well as prostatic cytotoxicity [21]. Similar reports on the protective role of MLT against EDC's alterations in the activities of antioxidant enzymes and the levels of ROS have been documented [4, 22].

Previous authors have reported similar prostatic lesions observed in this study as a result of maternal exposure to BPA doses of below the NOAEL in rats [12, 23]. The presence of these prostatic lesions correlate positively with the elevated levels prostate specific antigen as well as the observed increase in the levels of oxidative stress markers. Observations from the present study have shown that melatonin co-administered with BPA is capable of protecting against vascular congestion, prostatic atrophy and hyperplasia. In the present study, treatment with environmental relevant doses of BPA resulted in a dose-dependent increase in epithelial height with a marked reduction in luminal diameter. The observed alterations in prostatic epithelial and ductal luminal dimensions have been demonstrated in F1 rats following maternal exposure to BPA [12].

Alterations in prostatic epithelial and ductal luminal morphometry is capable of inducing a reduction in the secretion of prostate fluid, a major component of the mammalian semen. However, MLT co-administered with BPA protected against prostatic epithelial and duct alterations.

Conclusion

We report that exposure of pregnant rats from gestation day ten to twenty-one (GD10-21) to BPA (25 and 250 µg/kg) induced alterations in prostate function in the adult F1 generation in a dose-dependent manner, while its co-administration with MLT attenuated these alterations. We conclude that the mechanisms of action for MLT's protection against BPA-induced prostatic dysfunction are through the prevention of inflammation and oxidative stress.

Acknowledgement

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Conflict of interest statement

Authors do not have any conflict of interest.

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