CVJ CLUJ VETERINARY JOURNAL

Article

Melatonin Protects Rats Against Bisphenol A-Induced Testicular Dysfunction Through the Upregulation of Alpha-Smooth Muscle Actin, Vimentin, and S-100 Proteins

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Abstract Bisphenol A (BPA) is a widely used chemical in the plastic industry and a known endocrine disruptor which causes reproductive toxicity in animals. Also, melatonin is an antioxidant that can alleviate the toxicity caused by endocrine disruptors. Previous studies have demonstrated that melatonin protects male reproductive functions. However, the protective mechanisms of melatonin are not well elucidated. This study investigated how melatonin protects against BPA-induced testicular dysfunction in rats. Forty male Wistar rats were grouped randomly into four. Animals in group A (control) received 0.2 mL of olive oil orally, B: melatonin (10 mg/kg) intraperitoneally, C: BPA (10 mg/kg) orally, and D: co-exposed with BPA and melatonin. All rats were treated daily for 45 days. Testicular samples were harvested and analysed on the 46th day. This study showed that melatonin prevented the BPA-induced testicular necrosis and distortion of spermatozoa flagellar axoneme arrangement in the co-exposed rats. In addition, the induction of alpha-smooth muscle actin, vimentin, and S-100 proteins in the testes was significantly reduced in the BPA-alone-treated rats. The melatonin upregulated the proteins in the co-treated group. Increased expression of alpha-smooth muscle actin, vimentin, and S-100 proteins in normal tissue have been associated with effective regulation of fibroblast contractile activity, cell migration and metastasis, and apoptosis, proliferation, differentiation, and inflammation in different cell types, respectively. Therefore, our findings provide insights into the protective mechanisms of melatonin against bisphenol A-induced reproductive toxicity.

Received: 06.11.2024
Accepted: 20.11.2024
Published: 15.07.2025

DOI: 10.52331/v30i2512

Keywords: Bisphenol A, Melatonin, Spermatozoa, Testes, Protein expression

1. Introduction

Humans and animals are exposed to different kinds of toxic substances in the environment. These substances are found in the environment, synthetic materials, or chemical

products [1]. This exposure could be associated with several detrimental health consequences. Bisphenol A (BPA) [228 Da, (CH3)2C(C6H4OH)2] is a widely used product in the industry for the manufacture of plastics and food containers [2, 3]. However, despite its wide application in industries, Bisphenol A is an Endocrine-Disrupting Chemical (EDC) when exposed via ingestion, skin absorption, or inhalation, and causing lesions in the liver, adipose tissue, heart, and the reproductive system [2]. Its ability to disrupt the hormonal system holds a significant implication for reproductive function.

One of the mechanisms of BPA-mediated reproductive toxicity includes its ability to mimic oestrogen, a crucial hormone in the reproductive system [4]. BPA binds oestrogen receptors, thereby inducing an estrogenic effect in a way that disrupts the normal endocrine signalling pathway and also causes deleterious effects via its ability to bind to the gamma peroxisome proliferator-activated receptor and the orphan nuclear oestrogen-gamma receptor in other body cells [5]. BPA exposure can impair reproductive organ development, disrupt the hypothalamic-pituitary-gonadal axis function, which is critical for reproduction, reduce testosterone levels, and inhibit spermatogenesis, leading to infertility [6, 7].

BPA toxicity is associated with oxidative stress induction in the testes due to their high metabolic activities and polyunsaturated fatty acids in sperm cell membranes, which are prone to oxidation [4, 8, 9]. BPA can interfere with ovarian follicle development, oocyte maturation, and hormonal cycles, which are crucial for normal female reproductive function [10]. The role of BPA on male reproductive function has been extensively studied in animals, with deleterious effects observed on the various parameters monitored, including spermatozoa count and motility, antioxidant defence system, mitochondrial function, and androgen synthesis [11, 12].

Due to the detrimental health effects associated with exposure to BPA, there has been ongoing research to identify therapeutic agents that could serve as antidotes. One of these therapeutic agents is melatonin (N-acetyl-5-methoxytryptamine), a vital hormone in the body that has also been synthesized for medicinal uses [13]. Melatonin regulates the circadian rhythm, plays a critical role in energy metabolism and glucose homeostasis, functions as an antioxidant, and is involved in numerous biological processes such as immune modulation, cellular protection, and reproductive health [14, 15]. With melatonin being a free radical scavenger, it has been used as a therapeutic agent against numerous pathological conditions [9, 15, 16].

Since melatonin binding sites have been detected in the reproductive system of many species, [17] reported that melatonin influences the release of hormones, which are essential for reproductive function in both males and females. Furthermore, in a study by [18], defective sperm integrity was induced by high-fat diet-induced obesity in male Wistar rats, and this defect was ameliorated by melatonin supplementation at 4 mg/kg. As a result of these beneficial properties, melatonin can enhance reproductive health. Despite these reports, there has been a limited investigation of the role of melatonin on bisphenol A-mediated repro-toxicity. Therefore, this study aims to investigate how melatonin mitigates BPA-induced testicular dysfunction in rats. Findings from this study will be relevant to enhancing reproductive health among populations exposed to bisphenol A.

2. Materials and Methods

2.1. Chemicals and Reagents

Melatonin and Bisphenol A were obtained from Sigma-Aldrich Co. (USA). Melatonin (98% purity, dissolved in 0.5% ethanol in normal saline) was given at 10 mg/kg body weight (22). Bisphenol A was dissolved in DMSO, solubilized in canola oil, and given at 10 mg/kg body weight (22). All chemicals and reagents used were of standard analytical grade.

2.2. Animals

Forty male Wistar rats (160 ± 10 g) sourced from the Faculty of Veterinary Medicine, University of Ibadan, Laboratory Animal House were used. Rats in each group were housed in a cage measuring 60 × 60 × 50 cm and maintained under regulated environmental conditions of a temperature (25 ± 2.0°C), relative humidity (50 ± 15%), and photoperiod (12-hr light and 12-hr dark). The rats lived on a standard commercial diet with unlimited access to drinking water. All the procedures used in this study followed ethical standards and guidelines and the study was duly approved by the Institutional Ethics Committee (UI-ACUREC /17/0069).

2.3. Experimental Protocol

The animals were grouped randomly into four (n=10) and treated as follows.

Group A: Rats received 0.2 mL of olive oil orally for 45 days

Group B: 10 mg/kg body weight MLT administered intra-peritoneally, daily for 45 days.

Group C: 10 mg/kg body weight BPA administered orally, daily for 45 days

Group D: Concurrent oral administration of BPA (10 mg/kg) and intra-peritoneal administration of MLT (10 mg/kg) daily for 45 days.

The treatment modality was previously described [19, 20, 22].

2.4 Histopathology of the testis

Following diethyl ether anaesthesia and euthanasia by cervical dislocation, the testis was excised and observed for any sign of gross morphological changes. The testis was weighed and samples from the right testis were obtained, fixed in 4% buffered formalin solution, embedded in paraffin, and sectioned (5 μm-thick) for haematoxylin and eosin (H & E) staining. Stained slides were examined under a bright field light microscope (Olympus Corporation, Tokyo). Microscopy evaluations were performed as described for testicular toxicity [21].

2.5. Transmission Electron Microscopy

Fixed (glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, 4 h., 4 °C) testicular samples were rinsed several times and again fixed in 1% osmium tetroxide, and dehydrated in a graded ethanol solution. The clearing of the tissues was done using propylene oxide, infiltrated with both 1:1 propylene oxide: epoxy resin and 1:2 propylene oxide: epoxy resin solutions, and finally kept in 100% epoxy resin for 36 h under vacuum. This was followed by embedding the tissues in fresh epoxy resin and curing at 60 °C for two days. Semi-thin sections were stained using toluidine blue and viewed with a light microscope (Olympus BX63 fitted-DP72 camera). Ultra-thin sections (70-80 nm), double-stained with uranyl acetate and lead acetate were also viewed under a transmission electron microscope (Philips CM 10 TEM) which operates at 80 KV. The micrographs of the different testicular sections were captured (Gatan 785 Erlangshen digital camera, Gatan Inc., Warrendale, PA), analysed, and assembled (Adobe Photoshop CS5, Adobe Systems, San Jose, CA) [22].

2.6. Immunohistochemistry

The immunostaining for the detection of αSmooth Muscle Actin (αSMA), S-100 protein and Vimentin (Vm) was carried out according to the method described [22]. Briefly, super frosted slides containing testicular sections were de-waxed in xylene and rehydrated a graded concentration of alcohol. 3% hydrogen peroxide was used to block endogenous activity. Antigen retrieval was done by heating the citrate-buffered (0.1M, pH 6.0) tissue sections for 7 min (repeated three times) using a microwave at 750W. The slides were allowed to cool for 20 min after which they were washed with phosphate-buffered solution (PBS) (pH 7.2) thrice for 5 min each. Tissue permeabilization was carried out with 0.3% (v/v) Triton X-100 (Sigma, USA) in PBS for 10 min. Normal goat serum supplied with the Immunocruz mouse staining kit was used in blocking the slides for 1 h before incubation with primary antibodies, monoclonal mouse anti-α-smooth muscle actin 1:200, M085101; polyclonal rabbit anti-S-100, Dako, Z0311, 1:2000, and monoclonal mouse anti-vimentin 1:200, M072501, overnight at 4 °C in a humidified chamber. Following incubation, the slides were washed with PBS, and incubated again with biotinylated goat anti-mouse secondary antibody for 90 min. The slides were rinsed in PBS thrice for 5 min each, incubated again with a streptavidin horseradish peroxidase complex (Immunocruz kit) for another 30 min, and finally washed with PBS thrice for 5 min each. Following the addition of 0.05% (w/v) 3, 3, 9-diaminobenzidene (DAB) tetra-hydrochloride solution (Sigma, USA) and counterstaining using Mayer's Haematoxylin stain, the slides were mounted and visualized afterwards using a bright-field light microscope (Olympus BX63 fitted-DP72 camera. The protein expression level in area per cent was determined in the testes using an image analyser (Leica Qwin 500 C, Cambridge, UK) and 10 non-overlapping fields for each rat were taken (×400) as previously reported [23].

2.7. Statistical analyses

The One-way Analysis of Variance (ANOVA) was utilized in this study, with the significant level set at $p< 0.05$, using IBM SPSS Version 20. The data were presented as means plus standard deviation (SD).

3. Results

3.1. Histological changes in the testicular sections of exposed rats

In Fig. 1, the testicular sections of the control and MLT-exposed rats showed no visible lesions. The seminiferous tubules were intact including the normal succession of enclosed Sertoli cells and spermatogenic cells (Fig. 1). In addition, the testes interstitial was intact in the control and MLT-exposed rats, possessing Leydig cells as well as blood vessels. The rats of BPA-exposed groups displayed hyperaemia of the interstitial including sloughing of interstitial elements. There were testicular vacuolations within the seminiferous tubules in addition to a reduction in the number of elongated spermatids and disintegration of the basement membrane of seminiferous tubules in the BPA-exposed group (Figs. 1 and 2). Also, in the BPA-exposed group, the rats showed fewer spermatozoa in the lumen of the seminiferous tubules (Fig. 2). The MLT reversed these observations in the BPA and MLT co-treated group (Figs. 1 and 2).

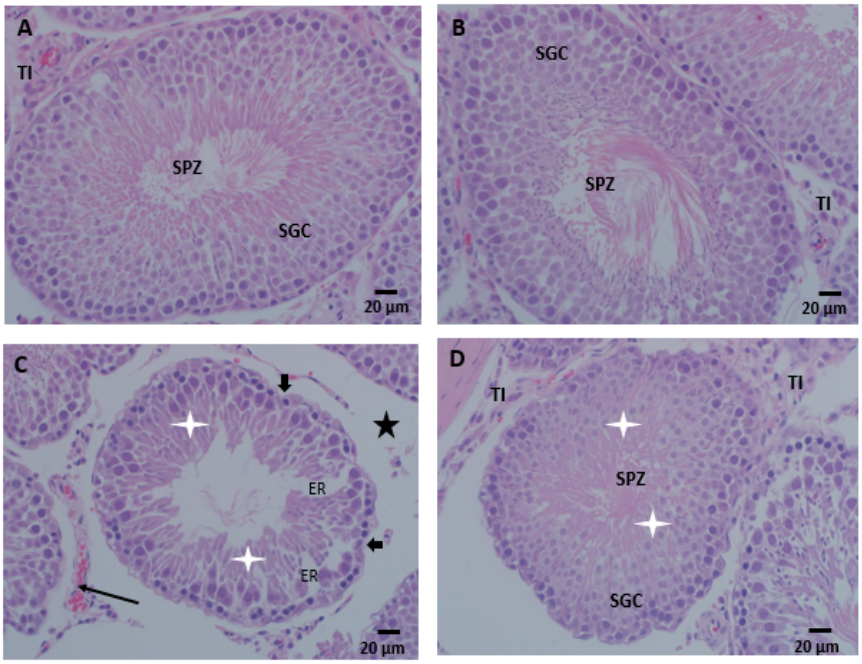

Fig. 1: Bisphenol A caused alterations in the seminiferous tubules of treated rats' testes.

Groups A and B showed no visible lesions. The seminiferous tubules were filled with normal germ cells (SGC), and spermatozoa (SPZ), and the interstitial are intact (IT). C. shows hyperaemia of the interstitial (arrow), sloughing of interstitial elements (black star), elongated spermatid (white star) reduction, and germinal cell degeneration (ER). D. shows intact interstitial (IT), spermatogenic cells (SGC), and spermatozoa (SPZ). A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 20µm (H & E).

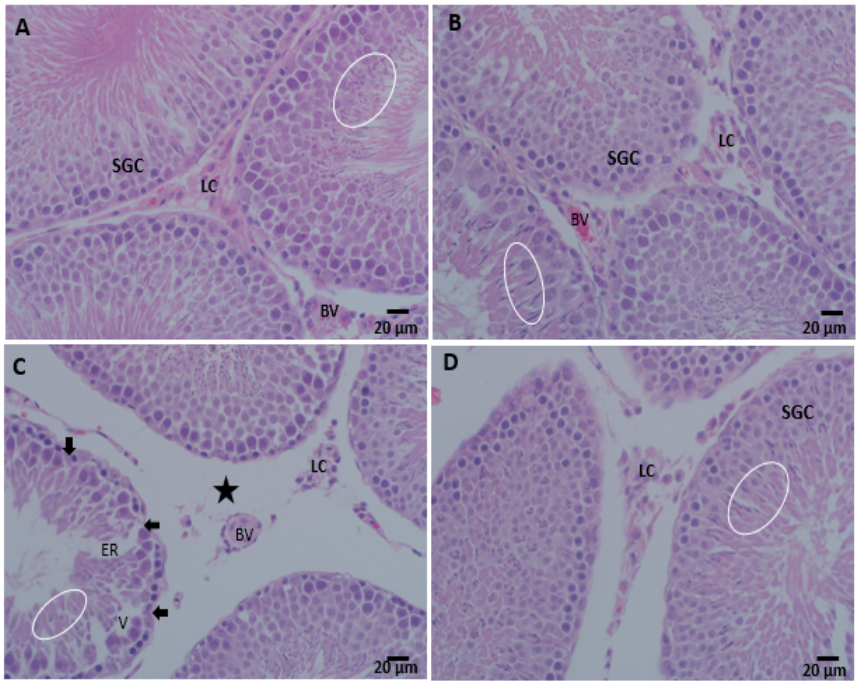

Fig. 2: Sections of the treated testes showing the various cell types.

Groups A and B: increased number of Leydig cells (LC), spermatozoa (SPZ), blood vessels (BV) and intact spermatogenic cells (SGC). Group C: shows testicular interstitial sloughing (star) with fewer Leydig

cells (LC), sloughing of germ cells (ER) and seminiferous tubules basement membrane disintegration (arrow-head) with fewer number of elongated spermatid (circle) and testicular vacuolations (V). Group D: shows normal elongated spermatids, spermatogenic cells (SGC) and sIn Fig 3., the seminiferous tubules that housed the spermatogenic cells and Sertoli cells in the control and MLT-exposed rats were intact with the spermatogonia found at the basement membrane in close opposition with Sertoli cells (Fig. 3). The BPA induced pyknotic nuclei formation and Sertoli cell cytoplasmic processes dissolution in-cluding testicular vacuolations, germinal cells sloughing, and distortion of the seminiferous tubules' basement membrane (Fig. 3). At higher magnpermatozoa (SPZ). A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 20µm (H & E).

3.2. Transmission Electron Microscope (TEM) of the testes of treated rats

Identification, numerous mitochondria and lipid droplets in the Sertoli cells cytoplasm were observed in the control and MLT-exposed groups (Fig. 4). In the co-treated MLT and BPA group, MLT reversed the effect of BPA to reflect the observations seen in the control group (Fig. 4).

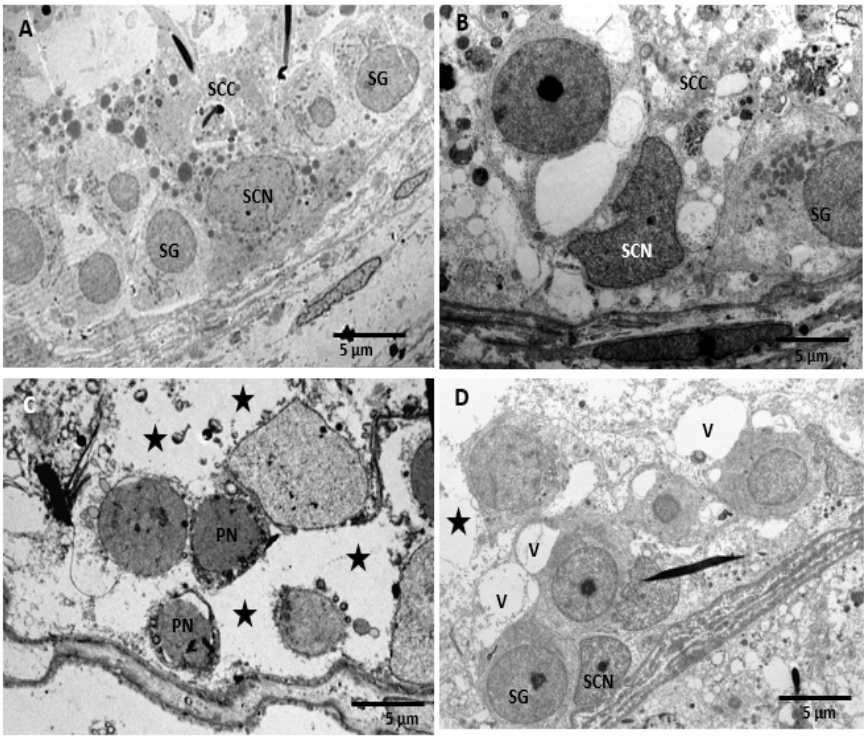

Fig. 3. Transmission Electron Microscopy sections of the seminiferous tubules of treated rats

Groups A and B showed intact spermatogonia (SG), Sertoli cell nucleus (SCN) and its cytoplasm (SCC), Group C: shows deranged basement membrane of the seminiferous tubules, pyknotic nucleus (PN) with se-vere germinal cell loss and the lack of Sertoli cell cytoplasmic processes (star), Group D: MLT reversed the derangement induced by BPA. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 5 µm.

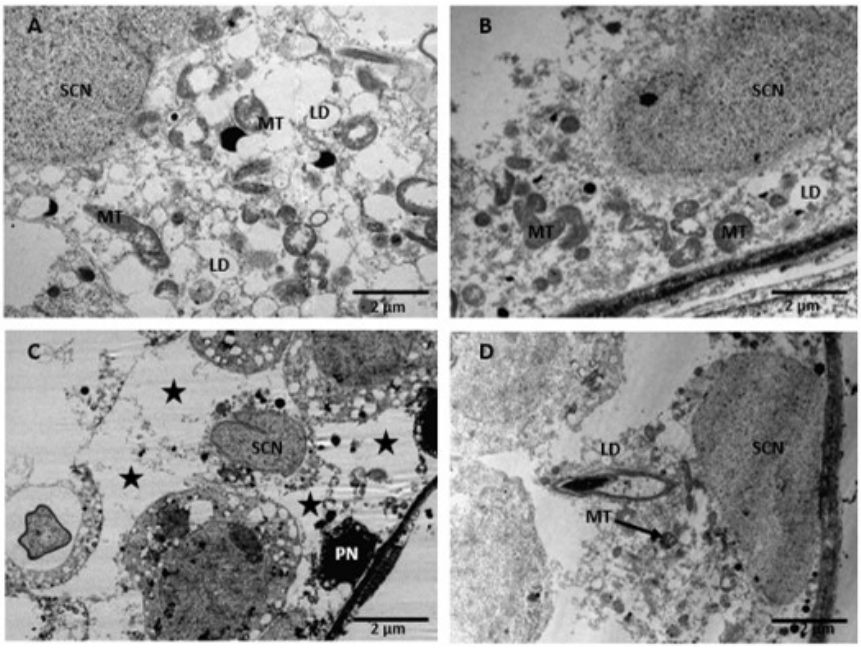

Fig. 4. Transmission Electron Microscopy sections of the Sertoli cells of treated rats.

Groups A and B displayed intact Sertoli cell nucleus (SCN) and Lipid droplets (LD) including numerous mitochondria (MT), Group C: there was a pyknotic nucleus of the Sertoli cells, Group D: the mitochondria (MT), lipid droplets (LD) and Sertoli cell nucleus (SCN) were intact. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 2µm

Fig. 5 shows an intact testicular interstitial of the control and MLT-exposed rats, with Leydig cells having no pathologic nucleus and cytoplasm, blood vessels, and lipid droplets. The TEM sections showed that BPA caused severe sloughing of the testicular interstitial with reduced Leydig cells which contained nuclei with no visible cytoplasm (Fig. 5). This was reversed in the BPA+MLT-exposed group (Fig. 5).

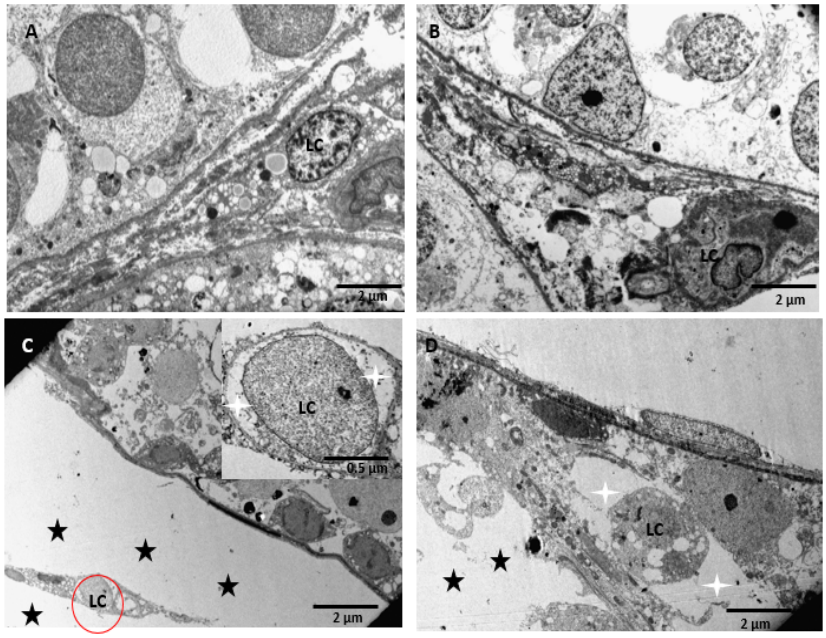

Fig. 5. Transmission Electron Microscopy sections of the Leydig cells of treated rats.

Groups A and B show no visible lesion of the Leydig cells (LC), Group C: shows Leydig cells (LC) having a dissolution of the cytoplasm and loss (star) of the interstitial (see Inset, Scale bar = 5μm), Group D: shows intact (arrow) interstitial and Leydig cell (LC). A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 2μm.

Fig. 6 shows that the control and MLT-exposed groups present with intact and round spermatids possessing normal acrosomal vesicles and granules and cytoplasm containing abundant mitochondria. There were round spermatids with deranged acrosomal vesicles and a lack of granules and mitochondria in the BPA-exposed group (Fig 6.). This was reversed in the BPA+MLT-exposed group (Fig. 6).

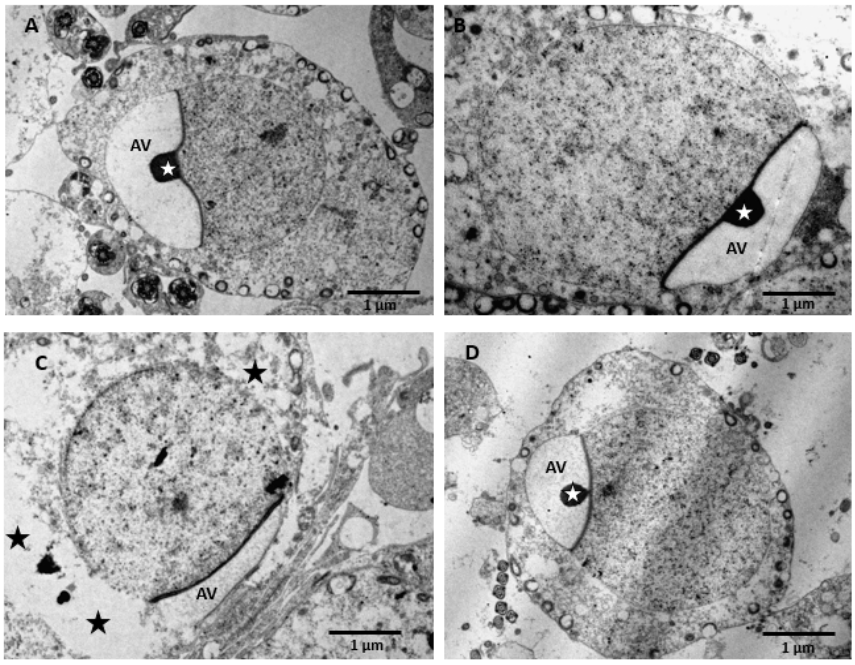

Fig. 6. Transmission Electron Microscopy sections of the acrosomal vesicle of treated rats.

Groups A and B: present an intact acrosomal vesicle (AV) and granule (star) with several mitochondria, Group C: presents a deranged acrosomal vesicle (AV) and reduced spermatid cytoplasm content (star), Group D: intact acrosomal vesicle (AV) and granule (star) with abundant mitochondria. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 1μm**.**

In Fig. 7, there was nuclear condensation and Sertoli cell cytoplasmic processes surrounding the elongated spermatids of the control and MLT-exposed rats. Conversely, the elongated spermatids showed karyorrhexis and dissolved Sertoli cell cytoplasmic processes in the BPA-exposed rats (Fig. 7). However, these lesions were reversed in the BPA+MLT-exposed group (Fig. 7).

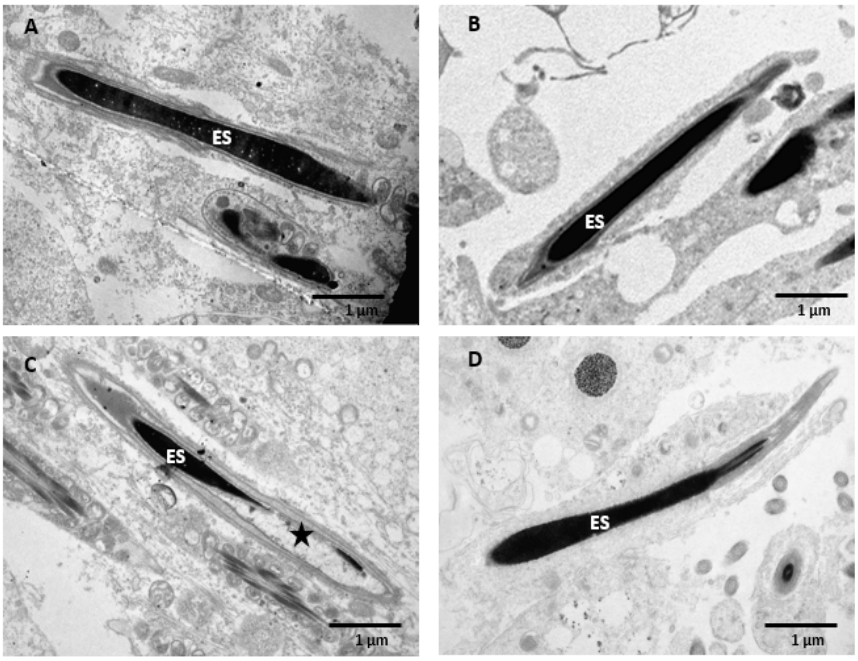

Fig. 7: Transmission Electron Microscopy sections of the spermatids of treated rats.

Groups A and B showed intact spermatid (ES), Group C: showed karyorrhexis (star) of elongated spermatid (ES), and Group D: shows elongated spermatid having normal nuclei. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 1 µm.

Fig. 8 revealed the 9+2 axoneme arrangement of the flagellar apparatus of spermatozoa in the control and MLT-exposed rats and a distortion of the 9+2 axoneme arrangement in the BPA-exposed rats (Fig. 8). However, there was a restoration of the 9+2 axoneme arrangement of flagellar apparatus of spermatozoa in the BPA+MLT exposed rats (Fig. 8).

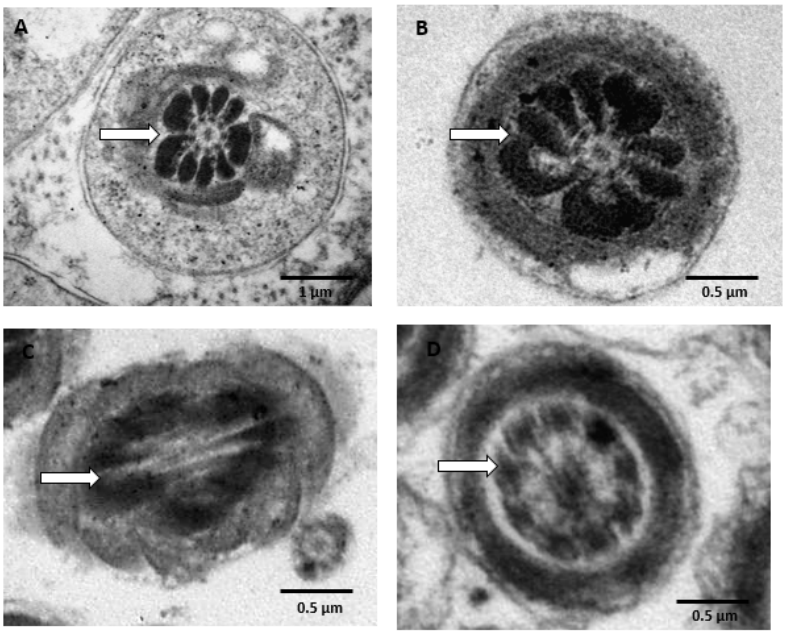

Fig. 8: Transmission Electron Microscopy transverse section of the Axoneme of Sperm cells.

Groups A and B showed an intact 9+2 Axoneme structure (white arrow), Group C: showed a deranged axoneme structure of the sperm cell, and Group D: showed an intact 9+2 Axoneme structure of the sperm cells. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 0.5 μm.

3.3. Immunostainings of the testes of treated rats

Table 1 shows the expression levels of alpha Smooth Muscle Actin (αSMA), S-100, and Vimentin (Vm) in the testes of rats exposed to the test samples. αSMA, S-100, and Vm proteins were significantly ($p<0.05$) downregulated in the BPA-exposed rats compared to the control (Table 1 and Figs. 9 to 10). There was no significant difference ($p>0.05$) in the expression level of the proteins between the control and MLT-exposed groups (Table 1 and Figs. 9 to 10). Although not significant, MLT enhanced the expression levels of αSMA, S-100, and Vm in the testes of the co-exposed rats (Table 1 and Figs. 9 to 10), especially at the Leydig cells, blood vessels, and peritubular membrane level (Figs. 9 to 10).

Table 1. Quantification of protein expression levels in the testes of treated rats

Proteins	**CONTROL**	**MLT**	**BPA**	**BPA+ML T**
αSMA (%)	8.85±0.55[a]	9.98±0.51[a]	4.48±0.37[b]	5.80±2.35[a]
S-100 (%)	4.72±0.51[a]	4.88±0.61[a]	3.40±0.01[b]	5.24±0. 26[a]
Vm (%)	7.87±1.57[a]	7.35±1.16[a]	4.39±1.13[b]	4.68±0.25[a]

Each result represents value of mean ± standard deviation. Values with similar superscript 'a' and 'b' within rows are significantly ($p<0.05$) different.

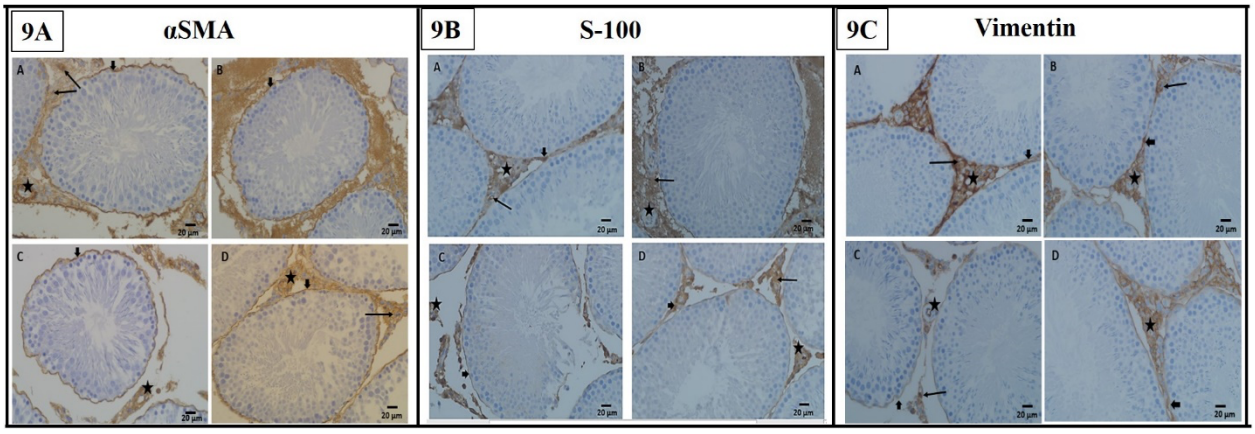

Fig. 9: Immunostaining for αSMA, S-100, and Vimentin proteins in the testes of treated rats.

In 9A, Groups A and B show higher immunopositivity for αSMA, especially at the basement membrane and blood vessels; αSMA immunopositivity was reduced at the basement membrane (arrowhead) and blood vessel (star) in Group C, Group D showed enhanced αSMA immunopositivity at the basement membrane (arrowhead) and blood vessel (star).

Similarly, in 9B, S-100 immunopositivity was pronounced in the Leydig cells (arrow), blood vessels (star), and peritubular membrane (arrowhead) of Groups A and B. S-100 reduced in intensity in Group C at the peritubular membrane (arrowhead) and blood vessel (star). Group D showed enhanced S-100 immunopositivity in

the peritubular membrane (arrowhead) and Leydig cells (star). In 9C, Vimentin expression was observed in the blood vessels and Leydig cells of rats in Groups A and B. Group C showed a reduction in vimentin staining intensity. Group D showed improved vimentin staining intensity in the blood vessels and peritubular membrane. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 20μm.

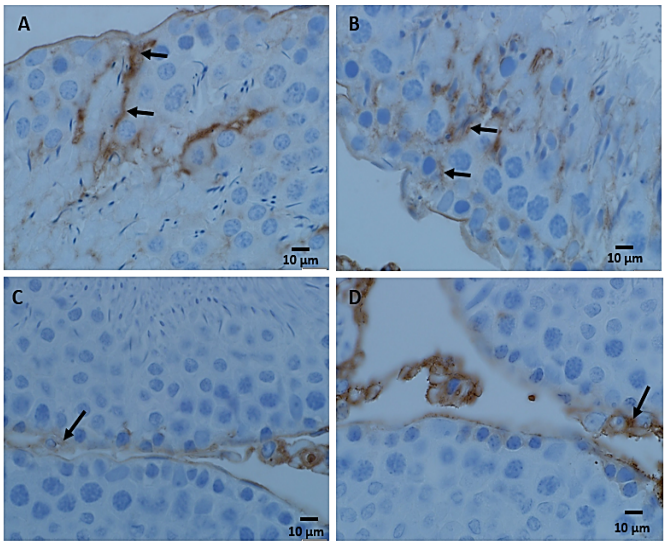

Fig. 10: Immunostaining for Vimentin in the testes of treated rats at higher magnification. Groups A and B showed vimentin-positive-Sertoli cells, Group C: shows a significant reduction in vimentin expression in the Sertoli cell level, and Group D: shows an improved vimentin expression. A = Control, B = MLT exposed, C = BPA exposed, and D = BPA + MLT exposed. Scale bar = 10μm.

4. Discussion

Bisphenol A was described as an endocrine-disrupting chemical which alters normal reproductive function [12]. [24] reported that the exposure of rats to Bisphenol A results in a decrease in sperm motility, which may be due to an increase in reactive oxygen species level. In addition, in a study by [11], the exposure of BPA at a concentration of 50 μM was associated with early DNA damage responses and perturbed cytoskeleton in the C18–4 spermatogonia cell line, further emphasizing the reproductive toxicity of BPA. We have seen in this study that exposure of rats to 10 mg/kg BPA for 45 days is capable of causing testicular dysfunction, which could subsequently precipitate infertility.

The histological assessment of the testes in the current study showed that exposure of rats to BPA caused testicular damage, including a reduction in germ cell number (Figs. 1 to 2). This reflects the capability of BPA to alter spermatogenesis in rats with an attendant effect on reproductive function. This corroborates the findings of [25], who exposed mice to graded doses of BPA. Some reports on the role of BPA in the reproductive performances of male rats have shown that bisphenol A impaired male fertility. Bisphenol A causes testicular dysfunctions, including induction of death of testicular germ cells, disruption of the junctional proteins of the blood-testis barrier, and distortion in androgen binding protein and steroidogenic enzymes levels [8, 9, 25, 26, 27, 28]. The morphological injuries in the testes induced by BPA in our study (Figs. 3 to 8) are similar to the previous reports in the prostate gland, adrenal gland, and cardio-renal system [22, 38, 39]. Other

studies further corroborate the observed BPA-induced histological derangement in the testes. However, the experimental animal model used was mice, with higher doses, and for longer periods [9, 25].

Several proteins, such as α-SMA, S-100, and Vm, are relevant biomarkers for investigating the reproductive toxicity of BPA and were used in this study. In this study, the administration of bisphenol A was associated with the downregulation of α-SMA, S-100, and Vm in the testes of male Wistar rats (Fig. 9 to 10). This finding aligns with the findings of [29], who reported a decrease in vimentin and SMA expression in the mammary glands of rats prenatally exposed to BPA. [22] also reported a decreased localization of α-Smooth muscle actin, vimentin, and S100 proteins in the prostate of BPA-exposed rats. This multi-reproductive organ toxic effect of BPA presents a significant reproductive implication.

S-100 belongs to the Ca^{2+} binding protein subfamily which has been reported to play a significant role in motility chemotaxis, and secretion in living systems [30]. Both S-100 and α-SMA have been widely considered biologically active proteins of the male reproductive organ. They possess functional relevance in absorption, secretion and contractile activities [31]. Similarly, Vimentin is a type III intermediate filament which maintains the structural integrity and mechanical resilience of cells and assists in keeping normal differentiating germ cell morphology [32]. The downregulation of these proteins in the testes of male Wistar rats by BPA could imply an impaired testicular secretion. This might also be associated with reduced sperm motility, as suggested by [22]. Since these proteins play a tremendous role in structural integrity, their downregulation is also a suggestion of the susceptibility of the testes to cellular damage. According to [33], α-SMA, once downregulated, might result in impaired smooth muscle function, which could compromise sperm transport and reduce overall reproductive efficiency.

However, melatonin demonstrated a therapeutic effect on the testes of the rats by upregulating the expression of α-SMA, S-100, and Vm (Figs. 9 to 10). Melatonin has been previously reported to possess antioxidative properties, making it a potential therapeutic agent in numerous pathological conditions. [14] reported that melatonin functions by forming a chelate with transition metals, which are involved in the Fenton/Haber-Weiss reactions. As a result, this prevents the formation of hydroxyl radicals, which play a significant role in oxidative stress. [34] also reported that melatonin directly scavenges free radicals and exhibits anti-inflammatory properties.

The upregulation of α-SMA, S-100, and Vm in the testes of male Wistar rats by melatonin was in alignment with the findings of [22], who reported a modulating effect of melatonin through the upregulation of vimentin, S-100, and α-smooth muscle actin. In addition, [9] have previously reported the therapeutic effect of melatonin in alleviating testicular damage caused by BPA. Therefore, melatonin could play a significant role in enhancing tissue integrity and cellular function. The upregulation of vimentin by melatonin in the testes of rats in this study could indicate its protective effect on the structural framework of the testes and epididymis, potentially stabilizing the cytoskeleton and promoting tissue repair or preservation [35].

Furthermore, S-100 has been reported to be involved in the inflammatory response [30], and its upregulation by melatonin could indicate the potential of melatonin to reduce oxidative stress and inflammatory damage implicated in impaired testicular and epidydimal function. Lastly, since melatonin upregulated the expression of α-SMA, melatonin could play a crucial role in regulating smooth muscle contraction needed for

sperm transport and general reproductive health [36]. The coadministration of both bisphenol A and melatonin was associated with the upregulation of vimentin, S-100, and α-smooth muscle actin in the testes of male Wistar rats. This suggests that melatonin can efficiently counteract the reproductive toxicity presented by bisphenol A, a finding supported by the report of [37].

Conclusions

We have reported that long-term exposure to low-dose BPA causes histological changes in the testes and downregulation of α-smooth muscle actin, S-100, and vimentin. The study has also demonstrated the ability of MLT to protect against BPA-induced testicular dysfunctions. Hence, MLT could be a therapeutic agent in preventing BPA-mediated male reproductive organ damage.

Authors Contributions

Conceptualization, O.S.A. S.G.O. and M.O.O; Methodology, O.S.A. S.G.O. and M.O.O; Software, O.S.A. and E.S.S.; Validation, O.S.A. S.G.O. M.O.O. and E.S.S.; Formal analysis, O.S.A. and E.S.S.; Investigation, O.S.A. S.G.O. and M.O.O.; Resources, O.S.A. S.G.O. and M.O.O.; Data curation, O.S.A.; Writing-original draft preparation, O.S.A. and E.S.S.; Writing-review and editing, O.S.A. S.G.O. M.O.O. and E.S.S.; Visualization, O.S.A. S.G.O. M.O.O. and E.S.S.; Supervision, S.G.O. and M.O.O.

Conflict of Interest

Authors declared none.

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