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Melatonin averts BPA-induced perturbations of the epididymis of Wistar rats

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Abstract

The protective effect of Melatonin (MLT) against Bisphenol-A (BPA) induced perturbation of the epididymis of Wistar rats was studied. Forty adult male rats assigned into four groups A-D (n=10) were used for the study. Group A (Control rats) received 0.2 ml of olive oil orally. Group B (MLT-treated rats): 10 mg/kg per day body weight MLT administered intra-peritoneally. Group C (BPA-treated rats): oral 10 mg/kg per day body weight BPA suspended in 0.2 ml olive and Group D (BPA+MLT-treated rats): All treatments lasted for 45 days. The results showed a significant reduction in epididymal weight, decreased sperm motility, livability and counts, decrease in catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase activities while hydrogen peroxide generation and lipid peroxidation were significantly increased compared with the control. Exposed rats also showed induced necrosis and epithelial sloughing as well as decreases in the expression of alpha Smooth Muscle Actin (αSMA), Vimectin (Vm) and S-100 proteins in the epididymidis. However, co-administration of MLT alongside BPA averted BPA-induced alterations in biochemical markers of oxidative stress, semen quality, protein expression and tissue architecture. Hence, MLT averts BPA-induced perturbations of the epididymis in Wistar rats.

Keywords: Bisphenol A, epididymis, melatonin, oxidative stress, sperm.

Introduction

Bisphenol A (BPA), has been the focus of increasing environmental toxicological research due to its widespread presence in several consumer products and its adverse effects on reproduction (Erler and Novak, 2010; Dekant and Colnot, 2013; Ahbab *et al*., 2017). BPA is a major constituent of epoxy and polystyrene resin. It is used in the manufacture of polycarbonate plastic making it present in several consumer products including the interior coatings of food cans, milk containers, and baby formula bottles as well as in dental sealants (Welshons *et al*., 2006; Doerge *et al*., 2011). However, BPA possesses certain hormone-like properties and these have raised health concerns about its suitability in consumer products and food containers. Reproductive toxicities induced

by BPA have been associated with increasing cases of male infertility resulting from alterations in the process of spermatogenesis (Qiu *et al*., 2013), perturbations in the blood-testis barrier (Li *et al*., 2009) and androgen/estrogen receptors (Matthews *et al*., 2001). BPA, like other environmental contaminants, have been shown to induce reactive oxygen species (ROS) generation in cells, resulting in cell death and tissue injury (Ho *et al*., 1998). There are plethoras of evidence that environmental contaminants alter the antioxidant system in the testis (Sujatha *et al*., 2001; Latchoumycandane *et al*., 2002) and epididymis of rats (Chitra *et al*., 2001, 2002; Latchoumycandane *et al*., 2002).

Melatonin (N-acetyl-5-methoxytryptamine, MLT), a neuro-hormone derived from tryptophan and mainly released from the pineal gland, possesses antioxidant and prophylactic properties against oxidative stress (Reiter *et al*., 2000). The antioxidant properties of MLT have been demonstrated in several experimental and clinical conditions with a large safety margin (El-Missiry, 2000; Reiter *et al*., 2004). Studies 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). Anjum *et al*. (2011) demonstrated the ameliorative role of MLT in BPA-induced biochemical toxicity in testicular mitochondria of the mouse. There is a paucity of research information on the preventive role of MLT on BPA-induced toxicity of epididymal spermatozoa. This study investigated the effects of the co-administration of Melatonin in BPA-induced perturbations of the epididymis of Wistar rats

Materials and Methods

Chemicals

Bisphenol-A (BPA) and Melatonin (MLT) were purchased from Sigma-Aldrich Co. (St Louis, Missouri, USA). Every other reagent used in this study was of standard grades.

Experimental Animals

Forty (40) male albino rats weighing 160 ± 10 g were used in the study. The rats were obtained from the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The rats were kept in cages (60 × 60 × 50 cm) under controlled conditions of temperature (25 ± 2.0°C), relative humidity (50 ± 15%) and normal photoperiod (12 hours light and 12 hours dark). The rats were fed with a standard rat diet (commercial pellet) and water was provided *ad libitum*. The procedures used in the study were performed following standard practices and were approved (Ref: UI-ACUREC /17/0069) by the Animal Care, Use and Research Ethical Committee, University of Ibadan, Nigeria.

Experimental Protocol

The rats were randomly assigned into four experimental groups of ten animals each as follows:

Group A (Control rats): 0.2 ml of olive oil orally administered. Group B (MLT-treated rats): 10 mg/kg per day of bodyweight MLT

(dissolved in 0.5% ethanol in normal saline) administered intra-peritoneally for 45 days (Anjum *et al.,* 2011). Group C (BPA-treated rats): 10 mg/kg per day bodyweight BPA suspended in 0.2 ml olive oil, orally administered for 45 days (El-Beshbishy *et al*., 2012). Group D (BPA+MLT-treated rats): Orally administered BPA (10 mg/kg per day of bodyweight) with a concomitant MLT, 10 mg/kg of bodyweight intra-peritoneally administered (Anjum *et al*., 2011; El-Beshbishy *et al*., 2012).

Necropsy, organ and body weight of rats

The rats were fasted overnight before sacrifice, weighed, anaesthesized using an overdose of ether and slaughtered 24 hours after the last treatment (Chitra *et al*., 2003). After taking the body weight of each rat, the right and left epididymides were harvested, and a semen sample was collected thereafter from the tail of the left epididymis through an incision made with a scalpel blade while the right epididymis was stored at -20° C till the time of biochemical assays.

Epididymal sperm motility, liveability and count

As reported by Latchoumycandane *et al*., (2002); sperm motility was estimated by aspirating fluid from the cauda epididymides. The aspirate was diluted to 2 ml of Ham's F-12 medium at 32° C. Using the Neubauer-type hemocytometer (Deep 1/10 mm, LABART, Germany), motile and non-motile sperm were counted and sperm motility was expressed as a percentage of motile sperm of the total sperm counted (Chitra *et al*., 2003). For sperm liveability assessment, a drop of semen sample was placed on a warm glass slide and stained with Eosin-Nigrosin stain after which a thin smear was then made, air-dried and observed under the light microscope (Oyeyemi *et al.,* 2011). Spermatozoa were counted by using the Neubauer-type hemocytometer (Deep 1/10 mm, LABART, Germany) chamber as described by Pant and Srivastava (2004).

Biochemical assays

The procedure for epididymal tissue homogenization was carried out as reported by El-Beshbishy *et al*., 2012. Cauda epididymides (1g/ sample) were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride. The epididymal homogenate was centrifuged at 4000 r/min for 10 min at 4 °C. The supernatant obtained was used as an enzyme source for biochemical assays. Estimation of catalase activity was by the method of Claiborne (1985) using H2O2 as substrate while the method of Marklund and Marklund (1974) was used in estimating Superoxide dismutase (SOD) activity. An assay for glutathione peroxidase (GPX) was carried out according to the method of Mohandas *et al*. (1984). Lipid peroxidation was measured by the method of Ohkawa *et al.* (1979). The assay for hydrogen peroxide (H2O2) generation was by the method of Pick and Keisari (1981) while glutathione peroxidase activity was estimated as described by Mohandas *et al.* (1984).

Histopathology and histomorphometry

Samples of the epididymis were fixed in buffered neutral formalin and embedded in paraffin blocks. Sections 2-4 µm thick were cut and stained with Haematoxylin and

Eosin, H&E (Olukole *et al.,* 2014). The slides were then viewed under a light microscope (Olympus BX63 with a DP72 camera) and sections were observed for lesions across the groups. Morphometric analyses of sections were performed with the aid of GIMP 2 Software using five serial sections of each of ten epididymal tissues stained with H&E, totalling 50 serial sections per parameter studied.

Immunohistochemistry

Immunohistochemistry for the localization of Smooth Muscle Actin (SMA), S-100 protein and Vimentin (Vm) was carried out as reported by Salian *et al*. (2009). Briefly, epididymal sections were placed on super frost slides, de-waxed in xylene and rehydrated through decreasing concentrations of alcohol. Following the initial blocking of endogenous activity using 3% hydrogen peroxide, antigen retrieval from slides was carried out by heating epididymal sections in 0.1M Citrate buffer (pH 6.0) in a microwave at 750 W for three cycles of 7 minutes each. Slides were then allowed to cool for 20 minutes after which they were washed with Phosphate-buffered solution (PBS, pH 7.2) thrice for 5 minutes each. Further permeabilization of slides was carried out in 0.1 M phosphate buffer containing 0.3% (v/v) Triton X-100 (Sigma, USA) for 10 minutes. Normal goat serum supplied with the Immunocruz mouse staining kit was used in blocking the slides for 1 hour before incubation with primary antibodies to SMA (1:200), S-100 (1:2000) and Vm (1:200), being incubated overnight at 4 °C in a humified chamber. For the negative control, the adjacent section on the same slide was incubated with preimmune sera. This was followed by washing of slides with PBS after which slides were incubated with biotinylated goat anti-mouse secondary antibody for 90 minutes. The slides were then washed with PBS thrice for 5 minutes each followed by incubation with a streptavidin-horseradish peroxidase complex (Immunocruz kit) for 30 minutes. Again, sections were then washed with PBS thrice for 5 minutes each and immunostaining was visualized following the addition of 0.05% (w/v) 3, 3, 9-diaminobenzidine (DAB) tetra-hydrochloride solution (Sigma, USA). Counterstaining of sections was carried out using Mayer's haematoxylin, mounted and visualized using a bright-field light microscope. Thereafter, images were captured using a light microscope (Olympus BX63 with a DP72 camera). Using 10 non-overlapping fields for each animal, the percentage positive contributions of each antibody in the epididymis was determined with the aid of the image analyser computer system, Lecia Qwin 500 C (Cambridge, UK) as reported by El-Ghamrawy et al. (2014).

Statistical analyses

Data were expressed as means and standard deviation (SD) while analyses were performed using One-way Analysis of Variance (ANOVA) with significance set at $P < 0.05$ using the GraphPad Prism, version 6.

Results

Effect of BPA and MLT on the body and epididymal weights of rats

There was no significant difference between the control and MLT groups in terms of epididymal and body weights (Table 1). However, treatment with BPA resulted in a significant decrease (P> 0.05) in body and epididymal weights in the rats (Table 1). Melatonin did not significantly affect the body weight of the BPA-treated rats whereas it caused a significant increase in epididymal weight in the BPA-treated group.

Effect of BPA and MLT on the sperm motility, livability and counts

Also, there was no significant difference between the control and MLT groups in terms of sperm motility and livability (Figures 1 and 2). However, sperm motility and livability differed significantly between BPA-treated rats and the control group. Sperm motility and livability differed also differed significantly between the BPA and BPA+MLT groups. There was no significant difference in sperm count between the control and MLT groups (Figure 3). Nevertheless, BPA resulted in a significant decrease in sperm count when compared to the control while MLT attenuated the decreased sperm count caused by BPA.

Effect of BPA and MLT on epididymal sperm oxidative stress markers

The results of the effect of MLT on BPA-induced oxidative stress markers are given in Figures 4-9. In BPA-treated rats, there were significant decreases ($p > 0.05$) in the activities of catalase, SOD, GSH and GPX compared to the control rats (Figures 4, 5, 6 and 9). However, BPA-MLT intake resulted in significant increases ($p > 0.05$) in the activities of catalase, SOD, GSH and GPX compared to the BPA-treated rats. Conversely, BPA-treated rats exhibited significant increases ($p > 0.05$) in LPO and H2O2 activities (figures 7 and 8). On concomitant treatment with MLT, these activities were significantly ($p > 0.05$) reduced. In comparison with the control group, MLT caused significant increases ($p > 0.05$) in the activities of GSH and H2O2 (Figures 6 and 8). In all the oxidative stress markers estimated, MLT demonstrated a significant ameliorative effect on BPA-induced epididymal sperm oxidative stress.

Effect of BPA and MLT on histopathology and histomorphometry of the epididymis

The epididymis of the control and MLT-treated rats showed intact epithelial cells, normal interstitium, normal duct membrane and intact lumen filled with spermatozoa. However, the BPA-treated rats revealed severe histopathological changes including the disintegration of the duct membrane, sloughing off of epithelial cells as well as spermatozoa within the lumen and moderate erosion of the epididymal interstitium (Figure 10). In terms of histomorphological parameters of the epididymis, BPA significantly reduced luminal diameter, ductal diameter and epithelial height when compared with the control rats (table 2). Though not significant, concomitant treatment with MLT increased the values for the luminal diameter, ductal diameter and

epithelial height compared to the BPA-treated group (Table 2).

Effect of BPA and MLT on Immunohistochemical localization of proteins

The percentage contributions of positive reactions to SMA, S-100 and Vm in the epididymis of rats in the present study are given in Table 3. BPA caused a collapse in the localization of SMA, S-100 and Vm in the caudal epididymis (figures 11-13). Concomitant treatment with MLT improved the percentage contributions of positive reactions to SMA, S-100 and Vm in the cauda epididymis of the rats.

Table I. Protective effect of MLT on BPA-induced epididymal toxicity in rats

Parameters	Control	MLT	BPA	BPA+MLT
Body weight of animals (g)	157.17±5.87	156.67±3.71	147.33±3.19	154.00±2.25
Epididymal weight (g)	0.59±0.04[a]	0.55±0.03[a]	0.38 ± 0.03[b]	0.41±0.06[a]

Values expressed as Means ± Standard Deviation (SD)
Means with different superscripts within row are significantly different (P<0.05).

Table II. Effect of MLT on BPA-induced changes in epididymal morphometry in rats

Parameters	Luminal diameter	Tubular diameter	Epithelial height
Control	295.34±11.62[a]	382.31±27.37	28.48±2.67[a]
MLT	285.14±17.53[a]	394.74±17.56	24.15±3.17[a]
BPA	255.72 ±14.61[b]	365.72±22.35	21.43±3.21[b]
BPA + MLT	260.13 ±11.73[a]	370.19±17.23	25.93±4.34[a]

Values expressed as Means ± Standard Deviation (SD)
Means with different superscripts within row are significantly different (P<0.05).

Table III. Percentage contribution of positive reactions to proteins in the epididymis of rats

Proteins	CONTROL	MLT	BPA	BPA+MLT
SMA (%)	9.14±0.65[a]	8.32±0.61[a]	3.74±0.37[b]	5.72±2.25[a]
S-100 (%)	5.30±0.52[a]	5.83±0.61[a]	1.93±0.02[b]	4.36±0.25[a]
Vm (%)	8.87±1.47[a]	11.26±1.15[a]	2.28±1.12[b]	6.81±0.25[a]

Values expressed as Means ± Standard Deviation (SD)
Means with different superscripts within row are significantly different (P<0.05).

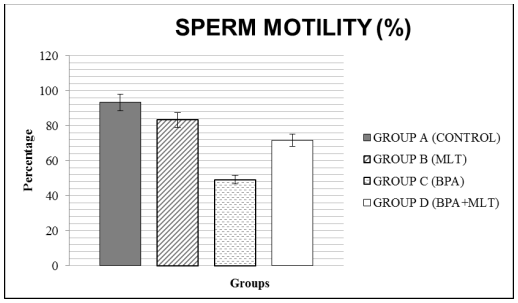

Figure 1. Protective effect of MLT on BPA-induced reduced sperm motility in rats

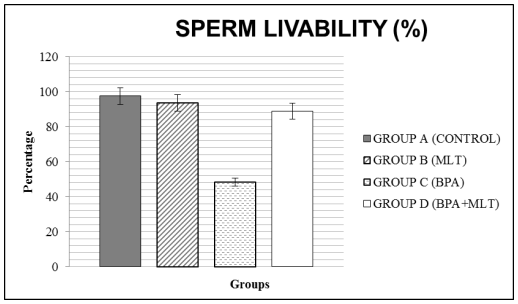

Figure 2. Protective effect of MLT on BPA-induced reduced sperm livability in rats

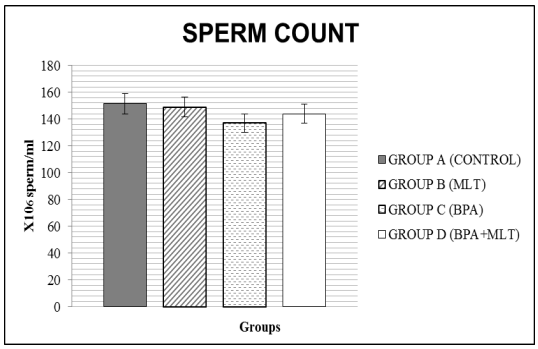

Figure 3. Protective effect of MLT on BPA-induced reduced sperm count in rats

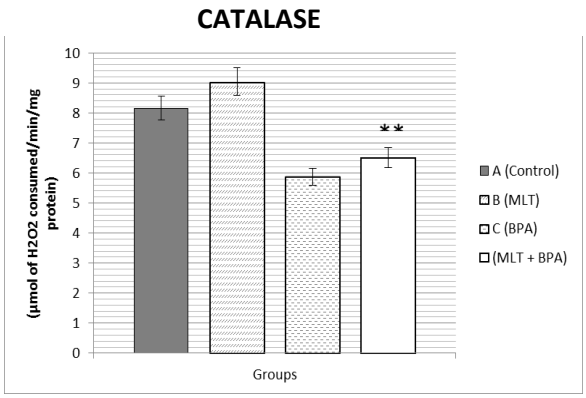

Figure 4. Protective effect of MLT on BPA-induced catalase activity in rats

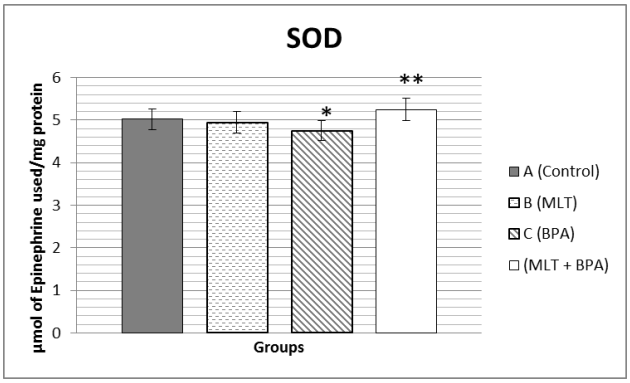

Figure 5. Protective effect of MLT on BPA-induced SOD activity in rats

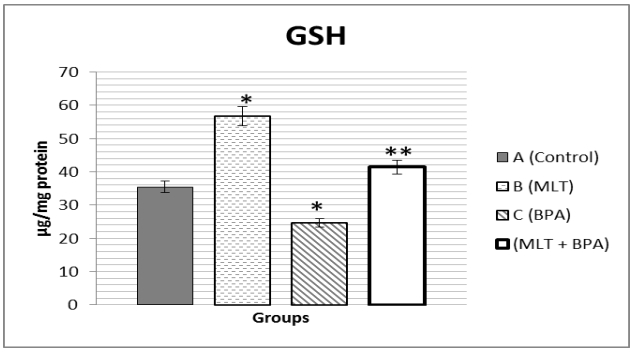

Figure 6. Protective effect of MLT on BPA-induced GSH content in cauda epididymis of rats

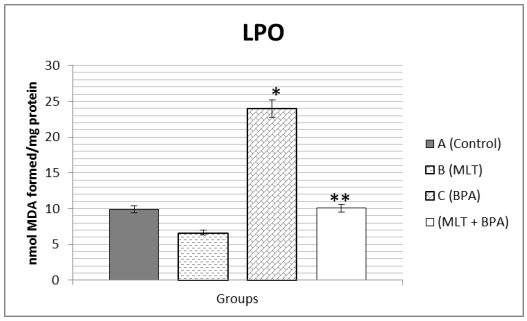

Figure 7. Protective effect of MLT on BPA-induced LPO content in cauda epididymis of rats

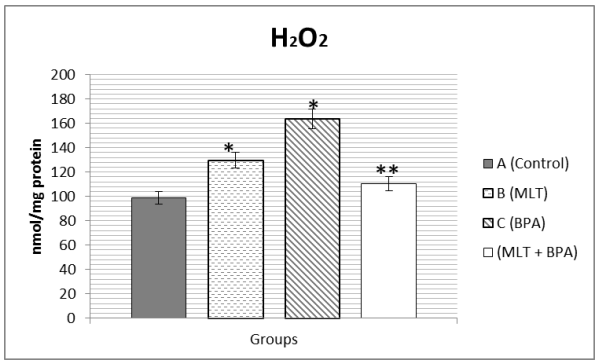

Figure 8. Protective effect of MLT on BPA-induced H_2O_2 activity in cauda epididymis of rats

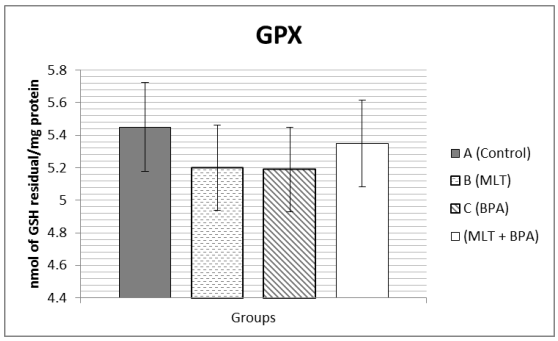

Figure 9. Protective effect of MLT on BPA-induced GPX activity in cauda epididymis of rats

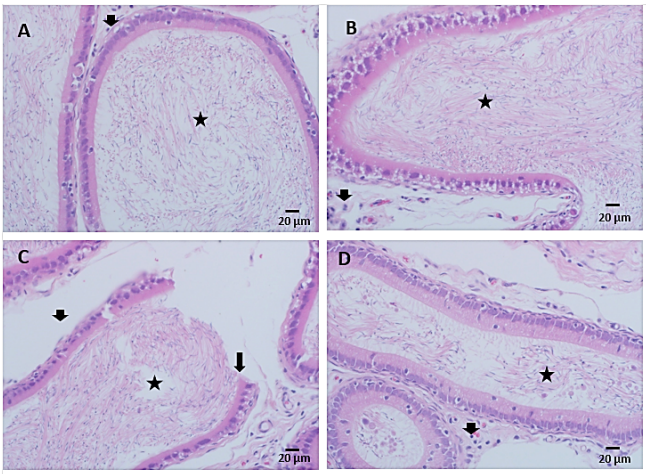

Figure 10. Cross section of the cauda epididymis (H&E). A. Control group showing epididymal lumen filled with spermatozoa (star) with normal interstitium (arrow head). B. MLT treated group showing the lumen of epididymis with more compactly packed spermatozoa (star) and normal interstitium (arrow head). C. BPA treated group showing eroded interstitium (arrow head), disintegration of the duct membrane of epididymis (arrow) as well as sloughing off of spermatozoa (star) within the lumen of the epididymis. D. BPA + MLT group showing normal interstitium (arrow head) and spermatozoa (star) within the lumen of the epididymis.

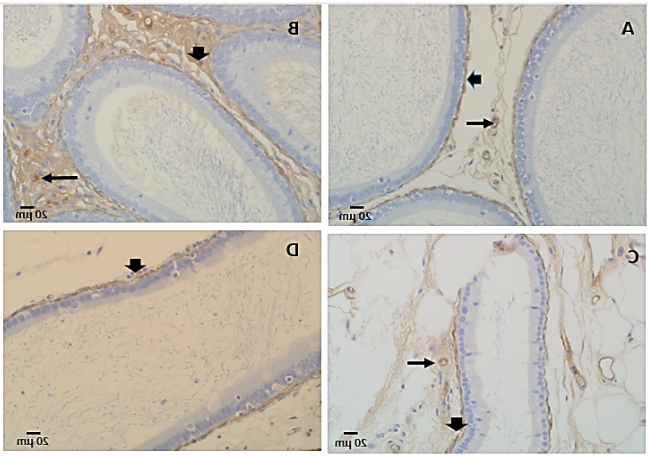

Figure 11. Immunohistochemical localization of SMA in the cauda epididymis. A. Control groupshowing SMA-positive epididymal duct membrane (arrow head) and endothelium (arrow). B. MLT treated group showing SMA-positive interstitium (arrow head) and endothelium (arrow) C. BPA treated group showing weakly SMA-positive epididymal duct (arrow head) and endothelium (arrow) D. BPA + MLT group showing SMA-positive epididymal duct membrane (arrow head).

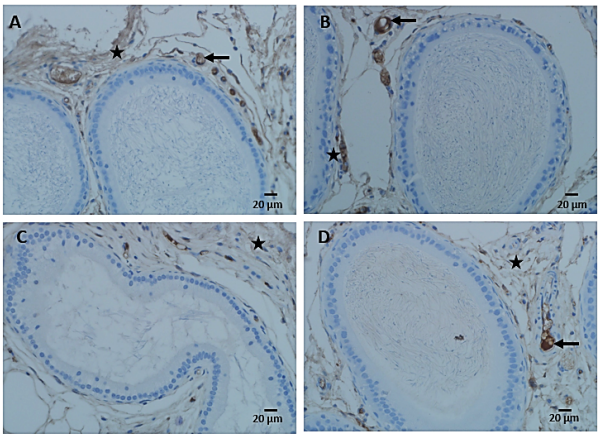

Figure 12. Immunohistochemical localization of S-100 protein in the cauda epididymis. A. Control group showing S-100-positive interstitium (star) and endothelium (arrow). B. MLT treated group showing S-100-positive interstitium (star) and endothelium (arrow) C. BPA treated group showing weakly S-100-positive interstitium (star). D. BPA + MLT group showing S-100-positive interstitium (star) and endothelium.

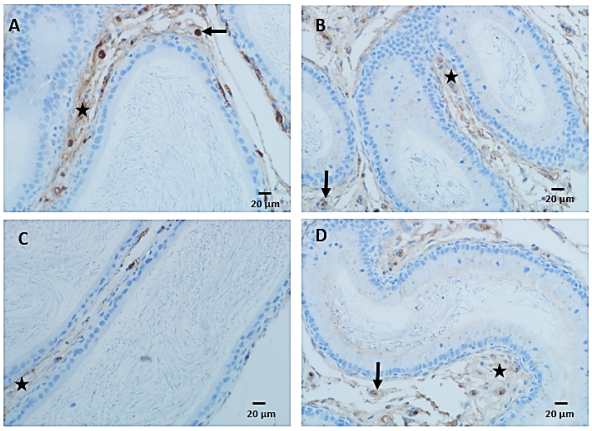

Figure 13. Immunohistochemical localization of Vm in the cauda epididymis. A. Control group showing Vm-positive interstitium (star) and endothelium (arrow). B. MLT treated group showing Vm-positive interstitium (star) and endothelium (arrow) C. BPA treated group showing weakly Vm-positive interstitium (star). D. BPA + MLT group showing Vm-positive interstitium (star) and endothelium.

Discussion

In the present study, the absence of significant differences in the body weight of the rats in the control and treatment groups suggests that BPA, as well as MLT, did not change the metabolic activities of the rats. This conforms to the report of Chitra *et al*. (2003). However, the BPA-induced reduction in epididymal weight observed in the present study is indicative of BPA toxicity in the epididymis. Previous authors have demonstrated a significant decrease in testicular and epididymal weights in response to BPA toxicity even without any significant increase in body weight (Thiele *et al*., 1995; Takahashi and Oishi, 2001; Chitra *et al.,* 2003; Yang *et al*., 2010). BPA-induced decrease in the weight of male reproductive organs has been linked to the ability of BPA to inhibit spermatogenesis, decrease elongated spermatids and alter steroidogenic enzyme activities (Takahashi and Oishi, 2001).

Recent research on the effect of BPA on the fertility of male rats has consistently shown that it impaired male fertility causing severe testicular dysfunction, including induction of apoptosis of testicular germ cells, disruption of the junctional proteins of the blood-testis barrier, alterations in the levels of androgen binding protein and steroidogenic enzymes (Peretz *et al*., 2014; Wang *et al*., 2015; Quan *et al*., 2016; Durando *et al*., 2016; Tian *et al*., 2017). Conversely, the significant increase in epididymal weight in the BPA+MLT group compared to the BPA group suggests the

ameliorative effect of MLT on BPA-induced male reproductive toxicity.

The BPA-induced significant decrease in epididymal sperm motility, livability and count observed in the present study shows that oral administration of BPA (10 mg/kg per day body weight) in adult rats for 45 days can result in male reproductive perturbations. Thiele et al. (1995) have suggested that BPA-induced decrease in epididymal sperm motility, livability and count are a result of increased lipid peroxidation. Our present finding of increased lipid peroxidation after BPA intoxication in rats confirms this. In the present study, MLT appears to have ameliorated BPA-induced decrease in epididymal sperm motility, livability and count, showing its ability to prevent BPA toxicity. This claim is supported by previous authors that the prevention of BPA-induced toxicity has been mediated through the use of several antioxidants (Anjum *et al.,* 2011; Jain *et a*l., 2011).

The significant decreases in the activities of catalase, SOD, GSH and GPX as well as the increase in LPO and H2O2 content in the epididymis due to BPA intoxication observed in the present study are inconsonant with the findings of Chitra *et al. (*2003) as well as those of Anjum *et al*. (2011). The ability of MLT to ameliorate these observed changes in the oxidative stress markers of epididymal sperm of adult rats is a clear indication of the antioxidant properties of MLT. In the present study, BPA induced an increase in LPO and H2O2 content in epididymal sperm. Authors have reported that increased levels of H2O2 mediate toxic effects through the formation of hydroxyl radical a potent activator of lipid peroxidation (Saradha and Mathur, 2006; Oyagbemi *et al.,* 2010). It has been demonstrated that a marked reduction in the activity of catalase reflects the inability of epididymal sperm to eliminate H2O2 generated due to BPA intoxication as glutathione peroxidase has been shown to directly act as antioxidant enzymes resulting in the inhibition of sperm lipid peroxidation (Lenzi *et al.,* 1994; Sikka, 2001; Chitra *et al*., 2003).

The results of the histopathological assessment of the epididymis showed that BPA perturbs the epididymis significantly and explains the observations on biochemical assays. The injury inflicted upon the epididymis by BPA in the present study confirms the findings of previous authors on the effect of BPA on male reproductive organs (Molina-Molina *et al*., 2013; Ullah *et al*., 2016; Feng *et al*., 2016). The sloughing off of epithelial cells of the cauda epididymis observed in the study is capable of affecting the secretory role of the epididymal epithelium. The mammalian epididymis has been demonstrated to induce biochemical changes in gametes by its catalytic activity of acid hydrolases secreted by the epididymal epithelium (Tulsiani et al., 1998; Dacheux *et al.,* 2005; Tulsiani & Abou-Haila, 2011; Ullah *et al.,* 2016). Authors have opined that the secretory activity of cauda epididymis is vital to sperm maturation (Dacheux *et al.,*2005; Tulsiani & Abou-Haila, 2011; Carvelli *et al*., 2014). Epididymal epithelial cells monitor the secretory and endocytotic activities of the epididymis and thus ensure

that an adequate amount of protein as well as water and ions required for sperm maturation are made available (Turner, 2002).

The observed BPA-induced injuries to the epididymis have great potential to affect the storage of spermatozoa in the cauda epididymis of the rats. The cauda epididymis not only stores the sperm but also makes available the enabling environment for holding sperm in a quiescent state of metabolism. The exact mechanisms, which maintain potentially motile mature sperm in a quiescent state, are not completely understood (Setchell *et al*., 1993). The BPA-induced decreased localization of SMA and Vm in the cauda epididymis observed in the study implies that the sperm maturation function of the cauda epididymis will be affected. For example, the BPA-induced collapse of SMA and Vm observed in the rats is capable of reducing the contractile activity of the epididymis and in turn negatively affecting sperm transport, a major function of the epididymis.

In the present study, BPA induced a decrease in the expression of S-100 protein. The S-100 protein is a multifunctional subfamily of Ca^{2+}-binding proteins that have been demonstrated in some metabolic functions including motility, chemotaxis, and secretion (Heizmann et al., 2002). Put together, the decreased expression of SMA, S-100 and Vm by BPA in the cauda epididymis of Wistar rats is capable of affecting the regulation of cell morphology, the dynamics of certain cytoskeleton constituents, and the reciprocal relationships of cytoskeleton elements via direct or indirect interactions with microtubules, intermediate filaments, microfilaments, myosin, and tropomyosin (Donato, 2001; Abd-Elmaksoud *et al.,* 2014). However, the upward regulation of these proteins by concomitant treatment with MLT shows its protective role against BPA-induced epididymal protein downward regulation.

Conclusions

Our results showed that MLT is a potent antioxidant, having prevented BPA-induced epididymal sperm oxidative stress, histopathological perturbations as well as protein downward regulation. Hence, MLT is recommended in the prevention of male reproductive toxicities due to endocrine disruptive agents.

References

Abd-Elmaksoud, A., Shoeib, M.B., Marei, H.E.S.: Localization of S-100 proteins in the testis and epididymis of poultry and rabbits. *Anatomy & Cell Biology*. 47, 180-187 (2014).

Ahbab, M.A., Barlas, N., Karabulut, G.: The toxicological effects of bisphenol A and octylphenol on the reproductive system of prepubertal male rats. *Toxicology and Industrial Health*, 33(2):133–146. (2017).

Anjum, S., Rahman, S., Kaur M., et al.: Melatonin ameliorates bisphenol A-induced biochemical toxicity in testicular mitochondria of mouse. *Food and Chemical Toxicology* 49: 2849–2854. (2011).

Carvelli, L.N., Bannoud1, A.C., Aguilera, T., et al.: Testosterone influences the

expression and distribution of the cation-dependent mannose-6-phosphate receptor in rat epididymis. Implications in the distribution of enzymes. *Andrologia*, 46, 224–230 (2014).

Chitra, K.C., Sujatha, R., Latchoumycandane, C., Mathur, P.P.: Effect of lindane on antioxidant enzymes in epididymis and epididymal sperm of adult rats. *Asian Journal of. andrology* 3, 205-208 (2001).

Chitra, K.C., Latchoumycandane, C., Mathur, P.P.: Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. *Archive of Toxicology*. 76, 545-551 (2002)

Chitra, K.C., Latchoumycandane, C., Mathur, P.P.: Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology*. 2003; 185(1-2): 119-27. PMID: 12505450 (2003).

Claiborne, A.: Catalase activity. In: Greenwald, R. (Ed.), *CRC Handbook of Methods For Oxygen Radical Research*. CRC Press, Boca Raton, Florida, pp. 283_/284 (1985).

Dacheux, J.L., Castella, S., et al.: Epididymal cell secretory activities and the role of proteins in boar sperm maturation. Theriogenology 63:319–341. *Erratum in: Theriogenology* (2005) 64, 1244 (2005)

Dekant, W., Colnot, T.: Endocrine effects of chemicals: aspects of hazard identification and human health risk assessment. *Toxicology Letters* 223: 280–286 (2013).

Doerge, D.R., Twaddle, N.C., Vanlandingham, M., et al.: Distribution of bisphenol A into tissues of adult, 1546 Toxicology and Industrial Health 32(9) neonatal, and fetal Sprague-Dawley rats. *Toxicology and Applied Pharmacology* 255: 261–270 (2011).

Donato, R.: S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *The International Journal of Biochemistry & Cell Biology*. 33, 637-68 (2001).

Durando, M., Canesini, G., Cocito, L.L., et al.: Histomorphological changes in testes of broad-snouted caimans (*Caiman latirostris*) associated with in ovo exposure to endocrine-disrupting chemicals. *Journal of Experimental Zoology*. 325A:84–96 (2016).

El-Beshbishy, H., Ali, H.A.A., El-Shafey, M .: Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. *Toxicology and Industrial Health* 29(10) 875–887 (2012).

El-Ghamrawy, T.A., Helmy, D., Elall, H.F.A.: Cadherin and vimentin immunoexpression in the testis of normal and induced infertility models of albino rats. *Folia Morphologica*. Vol. 73, No. 3, pp. 339–346 (2014).

El-Missiry, M.A., A.b.d., El-Aziz, A.F.: Influence of melatonin on proliferation and antioxidant system in Ehrlich ascites carcinoma cells. *Cancer Letter* 151: 119–125 (2000).

Erler, C., Novak, J.: Bisphenol a exposure: human risk and health policy. *Journal of Pediatric Nursing* 25: 400–407 (2010).

Feng, Y., Jiao, Z., Shi, J., et al.: Effect of bisphenol analogues on steroidogenic gene expression and hormone synthesis in H295R cells. *Chemosphere* 147, 9-19 (2016).

Heizmann, C.W., Fritz, G., Schafer, B.W.: S100 proteins: structure, functions and pathology. *Frontiers in Bioscience*. 7, 1356-68. (2002).

Ho, Y.S., Magnenat, J.L., et al.: The nature of antioxidant defense mechanisms: a lesson from transgenic studies. *Environmental Health Perspectives.* 106, 1219_/ 1228 (1998).

Jain, S., Mahendra, Kumar, C.H., et al.: Protective effect of N-acetylcysteine on bisphenol A-induced cognitive dysfunction and oxidative stress in rats.

Food and Chemical Toxicology 49: 1404–1409 (2011).

Kalkanli, S., Kalkanli, N., et al.: Effects of formaldehyde and vimentin expression in rat skin. *Int. Journal of Morphology, 31(3)*:845-848, 2013 (2013).

Kato, H., Furuhashi, T., Tanaka, M., et al.: Effects of Bisphenol A given neonatally on reproductive functions of male rats. *Reprodive Toxicology*. 22, 20–29 (2006).

Latchoumycandane, C., Chitra, K.C., Mathur, P.P.: The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the antioxidant system in mitochondrial and microsomal fractions of rat testis. *Toxicology* 171, 127_/135 (2002).

Marklund, S., Marklund, G.: Involvement of superoxide anion radical in antioxidation of pyrogallol and a constituent assay for superoxide dismutase. *European Journal of Biochemistry*. 47, 469_/474 (1974).

Mohandas, J., Marshall, J.J., et al.: Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. *Cancer Research*. 44, 5086_/5091 (1984).

Molina-Molina, J.M., Amaya, E., et al.: In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. *Toxicology and Applied Pharmacology*. 272, 127-136 (2013).

Ohkawa, H., Ohishi, N., Yagi, K.: Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. 95, 351-358 (1979).

Olukole, S.G., Oyeyemi, M.O., Oke, B.O.: Biometrical and histometrical observations on the testis and epididymis of the African sideneck turtle (*Pelusios castaneus*). *European Journal of Anat*omy, 18 (2): 102-108 (2014).

Oyagbemi, A.A., Adedara, I.A., et al.: Role of Oxidative Stress in Reproductive Toxicity Induced by Co-administration of Chloramphenicol and Multivitamin–Haematinics Complex in Rats. *Basic & Clinical Pharmacology & Toxicology*, 107, 703–708 (2010).

Oyeyemi, M.O., Olukole, S.G., et al.: Semen characteristics and sperm morphological studies of the West African Dwarf Buck treated with Aloe vera gel extract. *Iran Journal of Reproductive Medicine,* 9 (2) 83-88 (2011).

Pick, E., Keisari, Y.: Superoxide anion and H2O2 production by chemically elicited peritoneal macrophagesinduced by multiple nonphagocytic stimuli. *Cell Immunology*. 59, 301-318 (1981).

Peretz, J., Vrooman, L., et al.: Bisphenol A and reproductive health: update of experimental and human evidence, 2007–2013. *Environmental Health Perspectives,* 122:775–786; http:// dx.doi.org/10.1289/ehp.1307728 (2014).

Reiter, R.J., Tan, D.X., Gitto, E., et al.: Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. *Polish Journal of Pharmacology* 56: 159–170 (2000).

Reiter, R.J., D.X., Tan, M.P., et al.: "Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions," *Acta Biochimica Polonica*, 54 (1): 1–9, (2007).

Salian, S., Doshi., T., Vanage, G.: Neonatal exposure of male rats to bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. *Toxicology* 265:56–67 (2009).

Saradha, B., Mathur, P.P.: Induction of oxidative stress by lindane in epididymis of adult male Rats. *Environmental Toxicology and Pharmacology,* 22: 90–6 (2006).

Setchell, B.P., Sanchez-Partida, L.G., Chairussyuhur, A.: Epididymal constituents and related substances in the storage of spermatozoa: A review. *Reproduction, Fertility and Development*, 5 601-612 (1993**).**

Sikka, S.C.: Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*. 8, 851-862 (2001).

Sujatha, R., Chitra, K.C., et al.: Effect of lindane on testicular antioxidant system and steroidogenic enzymes in adult rats. *Asian Journal of Andrology*. 3, 135-138. (2001)

Takahashi, O., Oishi, S.:Testicular toxicity of dietary 2,2- bis(4 hydroxyphenyl) propane (bisphenol A) in F344 rats. *Archives of Toxicology*75, 42-51(2001).

Thiele, J.J., Freisleben, H.J., et al.: Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed semen. *Human Reproduction.* 10, 110-115 (1995).

Tian, J., Ding, Y., She, R., Ma, L., Du, F., Xia, K., Chen, L.: Histologic study of testis injury after bisphenol A exposure in mice: Direct evidence for impairment of the genital system by endocrine disruptors. *Toxicology and Industrial Health* 33 (1): 36–45 (2017).

Toyama, Y., Yuasa, S.: Effects of neonatal administration of 17˘-estradiol, ˘-estradiol 3-benzoate or Bisphenol A on mouse and rat spermatogenesis. *Reproductive. Toxicology*. 19, 181–188 (2004).

Tulsiani, D.R., Abou-Haila, A.: Molecular events that regulate mammalian fertilization. *Minerva Ginecol* 63:103–118 (2011).

Tulsiani, D.R., Orgebin-Crist, M.C., Skudlarek, M.D.: Role of luminal fluid glycosyltransferases and glycosidases in the modification of rat sperm plasma membrane glycoproteins during epididymal maturation. *Journal of reproduction and fertility. Supplement* 53: 85–97. (1998).

Turner, T.T.: Necessity's potion: Inorganic ions and small organic molecules in the epididymal lumen. In: Robaire, B.; Hinton, BT., editors. The epididymis*:* From molecules to clinical practice. *Kluwer Academic/Plenum Publishers; New York,* NY: 2002. p. 131-150 (2002).

Ullah, H., Jahan, S., et al.: Effect of bisphenol S exposure on male reproductive system of rats: A histological and biochemical study. *Chemosphere* 152: 383-391(2016).

Welshons, W.V., Nagel, S.C., Vom, Saal, F.S.: Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology*. 2006; 147: S56–69 (2006).

Wang, C, M., Fu, W.J., et al.: The role of Pten/Akt signaling pathway involved in BPA-induced apoptosis of rat Sertoli cells. *Environmental Toxicology* 30: 793–802 (2015).

Yang, Y.J., Le, S.Y., et al.: Acute testis toxicity of bisphenol A diglycidyl ether in Sprague-Dawley rats. *Journal of Preventive Medicine and Public Health* 43(2): 131–137 (2010).