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DOES ORAL EXPOSURE TO LOW DOSES OF BISPHENOL A IMPAIR THE MALE REPRODUCTIVE FUNCTION IN WISTAR RATS?

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ABSTRACT

The aim of this study was to evaluate the effects of Bisphenol A (BPA) exposure at different doses on male reproductive function in Wistar rats. To this end, animals were randomly divided into 6 groups (n=6). Gr1: control that received 1mL of ethanol (0.1%); Gr2: exposed to 0.0004µg/kg/day of BPA; Gr3: exposed to 0.004µg/kg/day of BPA; Gr4: exposed to 0.04 µg/kg/day of BPA; Gr5: exposed to 0.4 µg/kg/day of BPA; Gr6: exposed to 4 µg /kg/day of BPA. BPA was given daily by gavage for 60consecutivedays. The obtained results showed a significant decrease in the body weight and weight gain in the group exposed to the lowest dose (0.0004µg), while no significant changes were found in the relative testicular weight of rats. A significant decrease in sperm count, motility and viability was also observed in all BPA-treated groups compared to controls. A significant decrease in Testosterone levels was noted in all BPA-exposed groups but not the group exposed to 4 µg. The histological analysis showed many morphological changes in the BPA-treated groups e.g detachment of germ cells from the basal lamina, degenerative changes in the germinal layer, decline in spermatozoa number in the lumen, apparition of cell debris in the lumen of tubules, and vacuolization of Sertoli cells. In conclusion, our results demonstrate clearly that BPA exposure at different low doses (0.0004, 0.004, 0.04, 0.4,4 µg/kg/day) for 60 days induce serious adverse effects on the male reproductive function in Wistar rats.

Keywords: BPA, Sperm, testis, Testosterone, histology.

INTRODUCTION

Spermatogenesis is a complex process that mainly depends on the action of pituitary and gonadic hormones. The integrity of the endocrine system is necessary for the good regulation of this process (Corradi et al. 2016). In the last decades, the increasing prevalence of male infertility and the decline in sperm quality were correlated to the rapid industrialization and the release of excess synthetic substances into the environment (Selvaraju et al. 2021). Indeed, many exogenous substances known as endocrine-disrupting chemicals (EDCs) can interfere with the endocrine system and disrupt hormonal action, which may result in male reproductive disorders including poor sperm quality (Usman and Ahmad, 2016).Among 800 chemicals known or suspected to be endocrine disruptors, Bisphenol A (BPA) [4,4-dihydroxy-2,2-diphenylpropane, CAS80-05-7] is a monomer highly used in the polycarbonate plastics and epoxy resins manufacturing process as a plasticizer (Wetherill et al. 2007; Usman and Ahmad, 2016). Since BPA can be rejected into the environment and contaminate food and water, it is closely monitored as a potential environmental pollutant (Di Donato et al. 2017). Due to its increased production and widespread applications, BPA is omnipresent among the general population, and more than 90% of them have detectable levels of BPA in urine (Lehmler et al. 2018). This substance may penetrate the human body through dietary routes. Indeed, several studies have estimated that almost 80-90% of BPA exposure occurs by consuming food and drinks contaminated with BPA from bottles and cans coated with polycarbonate (Siddique et al. 2021). BPA is a xenoestrogen that received special

attention from researchers due to its ability to bind to several types of receptors such as estrogen receptor a/b (ER a/b), androgen receptor (AR), and thyroid hormone receptor (Wetherill et al. 2007). For example, BPA has been shown to compete with 5-dihydrotestosterone (DHT) for binding to AR. These results suggest that BPA may affect several stages of AR activation and function, resulting in an antagonistic action of BPA on it (Wetherill et al. 2007) playing so a pathogenic role in some types of endocrine disorders such as male infertility (Kazemi et al. 2017).

In 2006, the European Food Safety Authority (EFSA) set a Tolerable Daily Intake (TDI) for BPA of 50 µg/kg bw/day in order to reduce the related health risks (EFSA, 2010). In 2015, EFSA established a temporary tolerable daily intake (t-TDI) of 4 µg/kg bw per day due to uncertainties on low-dose effects on the mammary gland, reproductive, neurological, immune, and/or metabolic systems (EFSA, 2015). In 2021, EFSA's expert Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) reduced the TDI to 0.04 ng/kg/bw per day for reasons related to the adverse effects of BPA on the immune but not on the reproductive system (EFSA, 2021). Furthermore, EFSA estimated that reproductive-aged women compared with men of the same age have BPA dietary exposures up to 0.388 µg/kg bw/day, adolescent exposure of up to 1.449 µg/kg bw/day, infants and toddlers up to 0.875µg/kg bw/day (EFSA ,2015). Accordingly, EFSA concluded that the new TDI is exceeded in all age groups, indicating health concerns (EFSA, 2021).

Taking into account these data, the present study was carried out to evaluate the effects of subchronic exposure to BPA at different low doses on male Wistar rats reproductive function. For this purpose, five doses of BPA were tested (0.0004, 0.004, 0.04, 0.4, 4 µg/kg/day) and a multiparametric evaluation including sperm analysis, body weight monitoring, hormonal and histological study was performed.

MATERIALS AND METHODS

Chemicals

BPA (97% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). This chemical is poorly soluble in water. In this study, BPA was first dissolved in absolute ethanol at a concentration of 1 mg/ml and was then diluted several times in distilled water to reach a final concentration of 1, 0.1, 0.01, 0.001, and 0.0001µg/mL. BPA was then delivered by gavage according to the weight of each rat.

Animals

This study was performed on thirty-six male Wistar rats (*Rattusnorvegicus*) aged of ten weeks and weighing 231.69 ± 18.56 g. The animals were purchased from Pasteur Institute in Algiers, Algeria. They were acclimatized to laboratory conditions for one week prior to the experiment. The animals were housed in appropriate cages under a 12 h/12 h light/dark cycle at a room temperature of 22-24 °C. They were fed rodent standard diets and tap water *ad libitum*.

Experimental design

Animals were weighed and randomly divided into 6 groups (n=6). Groupe1: control group that received 1mL of ethanol (0.1%); Groupe2: exposed to 0.0004µg/kg/day of BPA; Groupe3: exposed to 0.004µg/kg/day of BPA; Groupe4: exposed to 0.04 µg/kg/day of BPA; Groupe5:exposed to 0.4 µg/kg/day of BPA; Groupe6:exposed to 4µg/kg/day of BPA. BPA was given daily by oral gavage for 60 consecutive days.

Specimens collection and analytical methods

At the designated time, the animals were fasted overnight and anesthetised with chloroform. Blood was collected via cardiac puncture and the serum was separated by centrifugation at 3000 rpm for 15 minutes. Testosterone level was determined by enzyme immunoassay with fluorimetric detection, using AIA-360 Automated Immunoassay Analyser. The cauda of epididymis was placed into a petri dish with 2 mL of physiological saline solution (NaCl 0.9%), then dissected with fine scissors, and incubated at 35-37 °C for 15 min to deliver the sperm suspension (Wang, 2002). Motility, viability, and sperm concentration were evaluated according to the protocol described by Dussault (2009). The testicular histological study was performed according to the standard

histological technique. Briefly, the testes were removed, weighed, and then fixed in formalin 10%. They were then dissected and placed in the cassettes, dehydrated with different grades of alcohol, and cleared with Xylene. Afterward, the cassettes were impregnated with paraffin. Paraffin sections were cut using a microtome (5 µm), and stained with Hematoxylin and Eosin (H&E).The stained slides were examined by light microscopy to study the different histological structures of testis. In this study, each test was performed in triplicate.

Statistical Analysis

Data statistical analysis was performed using IBM SPSS. 22. Data were expressed as mean±(SD)for all parameters. The parameters were analyzed by the ANOVA test followed by the Tukey HSD (Honest Significance Difference) test to estimate significant differences between groups. A value of $P<0.05$ was considered significant for all tests.

RESULTS

Effect of BPA on body weight, weight gain and relative testicular weight

The results showed that of all the different doses of BPA in our study, only the dose of 0.0004 µg/kg/day resulted in a significant decrease in body weight ($p < 0.01$) and weight gain ($p < 0.05$) compared to controls (Table 1). Furthermore, no significant difference was observed in relative testicular weight between controls and all experiment groups exposed to BPA at different doses.

Table.1: **Effect of BPA on body weight, weight gain, and relative testicular weight of rats.**

Groups	Initial BW (g)	Final BW (g)	Weight gain (g)	Testes RW (%)
Control	228,66 ± 16,09	318,00 ± 6,13	89,33 ± 16,45	1,05 ± 0,07
BPA 0.0004µg	233,66 ± 24,32	293,50 ± 7,34**	59,83 ± 18,98*	0,95 ± 0,07
BPA 0.004µg	230,83 ± 19,42	318,83 ± 13,96	88,00 ± 14,44	0,95 ± 0,13
BPA 0.04µg	233,16 ± 22,63	332,00 ± 12,24	98,83 ± 19,91	1,01 ±0,06
BPA 0.4µg	228,00 ± 14,12	311,83 ± 11,37	83,83 ± 6,70	1,07 ± 0,08
BPA 4µg	235,83 ± 20,54	327,00 ± 13,44	91,16 ± 14,31	0,99 ± 0,07

*A comparison between groups was made using the Tukey test. Results are expressed as mean ± standard deviation; (n=6). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001).*

Effect of BPA on sperm parameters

As shown in Table 2, sperm count was significantly decreased in the group exposed at 4 µg/kg/day ($p<0.05$). This decrease was more pronounced ($p<0.001$) in animals treated with BPA at 0.0004, 0.004, 0.04, and 0.4 µg/kg/day. Moreover, BPA administration at various doses significantly decreased sperm motility when compared to control. Also, the viability of spermatozoa in animals exposed to BPA at 0.004, 0.04, and 4 µg/kg/day was significantly decreased ($P<0.001$), while BPA exposure at 0,0004 and 0,4 µg/kg/day did not induce any significant decrease in viability as compared with the controls.

Table.2: Effect of BPA on sperm parameters.

Groups	Sperm count (10^6/mL)	Motility (%)	Viability (%)
Control	370,33 ± 17,61	60,38 ± 3,07	60,71 ± 1,95
BPA 0.0004µg	194,66 ± 21,12***	45,03 ± 6,07**	57,31 ± 2,68
BPA 0.004µg	201,66 ± 23,18***	37,87 ± 3,93***	47,13 ± 3,99***
BPA 0.04µg	198,91 ± 13,13***	45,67 ± 2,93**	46,22 ± 0,73***
BPA 0.4µg	196 ± 21,28***	41,19 ± 1,79**	54,95 ± 1,24
BPA 4µg	313,83 ± 15,78*	34,98 ± 4,09***	42,07 ± 1,80***

*A comparison between groups was made using the Tukey test. Results are expressed as mean ± standard deviation; (n=6). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001).*

Effect of BPA on serum testosterone levels

Our data showed that exposure of male rats to BPA at doses of 0.0004, 0.004, 0.04, and 0.4 µg/kg/day significantly reduced testosterone levels (p<0.001). The minimum testosterone level was observed in the group treated with BPA at 0.0004 µg/kg/day (Fig.1). However, there was no significant difference in testosterone levels between controls and animals exposed to BPA at 4 µg/kg/day.

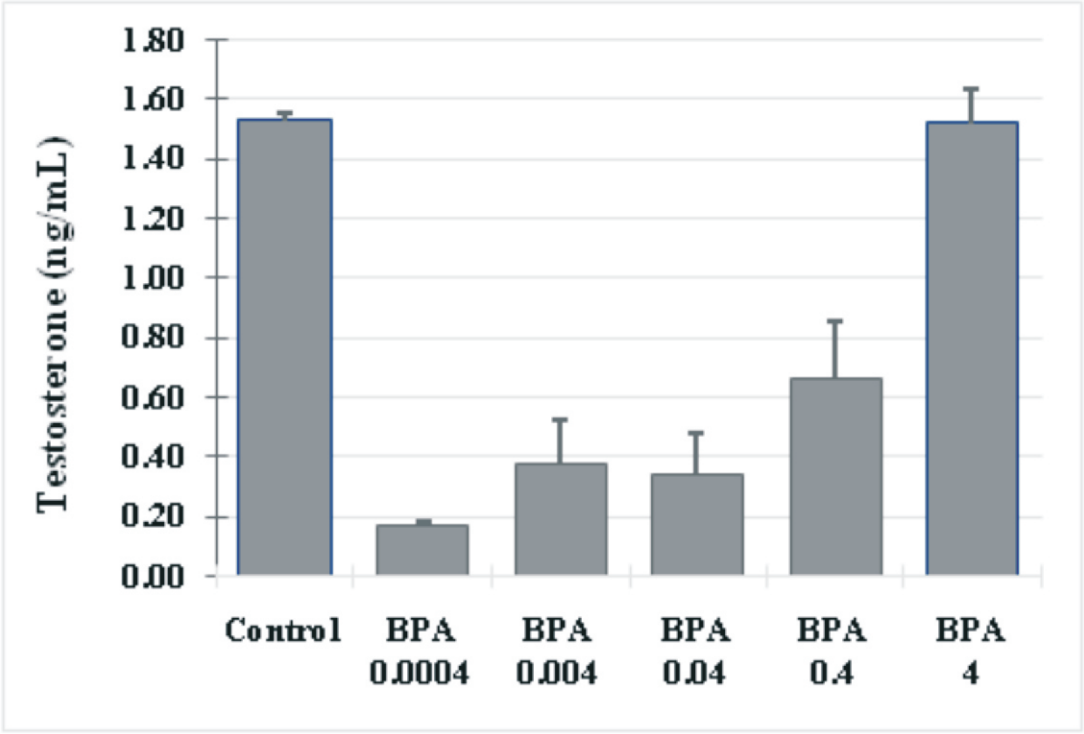

Figure.1: Evaluation of Testosterone levels in animals exposed to different doses of BPA.
*A comparison between groups was made using the Tukey test. Results are expressed as mean ± standard deviation; (n=6). * Significant difference (p<0.05), **very significant difference (p<0.01), ***highly significant difference (p<0.001).*

Effect of BPA on structural histology of testis

The light microscopic analysis of the testicular histology of all the study groups is illustrated in Figure 3. The testis of the control group shows normal morphology of seminiferous tubules and the presence of numerous Leydig cells in the interstitial space between the seminiferous. The germinal epithelium contains normal Sertoli cells and germ cells in different stages of development including spermatozoa present in the lumen of the tubules, which indicate a normal spermatogenesis process (Fig. 2A). However, morphological changes were found in the BPA-treated groups, especially the detachment of germ cells from the basal lamina (Fig. 2B,F), degenerative changes in the germinal layer (Fig. 2B,C), the decline in spermatozoa number in the lumen (Fig. 2B,C,D,E), apparition of cell debris in the lumen of the tubule (Fig. 2D,E), and vacuolization of Sertoli cells (Fig. 2D).

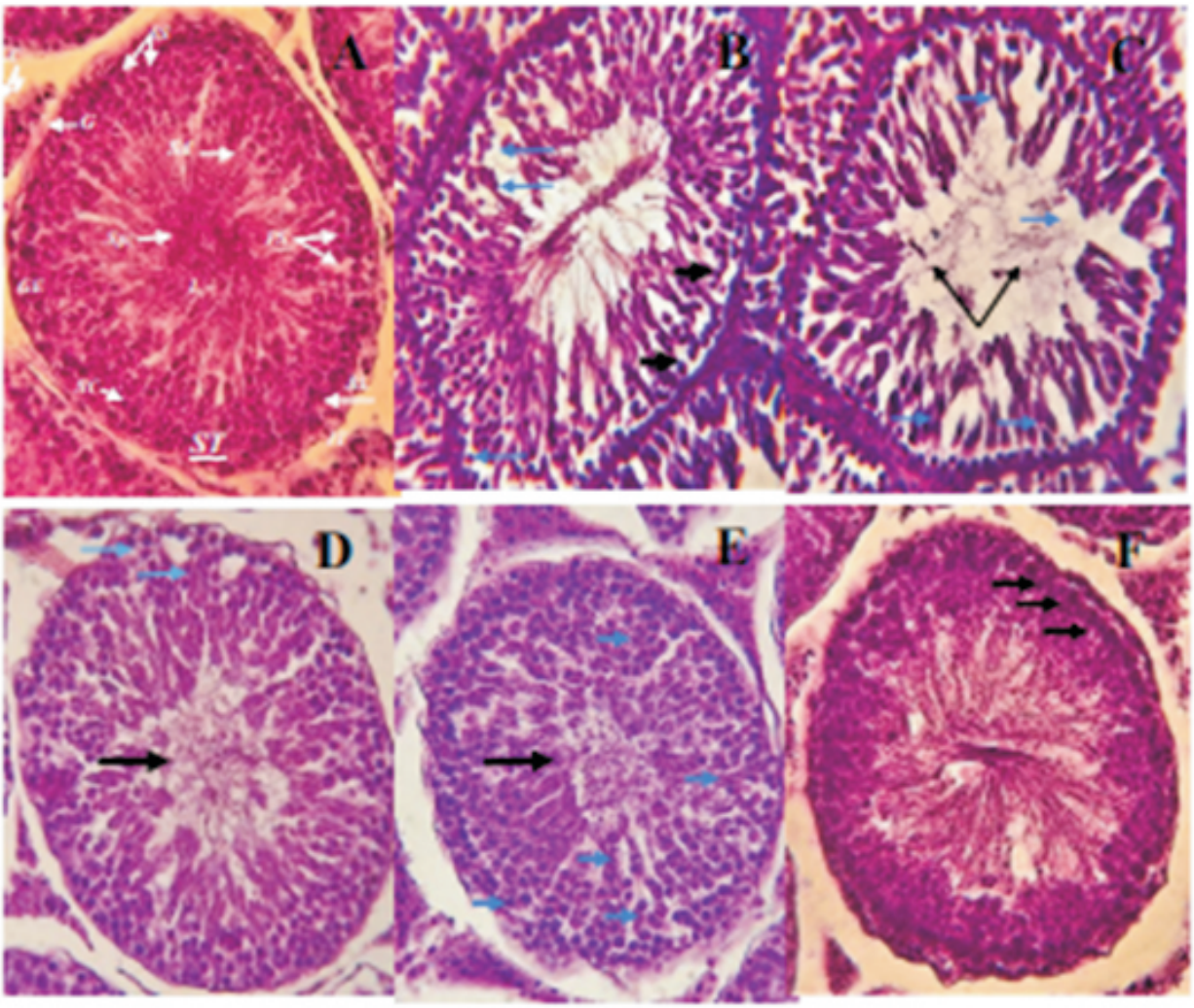

Figure.2: Microscopic observation of the Histological structure of rat testis in control and BPA-treated groups (H&E, Gr×40).

(**A**): control group. (**B**): 0.0004 µg BPA-group. (**C**): 0.004 µg BPA-group. (**D**): 0.04 µg BPA-group. (**E**): 0.4 µg BPA-group. (**F**): 4 µg BPA-group. (LC): Leydig cells. (IT): Interstitial tissue. (ST): Seminiferous tubule. (BL): Basal lamina. (SC): Sertoli cell. (GE): Germinal epithelium.(G): Spermatogonia. (PS): Primary spermatocytes. (SS): secondary spermatocytes. (Sd): Spermatid. (Spz): Spermatozoa. (L): Lumen of the seminiferous tubule. In (**B**): detachment of germ cells from the basal lamina (black arrows), irregular arrangement of germ cells (Blue arrows). In (**C**): germ cell loss in seminiferous tubules (Blue arrows), few spermatozoa in the lumen (black arrows). In (**D**): vacuolization of Sertoli cells (Blue arrows), cell debris in lumen of

tubule (black arrow). In (**E**): disturbed epithelium with separate germ cells are seen (Blue arrows), cell debris in lumen of tubule (black arrow).In (**F**): detachment of germ cells from the basal lamina (black arrows).

DISCUSSION

This present study focused on the effect of oral exposure to low doses of BPA on the reproductive function of male Wistar rats. Animals were treated with BPA at 0.0004, 0.004, 0.04, 0.4, and 4 µg/kg/day for two months. The results indicated a significant decrease in final body weight in the group treated with BPA at 0.0004 µg/kg/day. Similar results were found by Behmanesh et al. (2018) who reported a decrease in body weight in rats treated with BPA at 20 µg/kg/day for 8 weeks. This decrease in weight could be attributed to the decrease in testosterone levels that could reduce muscle mass and bone density (Isidori et al. 2005). Although, other study reported that exposure to BPA increased body weight (Ullah et al. 2018) or did not change it (Alboghobeish et al. 2019). In the present study, no significant difference in relative testicular weight was observed in all BPA treated groups. These findings are closely related to the results of Balci et al. (2020), while previous studies noted that BPA exposure lowered relative testicular weight (Park et al. 2018; Srivastava and Gupta, 2018).These variations could be explained by the difference in the exposure time, applied doses, administration routes, or animal strains used (Park et al. 2018).

Moreover, data obtained from this study indicate that exposure to BPA altered sperm characteristics in all BPA-treated groups. A decreases in sperm count, mobility, and viability was observed. These alterations are common with findings that were reported in study of Jiang et al. (2016). Furthermore, a study conducted on workers by Li et al. (2011) showed a highly significant linear correlation between increased urine BPA levels and reduced total sperm count, vitality and motility indicating the antispermatogenic effects of BPA. In this regard, it has been demonstrated that BPA disrupts spermatogenesis by several mechanisms; by increasing germ cells injury, inducing germs cell death by the mitochondrial apoptotic process (Qian et al. 2015), and activating the Fas/FasL pathway (Li et al. 2009). In addition, it has been shown that BPA inhibits spermiation process, disrupts meiosis, and supports the persistence of meiotic DNA strand ruptures in pachytene spermatocytes (Liu et al. 2013). On the other hand, motility is one of the principal characteristics of sperm maturation. Spermatozoa, as they leave the testis, are immature and immotile cells that require a maturation process during epididymal transit (Holt, 2018).This passage is mainly dependent on stimulation of the epididymis by testosterone and allows spermatozoa to acquire what is needed for their maturation (Cole, 2016). Wisniewski et al. (2015) reported that BPA caused faster epididymal transit, which could lead to insufficient maturation and sperm motility disorders.

Data of hormonal analysis performed in our study showed that BPA exposure decreased significantly testosterone levels. This result is consistent with a several studies on the reprotoxic effect of BPA at low doses (Srivastava and Gupta, 2018; Ullah et al. 2018). Testosterone is the main androgen secreted by Leydig cells in the testis under the influence of the Luteinizing Hormone (LH) (Tamilselvan et al. 2014). Testosterone is critical for spermatogenesis process because in a lack of its secretion, the spermatogenesis remains blocked in the prophase 1-leptotene stage of meiosis (Cole, 2016). These data contribute also to justify the decrease in the quantity and quality of sperm observed in this study. The reduction of the testosterone levels might be a result of a decrease in LH serum levels (Tamilselvan et al. 2014). Indeed, Akingbemi et al. (2004) reported a significant decrease in testosterone and LH levels in rats treated with BPA at 2.4µg/kg/day. Moreover, BPA has been found to inhibit expressions of the steroidogenic enzymes 3β-HSD and 17β-HSD, two key enzymes in testosterone biosynthesis (Jiang et al. 2016; Ahbab et al. 2017).

In this study, the testicular histology analysis showed histopathological abnormalities in the testis of all BPA-treated groups compared to controls. These histological changes are associated with diminution of spermatozoa number in the lumen, vacuolization of Sertoli cells, the apparition of cell debris in the lumen of the tubule, and germ cell loss indicating the

disruption of spermatogenesis. This results are consistent with the observations of Ahbab et al. (2017); Zahra et al. (2020). Moreover, Srivastava and Gupta. (2018) reported that exposure to BPA at low doses (5, 50,100 µg/100gmBW) caused degenerative changes in the germinal epithelium and increased apoptosis in germ cells. Furthermore, testosterone that constitute an indispensable factor for the control and maintenance of spermatogenesis could be the cause of these histopathological alterations when its levels is reduced. Other factors also seem to be involved such as oxidative stress and Sertoli cell dysfunction (Wang et al. 2016).

CONCLUSION

In conclusion, the present study demonstrates that BPA exposure at different doses (0.0004, 0.004, 0.04, 0.4 and 4 µg/kg/day) for 60 days induced adverse effects on the reproductive function of male Wistar rats. It impaired spermatogenesis, decreased testosterone levels, and disrupted testicular histology. However, further investigations regarding mechanisms of action by which BPA could induces these effects are needed to better understand its reprotoxic impacts on the male reproductive system.

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Conflict of interest

We declare that we have no conflict of interest.

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