Bisphenol F induces testicular toxicity via upregulation of xanthine oxidase/uric acid signalling and downregulation of testicular steroidogenic enzymes in male Wistar rats

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Article

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Abstract

OBJECTIVE: This study investigated the effect of bisphenol F (BPF) on testicular integrity and function. The roles of xanthine oxidase/uric acid signalling and steroidogenic enzymes were also probed.

METHODOLOGY: Male Wistar rats were randomized into vehicle-treated control, BPF-treated (10, 30, and 50mg/kg), and BPF-treated recovery (animals treated with varying BPF doses and allowed a 28-day exposure free period for recovery) (n= 6 rats per group). The administration was via gavage and lasted for 28 days. Animals in the recovery groups were allowed a 28-day exposure-free period after the initial 28 days of BPF exposure.

RESULTS: BPF resulted in the distortion of the testicular histoarchitecture, accompanied by a significant rise in testicular GGT and LDH activities but a decline in SDH activity. Also, BPF caused a significant reduction in plasma GnRH, LH, FSH, and testosterone, which was associated with the downregulation of testicular 3beta-hydroxysteroid dehydrogenase (3β-HSD) and 17beta-hydroxysteroid dehydrogenase(17β-HSD) activities. Furthermore, BPF induced testicular inflammation, redox imbalance, and apoptosis, accompanied by elevated xanthine oxidase activity and uric acid concentration. Again, the observed toxic effects of BPF were dose-dependent and not reversed by BPF exposure withdrawal.

CONCLUSION: Bisphenol F-induced gonadotoxicity by down-regulating the activities of steroidogenic enzymes and upregulation of xanthine oxidase/uric acid signalling.

1. Introduction

The endocrine system maintains homeostasis by releasing hormones into the bloodstream, where they are delivered to their target organs and magnified. The circulating level of male reproductive hormones can be disrupted by exposure to environmental pollutants or endocrine-disrupting chemicals (EDCs). Studies continue to show that male infertility is on the rise and that this is linked to an increase in EDCs exposure [1]. Correlations between increased EDC exposure and rising rates of male infertility demonstrate that the long-term decline in male reproductive health could be due to exposure to EDCs. [2].

Bisphenols (A, F, S, and AF) are the most widely used EDCs, with approximately 90% of the human population being exposed to them [3]. Between 2003 and 2012, in the United States alone, bisphenol A (BPA) was found in the urine of more than 90% of a studied population [4], highlighting the high rate of human exposure to this environmental toxicant. Studies have shown that BPA induces testicular injury and sperm damage via an oxidative stress-mediated pathway and estrogenic activities [5;6;7;8]. Following the discovery of the gonadotoxic effect of BPA, countries worldwide have begun to impose limitations on its use. As a result of this restriction, scientists are attempting to avoid using BPA and are looking for alternatives that would provide the same function. However, these alternatives are not without their side effects. Out of all the alternatives for BPA, bisphenol F (BPF) is the most widely approved alternative [8].

Cans, plastics, food containers, feeding bottles, mugs, water tanks, thermal paper receipts, and medical gadgets contain BPF. Dietary, inhalation, and cutaneous exposure are the three main routes of BPF exposure, but dietary exposure is the commonest.

Bisphenol F has been reported to induce hormonal imbalance and oxidative damage in testicular tissue and sperm cells [9]. However, the mechanism associated with testicular toxicity is yet to be fully explored. Whether or not

steroidogenic enzymes are affected is not known. Also, the role of xanthine oxidase (XO)/ uric acid (UA) signalling in BPF testicular toxicity has not been studied.

The XO/UA signalling has been established to be an inducer of oxidative stress [10]. XO is a cytosolic enzyme that catalyses the conversion of hypoxanthine to xanthine and then to uric acid. XO utilises oxygen molecules as an electron acceptor and generates superoxide anion and other reactive oxygen species (ROs) as the end products during UA degradation. Exposure to elevated UA promotes oxidative stress and inflammatory response, which is eventually accompanied by the suppressed antioxidant buffering capacity of the cell.

Steroidogenic enzymes are key players in testicular steroidogenesis [11]. Steroidogenic Acute Regulatory Protein (StAR) translocates cholesterol into the inner mitochondria membrane of the testicular tissue, where it is being converted to pregnenolone before being acted on by 3beta-hydroxysteroid dehydrogenase (3β-HSD) to be converted to progesterone. The progesterone is then converted to a series of metabolic substrates before finally being converted into testosterone by 17beta-hydroxysteroid dehydrogenase (17β-HSD). Studies have shown that the upregulation of these enzymes increases the biosynthesis of testosterone. However, inhibition of these enzymes consequently leads to the suppression of the circulating level of testosterone [11].

With the aforementioned scientific pieces of evidence, the gonadotoxic effect of BPF may be mediated by modulation of XO/UA signalling and /or activities of steroidogenic enzymes. Despite the wide exposure to BPF and its reported testicular toxicity, the roles of these mechanisms are yet to be explored. In addition, whether or not the withdrawal of BPF exposure reverses the toxic effect of BPF is not known. Therefore, this study was designed to investigate the role of XO/UA signalling and the activities of steroidogenic enzymes on BPF induced testicular toxicity. Moreso, the effect of withdrawal after BPF exposure was also probed. The findings from this study will not only shed light on the mechanism of action of BPF in regard to testicular toxicity but will also open a window for the possible therapeutic intervention.

2. Results

Histopathological Findings

Testicular histoarchitecture appeared normal in the control group. The seminiferous tubules appeared normal in shape with germ cells at varying degrees of maturation. The Sertoli cells appeared normal. The lumen of the seminiferous tubules showed normal sperm cells. The interstitial space appeared normal with normal Leydig cell mass. However, the testicular histoarchitecture showed some distortions following treatment with BPF; the seminiferous tubules showed germ cells at varying degrees of maturation with some degenerated cell lines. The Sertoli cells appeared normal. Unlike the low and medium doses, the lumen of some seminiferous tubules in the animals treated with a high dose of BPF had scanty sperm cells compared with the age-matched control (Fig. 1). Surprisingly, cessation of BPF exposure did not reverse BPF-induced distortions in testicular histoarchitecture.

Male Reproductive Hormones

Table 1 shows the effect of BPF administration on male reproductive hormones. BPF caused a significant decrease in plasma GnRH, testosterone, FSH, and LH compared with animals in the control group. The reduction in plasma GnRH was only observed at the high dose and in all the BPF treated recovery groups compared with the age-matched control counterpart. Plasma FSH and LH were significantly reduced following medium and high-dose BPF treatment compared with the animals in the age-matched control group. These observed reductions were not

reversed by BPF cessation. In addition, BPF significantly reduced plasma testosterone levels across all the BPF-treated groups in a dose-dependent manner when compared with the age-matched control.

Table 1
Effect of BPF on Reproductive Hormones

Parameters	Control	Control-R	BPF-L	BPF-M	BPF-H	BPF-LR	BPF-MR	BPF-HR
GnRH (mIU/mL))	12.07± 1.232	12.12± 1.002	10.6± 1.651	10.27± 1.159	7.544± 1.291^{ab}	7.207± 1.347^{ac*}	8.814± 1.101^{a}	6.678± 0.731^{abc}
LH (mIU/mL)	5.648± 0.3496	5.716± 0.2385	5.331± 0.2109	2.901± 0.1376^{ab}	1.958± 0.1051^{abc}	5.131± 0.3231	2.817± 0.2172^{ab}	2.592± 0.1122^{ab*}
Testosterone (ng/mL)	2.516± 0.1174	2.479± 0.2228	1.867± 0.1034^{a}	1.32± 0.114^{ab}	0.8365± 0.0906^{abc}	1.771± 0.1252^{a}	0.8595± 0.2144^{ab*}	0.3908± 0.1283^{abc*}

$^{a}p < .05$ versus age-matched control, $^{b}p < .05$ versus low dose of BPF, $^{c}p < .05$ versus medium dose of BPF, *p < .05 versus groups with corresponding doses at p < .05 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison.

Steroidogenic Enzymes

There was a significant decrease in testicular 3-beta-hydroxysteroid dehydrogenase(3beta-HSD) and 17-beta hydroxysteroid dehydrogenase (17-beta hydroxysteroid dehydrogenase, as well as cholesterol in a dose-dependent manner when compared with the age-matched control (Fig. 2). The observed decrease in these parameters was completely reversed following 28 days of withdrawal from BPF treatment

Testicular Injury Markers

The activities of testicular GGT and LDH were significantly increased following the administration of medium and high doses of BPF. In contrast, the testicular SDH level was significantly decreased compared with the animals in the age-matched control group (Fig. 3). Unlike the testicular SDH that was reversed following withdrawal, testicular GGT and LDH remained unchanged.

Inflammatory Markers

Testicular TNF-alpha, IL-6, and NO were significantly increased following BPF treatment, which was observed at medium and high doses (Fig. 4) compared with the age-matched control. The observed increase was not reversed following 28 days of withdrawal of BPF. Also, a significant increase in the level of testicular MPO was observed. However, unlike TNF-alpha, IL-6, and NO, a significant increase was noticed across all the BPF treated groups compared with the age-matched control. The observed increase was not reversed following the withdrawal of BPF treatment except in the animals in the BPF-MR group, where partial recovery was observed.

Oxidative Stress Markers

As shown in Fig. 5, testicular MDA was significantly increased following medium and high doses of BPF treatment compared with the age-matched control counterpart. The observed increase in animals treated with medium-dose

were reversed by withdrawal, and it remained unchanged in animals treated with high dose. Also, there was a significant reduction in testicular SOD, catalase, GSH, GST, GPX, and GST. While the significant decrease in testicular SOD was partially reversed in animals treated with a high dose of BPF following 28 days of recovery, the observed partial recovery in testicular catalase was at low and high doses. Unlike the other parameters, the recovery observed in testicular GST was total, and it cut across all the recovery groups.

XO/UA Signalling

Testicular xanthine oxidase and uric acid were significantly increased following BPF treatment (Fig. 6). Unlike testicular xanthine oxidase, where the observed significant difference was noticed across all the groups, a significant increase in testicular uric acid was noticed in animals administered a high dose of BPF compared with the age-matched control. In addition, total recovery was observed in both testicular xanthine oxidase and uric acid following 28 days of recovery.

Apoptosis

As shown in Fig. 7, a significant increase in testicular DNA fragmentation index was caused by BPF treatment compared with the age-matched control. The increase was observed in the animals administered a medium and high dose of BPF. While there was no significant increase in animals administered a low dose of BPF, the animals in the low dose recovery group exhibited a significant increase in DFI when compared with the age-matched control. Partial recovery was observed in the animals treated with high dose of BPF.

3. Discussion

The increasing rate of industrialization has been implicated in the global rise in male infertility [20] which is majorly due to the release of endocrine disruptors into the environment. Bisphenol F is an endocrine disruptor, and little information is available about its effect on male reproduction. Although [9] have shown the impact of BPF on some male reproductive hormones and oxidative stress markers, there is no available information on its effect on steroidogenic enzymes and xanthine oxidase/uric acid signalling. Also, there is no available information about the after-withdrawal impact of BPF on biochemical indices and testicular histoarchitecture in male rats.

The testicular histoarchitecture of the control and control-recovery animals appeared normal. The seminiferous tubules were normal in shape with germ cells at different degrees of maturation. The seminiferous tubules' lumen showed normal cells of the sperm, and the Sertoli cells appeared normal. The interstitial space was normal with normal Leydig cell mass. BPF treatments (low, medium, and high dose) led to the distortion of testicular histoarchitecture. Some degenerated germ cell lines with scanty spermatozoa in some seminiferous tubules' lumen. Furthermore, the testicular histoarchitecture of animals in the low and medium recovery groups was distorted, evidenced by some degenerated germ cell lines, widened seminiferous tubular lumen with scanty sperm cells, and widened interstitial space with mild congestion. In addition, a gross distorted testicular histoarchitecture was observed in animals in the BPF-HR group accompanied by altered spermatogenesis, coagulative necrosis of the germ cells, scanty Sertoli cells, widened seminiferous tubular lumen with scanty or no sperm cells. There was also widened interstitial space with mild congestion and reduced Leydig cell mass. The observed distortion in the testicular histoarchitecture agreed with previously reported findings on both bisphenol F and its other analogs [9] [21] [22].

BPF exposure significantly reduced the plasma GnRH, LH, testosterone, and FSH. BPF disrupted the physiological sequence of events along the hypothalamus-pituitary-testicular axis. Suppression of the hypothalamus-pituitary-testicular axis could significantly reduce the activity of GnRH secreting cells and, hence, the hormone's plasma level. The consequent weak stimulatory effect of GnRH on the anterior pituitary causes impaired secretion of FSH and LH and thus a significant reduction in testosterone. The reduced testosterone level could not instigate stimulatory positive feedback effects at the anterior pituitary and hypothalamic level to bring about a compensatory increase in the levels of GnRH and gonadotropins. These findings were similar to a previous study that reported a significant reduction in plasma testosterone, LH, and FSH following bisphenol F treatment [9] and may suggest that BPF induces a local toxic effect on the testis as suppression of the hypothalamus-pituitary-testicular axis.

BPF-induced suppression of steroidogenesis was associated with reduced steroidogenic enzymes, evidenced by the significant reduction in the activities of 3β-HSD and 17 β-HSD, which was accompanied by reduced testicular cholesterol, the precursor of testosterone. These observations corroborate previous findings [23], which reported similar observations following bisphenol A treatment. It is plausible to infer that BPF-induced impaired steroidogenesis was, at least in part, a consequence of BPF-induced downregulation of 3β-HSD and 17 β-HSD. Also, the reduced testicular cholesterol concentration may likely be due to impaired steroidogenic acute regulatory protein (StAR), responsible for cholesterol transportation to the inner mitochondrial membrane from the outer mitochondrial membrane.

Furthermore, BPF treatment significantly increases testicular lactate, LDH, and GGT and a significant reduction in testicular sorbitol dehydrogenase (SDH). The significant increase in testicular lactate could result from an observed increase in LDH. LDH is responsible for converting pyruvate to lactate during the anaerobic condition. It is important to note that there is no available data to compare our findings, but the significant increase in testicular LDH and lactate could be a compensatory adaptation to restore the observed BPF-induced testicular damage and oxidative stress. This agreed with previous study [24] that reported lactate to preserve germ cells by limiting the loss of spermatids and spermatocytes in rats. A primary index of Sertoli role is GGT, and its activity is parallel to Sertoli cell replication and maturation [25]. The significant increase in the activity of testicular GGT in animals treated with BPF in this present study suggests a less effective function of the Sertoli cell. SDH activities provide glycolysis and oxidative phosphorylation energy by converting sorbitol to fructose to form ATP [26]. The observed unignorable reduction in SDH in this study suggests that BPF-treatment might lead to an energy imbalance in the sperm cell.

The balance between the generation and scavenging reactive oxygen species (ROS) must be maintained under physiologic conditions. Under pathological conditions, excessive ROS are produced, altering the balance between ROS (pro-oxidants) production and removal (antioxidants). This condition is referred to as oxidative stress. In the testes of BPF administered animals, oxidative stress was noticed. This was made evidenced by a significant increase in the level of testicular MDA and a significant reduction in testicular CAT, GSH, GST, SOD, and GPx. The observed increase in pro-oxidant and decrease in antioxidants concurred with [9] and [27] with similar findings following administration of BPF and its analog.

In this present study, a significant increase in MPO, NO, TNF-α and IL-6 was observed after exposure to BPF. These findings is in agreement with a previous study [28], which reported a significant increase in pro-inflammatory markers after bisphenol-A administration. It is worthy to note that the significant increase in inflammatory markers could result from the increased generation of reactive oxygen species. The observed significant increase in

testicular NO corroborates a previous study [29] that associated BPF treatment with a significant increase in NO. The significant increase in testicular NO may react with O2- to generate peroxynitrite, a potent oxidant that can decompose to produce reactive hydroxyl radical, which can eventually lead to testicular damage [30]. The significant increase in testicular MPO might also contribute to the testicular toxicity observed in this study. This is in agreement with a study [31] that revealed that the MPO properties, which are autonomous of its enzymatic activity, and also oxidants that are MPO-derived, seem to take part in a series of events that assists the propagation and initiation of the inflammatory response and, as a result, are involved in tissue pathology in diseases depicted by oxidative stress and increase in inflammation.

The increase in XO and Uric acid could significantly increase ROS levels since XO and lipid peroxidation have been linked together [13]. Although, it is important to note that UA may act as an antioxidant [32], the ability to curtail radical forming systems [33]. The pro-oxidant activity of UA can result from its reaction with other oxidants to produce radicals that majorly target cellular [34]. The lipophilic environment created due to the accumulation of lipids, in turn, creates an unfavourable environment for the antioxidant activity of UA [35], and the uric acid is thereby converted to oxidants by the oxidized lipids [36]. The finding that BPF treatment significantly increased testicular uric acid level suggests that its toxic effect on the testis could be due to the accumulation of uric acid. The significant increase in testicular UA may result in the activation of a pro-inflammatory response [37], resulting in a significant increase in testicular NO and MPO activity. Also, it may result in the observed oxidative stress following BPF treatment through increased generation of reactive oxygen species [37]. Since BPF could stimulate both pro-inflammatory signalling and oxidative stress, it is logical to conclude that BPF mediates its gonadotoxic effect via XO/UA-dependent oxidative stress and inflammatory response.

Probing the mechanism behind BPF-induced oxido-inflammatory damage to the testis and investigating its effects is important in understanding the aetiopathogenesis of the observed testicular toxicity. Our observations that BPF-induced oxidative and inflammatory damage to the testis is accompanied by a significant increase in DNA fragmentation, a marker of apoptosis. This finding concurred with a previous study that observed that a significant increase in UA stimulates epithelial-to-mesenchymal transition and apoptosis through oxidative stress-driven inhibition of E-cadherin synthesis and promotes E-cadherin degradation [38]. BPF exposure-induced testicular toxicity may be associated with the observed oxido-inflammatory response and apoptosis. Hence, BPF-induced testicular toxicity may be multifaceted; upregulation of XO/UA signaling may promote oxidative stress and inflammatory response, which are triggers of apoptosis [34] .Taken together, BPF exposure is a potential risk factor for testicular injury mediated by the upregulation of XO/UA signalling and DNA fragmentation.

Surprisingly, BPF withdrawal did not reverse the observed disruptive effect of BPF on the testis. The testicular toxicity of BPF lingers beyond expectation, evidenced by unrestored testicular homeostatic balance within 28 days of BPF withdrawal. It is expected that withdrawal of BPF for 28 days would ameliorate BPF-induced gonadotoxicity; astonishingly, it did not. This may be due to its high affinity for fatty tissues. Bisphenols can accumulate in fatty tissues [39] such as the testis, thus exerting a prolonged effect even after cessation of exposure.

4. Methods

4.1 Animals for the Experiment

Forty-eight (48) male Wistar rats weighing between 160-200g were housed in clean, well-ventilated plastic cages (6 rats/cage). Animals were maintained on a natural dark/light cycle and allowed free access to feed and water. The animals were humanely handled according to the Guidelines for Laboratory Animal Care of the National Institute of Health (NIH) and International Guiding Principles for Biomedical Research. Ethical approval was obtained from the University Ethical Review Committee (UERC), University of Ilorin, Kwara State, Nigeria and the study was reported in accordance with ARRIVE guidelines.

4.2 Experimental Procedure

The animals were allowed to acclimatize to their new environment for two weeks before allotting them randomly into eight groups (6 rats per group):

Group 1: control, vehicle-treated with 0.5ml normal saline

Group 2: low-dose BPF (BPF-L), received 10 mg/kg b.w of BPF

Group 3: medium-dose BPF (BPF-M), received 30 mg/kg b.w of BPF

Group 4: high-dose BPF (BPF-H), received 50 mg/kg b.w of BPF

Group 5: control recovery (Control-R), received treatment as the control group followed by a 28-day normal saline-free period

Group 6: low-dose of BPF recovery (BPF-LR), received 10 mg/kg b.w of BPF followed by a 28-dayBPF-free period

Group 7: medium-dose BPF recovery (BPF-MR), received 30 mg/kg b.w of BPF followed by a 28-day BPF-free period.

Group 8: high-dose BPF recovery (BPF-HR), received 50 mg/kg b.w of BPF followed by a 28-day BPF-free period.

All administrations were via oral route using an oro-pharyngeal cannula. The doses used in the present study were the human relevant safe doses as reported by Environmental Protection Agency [12].

At the end of the study, animals were euthanized via intraperitoneal administration of 4 mg/kg xylazine and 40 mg/kg of ketamine [13], and blood samples were collected via cardiac puncture. The blood samples were centrifuged at 3000rpm for 10minutes to obtain the plasma for hormonal analysis. The testes were harvested, separated from periphery structures and tissues, and weighed. The left testis was homogenized in cold phosphate buffer solution for biochemical assays, while the right testis was put in 10% formaldehyde for histological processing.

4.3 Histological Preparation

The testis was dehydrated with different levels of alcohol, and toluene was used to clear it.The testes were blocked in paraffin wax, and hematoxylin and eosin(H&E) stain were added. About 5 µm thick section was made for histological analysis.

4.4 Measurement of circulatory hormones

The plasma concentration of Growth releasing hormone (GnRH) (Melsin, China), luteinizing hormone (LH)(Bio-Inteco, UK), follicle stimulating hormone (FSH) (Bio-Inteco, UK), and testosterone (Bio-Inteco, UK) were estimated

following the instruction from the manufacturers.

4.5 Determination of steroidogenic enzymes

The effect of BPF on testicular3 beta-hydroxysteroid (3β-HSD) and 17 beta-hydroxysteroid (17 β-HSD) were estimated according to the method of [14]and [15] respectively.

According to the manufacturer's instruction, testicular cholesterol, which is the precursor of testosterone, was estimated using a standard ELISA kit (Randox, UK).

4.6 Estimation of testicular enzymes

Gamma-glutamyl transferase (GGT) activity was estimated according to the manufacturer's instructions (Pointe Scientific Inc., USA). Briefly, the working reagent was prepared based on the manufacturer's instruction, and 1.0 ml of the reagent was pipetted into well-labeled tubules. This reagent was pre-incubated for about 5 minutes at a temperature of 3700C. Water was used to zero the spectrophotometer at a 405 nm wavelength. About 100 µL of the sample was poured into the reagent; the mixture was returned to a thermo cuvette after being thoroughly mixed. After about 60 seconds, the absorbance was read and recorded at precisely 60 seconds intervals for 2 minutes. The mean absorbance difference was determined per minute, and to obtain the result in U/L, it was multiplied by 1158.

Lactate dehydrogenase activities were determined following the manufacturer's instructions (Agappe Diagnostics Ltd., India). The principle was based on pyruvate, NADH, and H + reaction to produce L-lactate and NAD+. Briefly, 1000 µL of working reagent was thoroughly mixed with 100 µL of the homogenate. The mixture was incubated for 1 minute at a temperature of 3700C, and absorbance was read every 20 seconds for 1 minute. Enzymatic activity was calculated enzyme by LDH-P activity (U/L) = (ΔOD/min) x 16030.

According to the manufacturer's instruction, the testicular lactate concentration was estimated using a colorimetry method, as stated by Abcam, China. The principle was founded on the oxidative reaction between LDH and lactate to yield a product that will produce colour when reacted with a probe.

4.7 Measurements of Markers of Inflammation

Standard ELISA kits (Solarbio, China) were used to assay the concentrations of interleukin-6 (IL-6) and tumour necrosis factor-α (TNF- α) in the testicular tissue following the manufacturers' guideline. Testicular myeloperoxidase (MPO) was determined based on established principle [16] [13]. This method involves guaiacol oxidation in the presence of hydrogen peroxide. Testicular nitric oxide was evaluated based on Griess reaction as previously documented [17].

4.8 Antioxidant enzymes and testicular oxidative stress measurement

Malondialdehyde (MDA), a marker of oxidative stress, was determined as previously documented [18] [13] based on the generated amount of thiobarbituric acid reactive substance (TBARS) during lipid peroxidation.

According to Ellman's method, the testicular glutathione (GSH) level was estimated. Also, catalase (CAT), glutathione peroxidase (GPx), Glutathione-S-transferase (GST), and testicular superoxide dismutase (SOD) activities were evaluated by the colorimetric method through the usage of standard laboratory reagents.

4.9 Xanthine Oxidase and Uric Acid Measurement

Testicular xanthine oxidase (XO) activities and concentration of testicular uric acid (UA) were estimated using colorimetric methods based on standard laboratory kits (Fortress Diagnostic, Antrim, UK and Precision, UK, respectively).

4.10 Estimation of testicular DNA Fragmentation Index (DFI)

To access the effect of BPF on apoptosis and testicular DNA damage, a spectrophotometric assay using diphenylamine (DPA) methods as previously described [19] was employed to determine the estimated percentage of DFI.

4.11 Analysis of the Statistics

One-way analysis of variance (ANOVA) followed by Tukey's post hoc test were conducted using GraphPad PRISM 5 software (GraphPad Software, La Jolla, California, USA). Data were presented as mean ± SD. P values < 0.05 were considered significant statistically.

5. Conclusion

BPF instigates gonadotoxicity by promoting pro-inflammatory response, oxidative stress, and apoptosis via the upregulation of XO/uric acid signalling. It also disrupts hypothalamic-pituitary-gonadal axis via the downregulation of steroidogenic enzymes. These effects were not restored after BPF cessation.

Declarations

Data availability statement

The manuscript's materials, including all relevant raw data, will be made freely available by the corresponding author (Odetayo Adeyemi Fatai) to any researcher who wishes to use them for non-commercial purposes while maintaining participant confidentiality.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authorship Contributions

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Conception and design of the study: OAF, OLA, AWJ

Analysis and/or interpretation of data: OAF and OLA

Drafting the manuscript: OAF

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References

1. Joensen, U. N., Bossi, R., Leffers, H., Jensen, A. A., Skakkebæk, N. E. and Jørgensen, N. Do Perfluoroalkyl Compounds Impair Human Semen Quality? Environmental Health Perspectives. 117(6): 923–927(2009).
2. Rehman, S. et al. Endocrine disrupting chemicals and impact on male reproductive health.TranslAndrolUrol 7(3):490–5(2018).
3. De Toni, L., De Rocco Ponce, M., Petre, G. C., Rtibi, K., Di Nisio, A., and Foresta, C.. Bisphenols and Male Reproductive Health: From Toxicological Models to Therapeutic Hypotheses. Frontiers in Endocrinology, 11 (2020).
4. Ye, X., Wong, L.-Y., Kramer, J., Zhou, X., Jia, T., and Calafat, A. M. Urinary Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S. Adults during 2000–2014. Environmental Science & Technology, 49(19): s11834–11839(2015).
5. Richter, C. A. et al. In vivo effects of bisphenol A in laboratory rodent studies. Reproductive Toxicology 24(2), 199–224 (2007).
6. Karnam, S., Ghosh, R., Mondal, S. and Mondal, M. Evaluation of subacute bisphenol-A toxicity on male reproductive system. Vet. World. 8(6), 738–744 (2015).
7. Ogo, F. M. et al. Low doses of bisphenol A can impair postnatal testicular development directly, without affecting hormonal or oxidative stress levels. Reproduction, Fertility and Development 29(11) 2245–2254 (2017).
8. Tomáš, J., Bistáková, J., Greifová, H., Tvrdá, E., and Lukáč, N. Male Reproduction: One of the Primary Targets of Bisphenol. Intechopen. 68629 (2017).
9. Ullah, A., Pirzada, M., Afsar, T., Razak, S., Almajwal, A., and Jahan, S. Effect of bisphenol F, an analog of bisphenol A, on the reproductive functions of male rats. Environmental Health and Preventive Medicine. 24(1): 41–52(2019).
10. Hamed, M. A., Aremu, G. O., & Akhigbe, R. E. Concomitant administration of HAART aggravates anti-Koch-induced oxidative hepatorenal damage via dysregulation of glutathione and elevation of uric acid production. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapy. 137, 111309(2021).
11. Barbagallo, F. et al. Effects of Bisphenols on Testicular Steroidogenesis. Frontiers in endocrinology, 11, 373 (2020).
12. Wisniewski, P. et al. Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic-pituitary-testicular axis. Toxicology, 329, 1–9 (2015).
13. Akhigbe, R. E., Hamed, M. A., Odetayo, A. F., Akhigbe, T. M., Ajayi, A. F. and Ajibogun, F. A. H. Omega-3 fatty acid rescues ischaemia/perfusion-induced testicular and sperm damage via modulation of lactate transport and xanthine oxidase/uric acid signaling. Biomedicine & Pharmacotherapy, 142, 111975 (2021).
14. Talalay, P. Enzymatic Analysis of Steroid Hormone Methods. Biochem Anal. 8,119 (1960).
15. Jarabak, J., Adams, J. A., Williams-Ashman, H. G., and Talalay, P. J. Purification of a 17beta-hydroxysteroid dehydrogenase of human placenta and studies on its transhydrogenase function. Biol. Chem. 245: 237,345 – 35 (1962).

16. Desser, R.K, Himmelhoch, S.R, Evans, W.H, Januska, M, Mage, M and Shelton, E. Isolation procedure and some properties of myeloperoxidase from human leucocytes. Arch. Biochem. Biophys. 148: 452–465 (1972).
17. Ridnour, L. A. et al. A Spectrophotometric Method for the Direct Detection and Quantitation of Nitric Oxide, Nitrite, and Nitrate in Cell Culture Media. Analytical Biochemistry, 281(2), 223–229 (2000).
18. Ohkawa, H, Ohishi, N and Yagi, K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry 95:.351–358(1979).
19. Perandones, C.E.,Illera, V.A., Peckham, D., Stunz, L.L. and Ashman, R.F. Regulation of apoptosis in vitro in mature murine spleen T cells. J Immunol. 151(7):3521–9(1993).
20. Selvaraju, V., Baskaran, S., Agarwal, A. and Henkel, R. Environmental contaminants and male infertility: Effects and mechanisms. Andrologia, 53(1) (2020).
21. Malmir, M., Mehranjani, M.S., Faraji, T. and Noreini, S.N. Antioxidant effect of Vitamin E on the male rat reproductive system by a high oral dose of Bisphenol-A. Toxicology Research and Application (2021).
22. Adegoke,E.O. et al. Bisphenol A damages testicular junctional proteins transgenerationally in mice. Environmental Pollution, 302 (1) (2022).
23. Samova, S., Patel, C. N., Doctor, H., Pandya, H. A. and Verma, R. J. The effect of bisphenol A on testicular steroidogenesis and its amelioration by quercetin: an in vivo and in silico approach. Toxicology Research, 7(1): 22–31 (2018).
24. Courtens, J.L. and Ploen, L. Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. Biol. Reprod. 61, 154–161 (1999).
25. Sherins, R. J., and Hodgen, G. D. Testicular gamma glutamyl-transpeptidase: an index of Sertoli cell function in man. Reproduction. 48(1), 191–193 (1976).
26. Cao, W., Aghajanian, H. K., Haig-Ladewig, L. A. and Gerton, G. L. Sorbitol Can Fuel Mouse Sperm Motility and Protein Tyrosine Phosphorylation via Sorbitol Dehydrogenase1. Biology of Reproduction. 80(1), 124–133 (2009).
27. Olukole,S.,Ola-Davies, E.,Lanipekun, D. and Oke, B. Chronic exposure of adult male Wistar rats to bisphenol A causes testicular oxidative stress: Role of gallic acid. Endocrine Regulations, 54 (1): 14–21(2020).
28. Moon, M. K. et al. Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. Journal of Korean medical science, 27(6): 644–652(2012).
29. Qiu, W. et al. Immunotoxicity of bisphenol S and F are similar to that of bisphenol A during zebrafish early development. Chemosphere, 194, 1–8 (2018).
30. Aprioku, J. S. Pharmacology of free radicals and the impact of reactive oxygen species on the testis. Journal of reproduction & infertility, 14(4), 158–172 (2013).
31. Van der Veen, B. S., de Winther, M. P. J., andHeeringa, P. Myeloperoxidase: Molecular Mechanisms of Action and Their Relevance to Human Health and Disease. Antioxidants & Redox Signaling, 11(11), 2899–2937 (2009).
32. Kellogg, E. W., & Fridovich, I. Liposome oxidation and erythrocyte lysis by enzymically generated superoxide and hydrogen peroxide. Journal of Biological Chemistry, 252, 6721–6728(1977).
33. Maples, K. R., & Mason, R. P. Free radical metabolite of uric acid. Journal of Biological Chemistry, 263, 1709–1712 (1988).
34. Akhigbe, R. E., Oladipo, A. A., Oyedokun, P. A., Hamed, M. A., Okeleji, L. O., & Ajayi, A. F. Upregulation of Uric Acid Production and Caspase 3 Signalling Mediates Rohypnol-Induced Cardiorenal Damage. Cardiovascular

toxicology, 10.1007/s12012-022-09723-z (2022).

35. Muraoka, S., & Miura, T. Inhibition by uric acid of free radicals that damage biological molecules. Pharmacology and Toxicology, 93, 284–289 (2003).
36. Bagnati, M., Perugini, C., Cau, C., Bordone, R., Albano, E., & Bellomo, G. When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: A study using uric acid. The Biochemical Journal, 340(Pt 1), 143–152 (1999).
37. Kang, D. H., Park, S. K., & Lee, I. K. Uric acid-induced C-reactive protein expression: Implication on cell proliferation and nitric oxide production of human vascular cells. Journal of the American Society of Nephrology, 6(12), 3553–3562 (2005).
38. Yu, E. S., Kim, M. J., Shin, H. S., Jang, Y. H., Choi, H. S., & Jo, I. Uric acid-induced phenotypic transition of renal tubular cells as a novel mechanism of chronic kidney disease. American Journal of Physiology. Renal Physiology, 304, F471–F480 (2013).
39. Csanády, G., Oberste-Frielinghaus, H., Semder, B., Baur, C., Schneider, K. and Filser, J. Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein. Archives of Toxicology. 76(5–6):299–305 (2002).

Figures

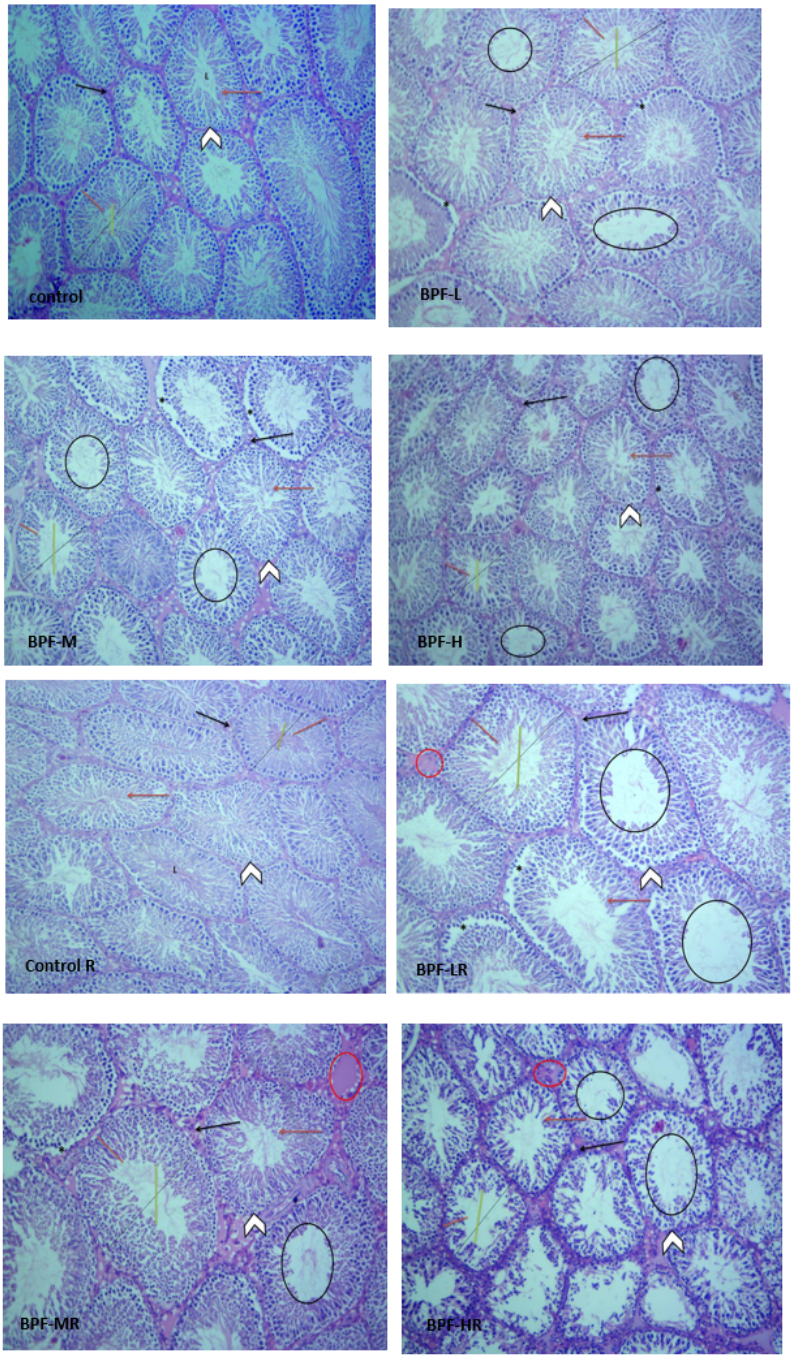

Figure 1

Control and Control-R: Testicular histoarchitecture appears normal. The seminiferous tubules appear normal in shape with germ cells at varying degree of maturation (arrow head). The Sertoli cells appear normal (red arrow). The lumen of the seminiferous tubules shows normal sperm cells (L). The interstitial space appears normal with normal Leydig cell mass (black arrow). BPF-L and BPF-M: The testicular histoarchitecture shows some distortions. The seminiferous tubules show germ cells at varying degree of maturation (arrow head) with some degenerated cell lines (*). The Sertoli cells appear normal (red arrow). The lumen of some seminiferous tubules shows scanty sperm cells (black circle). The interstitial space appears normal with normal Leydig cell mass (black arrow). BPF-H: The testicular histoarchitecture shows some distortions. The seminiferous tubules show germ cells at varying

degree of maturation (arrow head) with some degenerated cell lines (*). The Sertoli cells appear scanty (red arrow). The lumen of most seminiferous tubules shows scanty sperm cells (black circle). The interstitial space appears normal with normal Leydig cell mass. BPF-LR and BPF-MR: The testicular histoarchitecture shows some distortions. The seminiferous tubules show germ cells at varying degree of maturation (arrow head) with some degenerated cell lines (*). The Sertoli cells appear scanty (red arrow). The lumen of most seminiferous tubules appears widened with scanty sperm cells (black circle). The interstitial space appears widened with mild congestion (red circle). The Leydig cell mass appears normal (black arrow). BPF-HR: The testicular histoarchitecture shows gross distortions. The seminiferous tubules show grossly altered spermatogenesis with coagulative necrosis in the germ cells (arrow head). The Sertoli cells are markedly scanty (red arrow). The lumen of almost all the seminiferous tubules appears widened with very scanty or no sperm cells (black circle). The interstitial space appears widened with mild congestion (red circle). The Leydig cell mass is reduced (black arrow).

Black span: diameter of the seminiferous tubules; red span: epithelial height; green span: diameter of the seminiferous lumen.

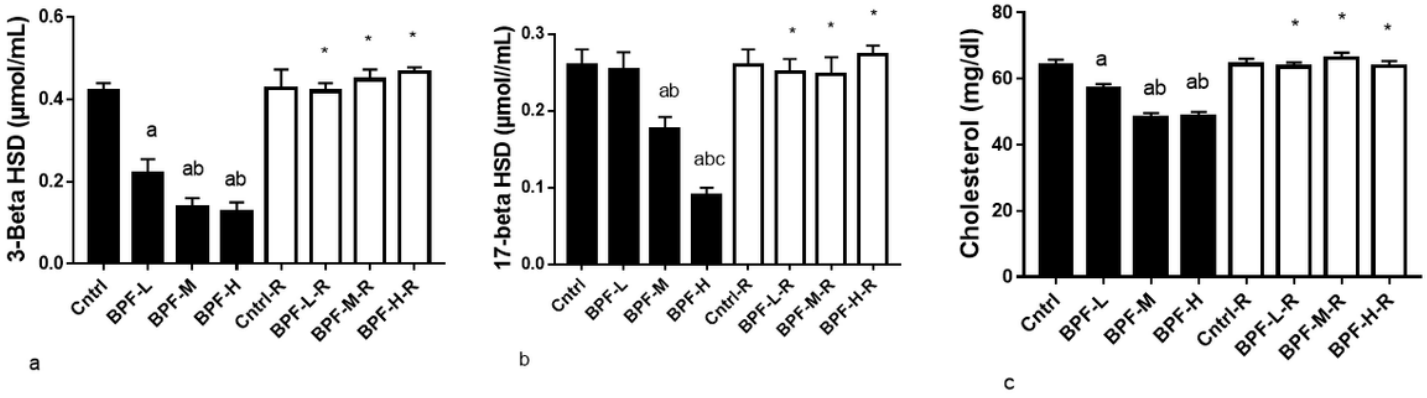

Figure 2

Effect of BPF on testicular (a) 3-beta HSD (b) 17-beta HSD (c) Cholesterol. [a]p < .05 versus age-matched control, [b]p < .05 versus low dose of BPF, [c]p < .05 versus medium dose of BPF, *p < .05 versus groups with corresponding doses at p < .05 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison

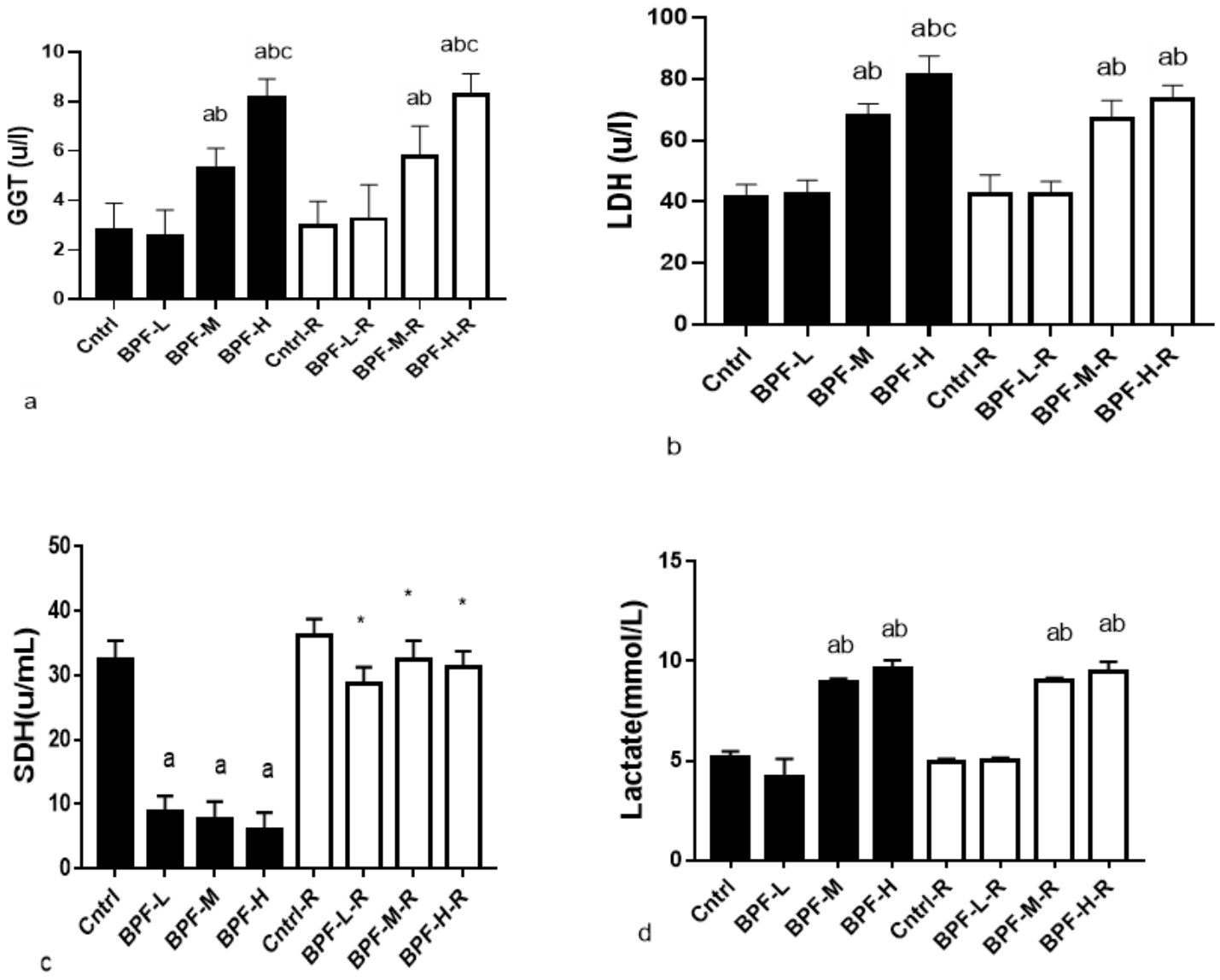

Figure 3

Effect of BPF on testicular (a) GGT (b) LDH (c) SDH (d) Lactate. [a]$p < .05$ versus age-matched control, [b]$p < .05$ versus low dose of BPF, [c]$p < .05$ versus medium dose of BPF, *$p < .05$ versus groups with coresponding doses at $p < .05$ using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison.

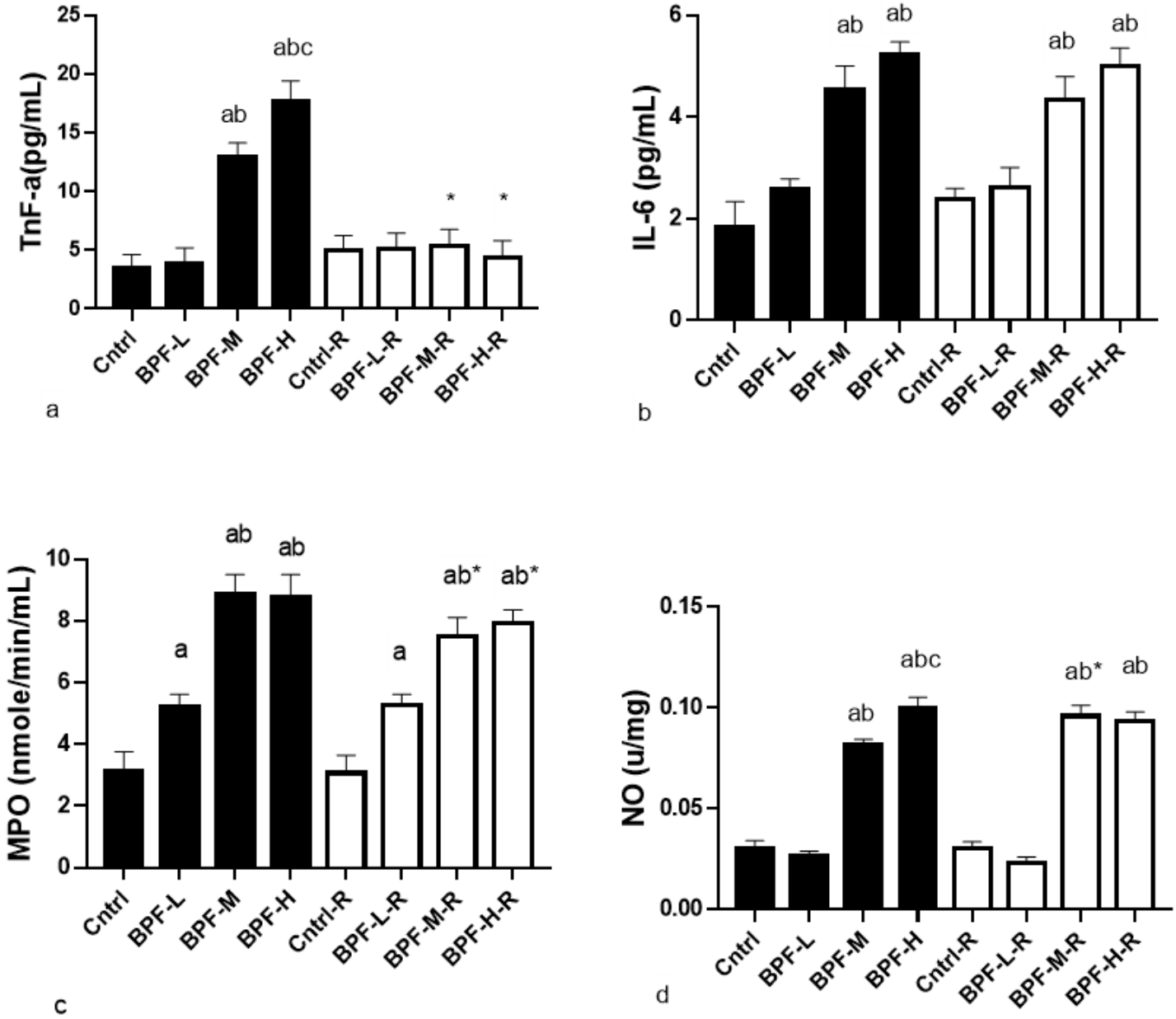

Figure 4

Effect of BPF on testicular (a) TnF-alpha(b) IL-6 (c) MPO (d) NO. [a]p < .05 versus age-matched control, [b]p < .05 versus low dose of BPF, [c]p < .05 versus medium dose of BPF, *p < .05 versus groups with corresponding doses at p < .05 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison.

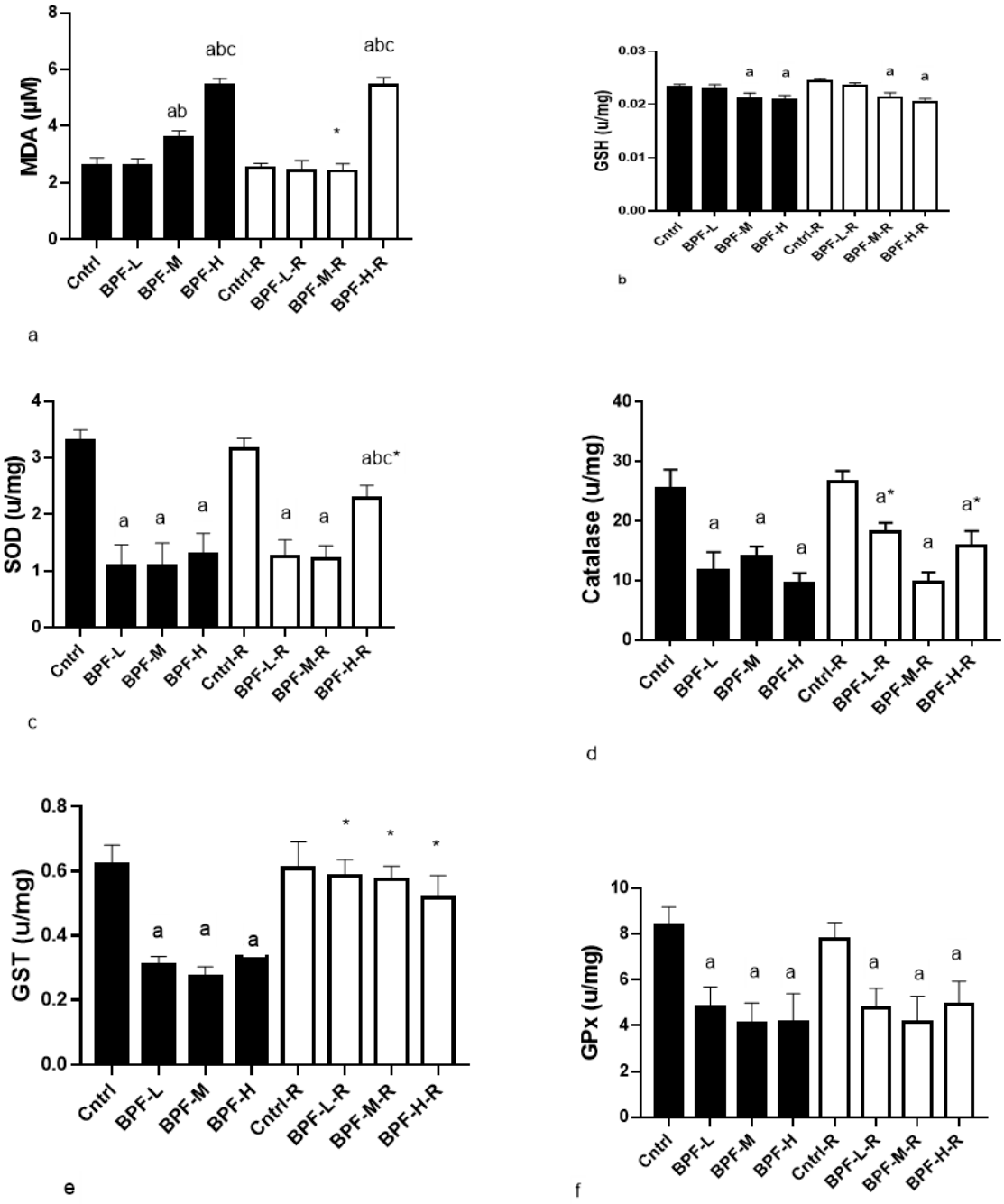

Figure 5

Effect of BPF on testicular (a) MDA (b) GSH (c) SOD (d) Catalase (e)GST (f) GPx. [a]$p < .05$ versus age-matched control, [b]$p < .05$ versus low dose of BPF, [c]$p < .05$ versus medium dose of BPF, *$p < .05$ versus groups with corresponding doses at $p < .05$ using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison

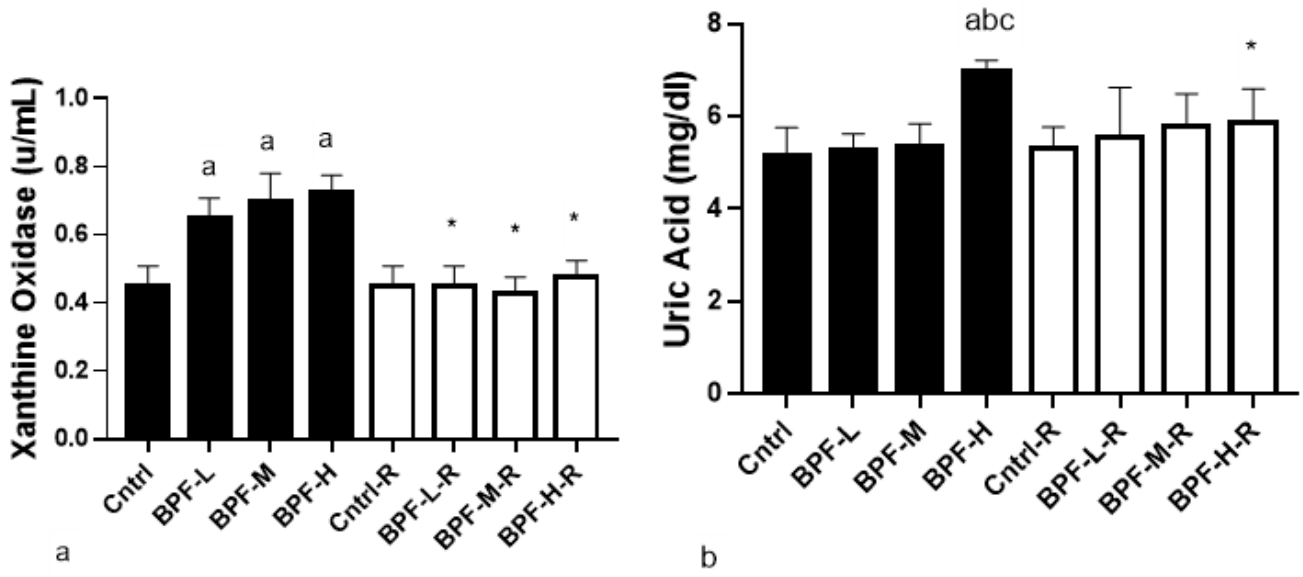

Figure 6

Effect of BPF on testicular (a) XO (b) UA. [a]p < .05 versus age-matched control, [b]p < .05 versus low dose of BPF, [c]p < .05 versus medium dose of BPF, *p < .05 versus groups with corresponding doses at p < .05 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison.

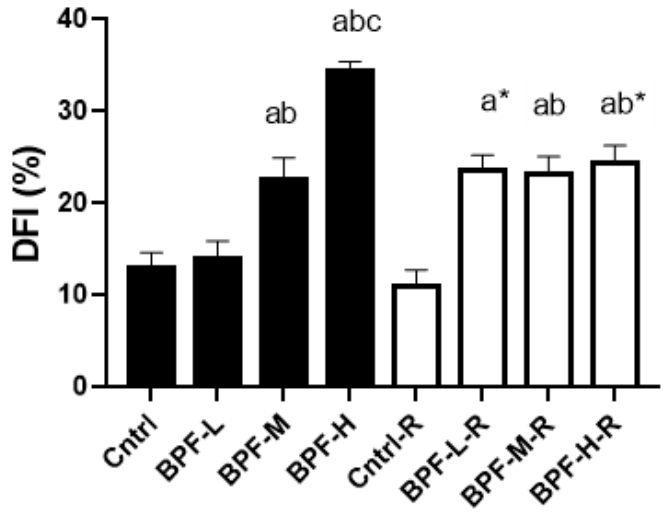

Figure 7

Effect of BPF on testicular DNA fragmentation Index (DFI). [a]p < .05 versus age-matched control, [b]p < .05 versus low dose of BPF, [c]p < .05 versus medium dose of BPF, *p < .05 versus groups with corresponding doses at p < .05 using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison.