NJBMB (2022) 37(4) 272-281

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

Cucumeropsis mannii (African White Melon) Seed Oil Mitigates Dysregulation of Redox Homeostasis, Inflammatory Response, and Apoptosis in Testis of Bisphenol A Exposed Male Rats

Patrick M. Aja[1], Peter C. Agu[1, 6*], Hilary A. Ogwoni[1], Ugbala E. Ekpono[2], Ernest M. Ezeh[5], Onyedika G. Ani[1, 4], Atamgba A. Asuk[3], Joshua N. Awoke[1], Boniface A. Ale[7], Felix E. Nwite[1], Oliver U. Ukachi[1], Obasi U. Orji[1], Peter C. Nweke[1], Ugbala E. Ekpono[1], Gift O. Ewa[1], Ikechuku O. Igwenyi[1], Esther U. Alum[1], Darlington C. Chukwu[1], Ekenechukwu, Maduagwuna[1], Francisca C. Nwiziogo[6]

[1]*Department of Biochemistry, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria.*
[2]*Department of Science Laboratory Technology, Biochemistry Option, Federal Polytechnic Oko, Nigeria.*
[3]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Nigeria*
[4]*Department of Clinical Sciences and Nutrition, University of Chester, Parkgate Road Tower Building, United Kingdom*
[5]*Department of Chemical Engineering, Caritas University, Amorji-Nike, Nigeria.*
[6]*Department of Science Laboratory Technology, Biochemistry Option, Our Savior Polytechnic Enugu, Nigeria.*
[7]*Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*

OPEN ACCESS

CORRESPONDENCE
Agu P.C.
sirpfoundation@gmail.com
+234-816-267-4263

ARTICLE HISTORY
Received: 16/06/2022
Reviewed: 02/09/2022
Revised: 05/09/2022
Accepted: 24/10/2022
Published: 30/12/2022

CITATION
Aja, P.M., Agu, P.C., Ogwoni, H.A., Ekpono, U.E., Ezeh, E.M., Ani, O.G., Asuk, A.A., Awoke, J.N., Ale, B.A., Nwite, F.E., Ukachi, O.U., Orji, O.U., Nweke, P.C., Ekpono, U.E., Ewa, G.O., Igwenyi, I.O., Alum, E.U., Chukwu, D.O., Maduagwuna, E. and Nwiziogo, F.C. (2022). *Cucumeropsis mannii* (African White Melon) Seed Oil Mitigates Dysregulation of Redox Homeostasis, Inflammatory Response, and Apoptosis in Testis of Bisphenol A Exposed Male Rats. *Nigerian Journal of Biochemistry and Molecular Biology*. 37(4), 272-281

ABSTRACT

Testicular toxicity is a prime threat to male reproductive health. Humans are constantly exposed to Bisphenol A (BPA) via oral route, thus inducing reproductive toxicity. *Cucumeropsis mannii* seed oil (CMSO) has nutraceuticals with antioxidant potential. This present study investigated the potential of CMSO to mitigate testicular oxidative damages induced by exposure to BPA. Thirty-six (36) male Wistar albino rats, 2-3 months old, were randomly assigned into six (6) study groups; 3 control groups (CG1, CG2, and CG3) and 3 test groups (TG1, TG2, and TG3), respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg body weight of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg body weight of BPA and 7.5 ml/kg bodyweight of CMSO. TG2 rats were co-administered with 100 mg/kg body weight of BPA and 5 ml/kg body weight of CMSO. TG3 rats were co-administered with 100 mg/kg body weight of BPA and 2.5 ml/kg body weight of CMSO. After the trial, the rats' testes were extracted and taken for biochemical analysis. The result showed that the exposure of rats to BPA significantly ($p < 0.05$) increased the levels of ROS and MDA but considerably decreased the levels of GSH, CAT, SOD, GPx, IL-1β, IL-6, TNF-α, NF-κB, and Caspase-3 activity in the testicular homogenate. However, co-administration of BPA with CMSO significantly ($p < 0.05$) reversed all the dysregulations. This study reported that CMSO is a potential nutraceutical for mitigation of BPA-induced testicular oxidative damages.

Keywords: *CMSO, BPA, Testicular toxicity, Redox Homeostasis, Inflammations, Apoptosis*

INTRODUCTION

Environmental pollution, particularly from plastics, has become a global health challenge in recent years. Bisphenol A (BPA), a well-known plasticizer, has been linked to several reproductive diseases, including testicular damage, leading to male reproductive health decline (Huang *et al*., 2012; Agu *et al.*, 2022). When ingested into the body, BPA impairs testicular functions by disrupting the pro-oxidant/antioxidant balance of testicular cells, triggering downstream pathways like apoptosis (Ho *et al*., 1998; Mathur *et al*., 2008; Wang *et al*., 2017). While physiological levels of reactive oxygen species (ROS) and apoptosis are necessary for normal testicular function, pathological levels can be detrimental (Cimmino *et al*., 2020).

Surprisingly, BPA is present in our environment due to its numerous applications, including the manufacturing of food packaging materials and household items. Moreover, poor storage facilities available to beverage sellers in developing countries may have significantly contributed to the constant release of BPA into food stored at high temperatures. Thus, humans are explicitly or implicitly susceptible to several levels of BPA through oral ingestion (Ohore and Songhe, 2019), and exposed male workers are at a higher risk of male sexual dysfunction across all domains of sexual function (Ribeiro *et al*., 2017).

The potential of plant products to treat various diseases has a link to the varying levels of antioxidant components found in them. Several melon species, for example, are planted for their food value in West African countries. *Cucumeropsis mannii*, a species of melon known as the African white melon, has been reported to contain a wide range of nutrients with therapeutic potential and can thus be used as a nutraceutical. The seeds, which contain 72% essential oil (Jack *et al*., 1972), are also high in protein (31.4%), essential amino acids, fat (52.5%), essential fatty acids, minerals, and vitamins (Anhwange *et al*., 2010; Ogunbusola et al., 2010; Besong *et al*., 2011). Essential oils are valuable natural products for perfumes, cosmetics, aromatherapy, phytotherapy, spices, nutrition, and insecticides (Buchbauer, 2000). Recent evidence has shown that essential oils of plant origin, such as omega-3-polyunsaturated fatty acids (PUFAs), vitamin E, and beta-carotene, among other things, have preventive and ameliorative potential against toxicants (Famurewa *et al*., 2019), possibly through oxidative stress mitigation. Furthermore, flaxseed oil derived from *Linum usitatissimum* seeds, for example, has been shown to successfully reduce BPA-induced genotoxicity in male mice (El-Makawy *et al*., 2018).

Similarly, Elhamalawy *et al*. (2018) demonstrated that oil derived from *Sesamum indicum* seeds has promising antioxidant potential for protecting against the harmful effects of BPA. The reported nutraceutical contents of *C. mannii* seed, on the other hand, influenced the choice of its exploration for similar potential in the present study. Although other researchers have reported on the chemical characterization of the *C. mannii* seeds there are no pieces of literature on the effect of the seed oil on the BPA-induced testicular oxidative damages. As a result, this study investigated the antioxidant, anti-inflammatory, and antiapoptotic properties of *C. mannii* (African White Melon) seed oil in the testes of male rats exposed to BPA. Data from the study have further revealed the potential of *C. mannii* seed oil with insight into its utilization as nutraceuticals.

MATERIALS AND METHODS

Chemicals and reagents

Pure pellets of 2, 2-bis (4-hydroxyphenyl) propane (Bisphenol A) were obtained from Sigma Aldrich Company, the UK, via Bristol Scientific, and assay kits from Randox Laboratories, Ltd., the UK.

Cucumeropsis mannii (African White Melon) seed collection and authentication

A peasant farmer in Ndingwuta Village, Amachi-Ndebor, Abakaliki L.G.A., Ebonyi State, was the source of sun-dried *Cucumeropsis mannii* seeds. The seeds and vegetative section of the plant were brought to Ebonyi State University's Department of Applied Biology in Abakaliki, Nigeria, for authentication (EBSU-H-394).

Extraction of Essential oil

The seed oil was extracted by Kate *et al.* (2014) techniques and modified according to Agu *et al.* (2022). For an improved efficiency of the extraction, drops of water were introduced during the extraction to enhance the release of the oil. Further, the extracted oil was left to settle and purer form of the oil was recovered by decantation.

Animal handling

The animals were utilized following the Departmental Ethical Review Committee's recommendations (approval number: EBSU/BCH/ET/20/002) and the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, amended in 1996).

Acute Toxicity Study of CMSO

The acute toxicity research was conducted using the limit dosage up and down approach, as recommended by OECD/OCDE Guidelines No. 425 (OECD, 2008). In this study, the limit for the test dosage set for CMSO was 50 ml/kg. The medium/intermediate dosage was set at 10% of the limit dose (5 ml/kg), half of it (2.5 ml/kg) was set as the lower dose, and 1.5 times the middle dose (7.5 ml/kg) was set as the higher dose, according to OECD guideline No. 425.

Experimental Design

Thirty-six (36) male albino Wistar rats were randomly clustered into six (6) experimental groups of 1, 2, 3, 4, 5, and 6 (n = 6 rats). Groups 1, 2, and 3 were control groups designated CG1, CG2, and CG3, whereas groups 4, 5, and 6 were the treatment groups designated TG1, TG2, and TG3, respectively. Rats were administered both BPA and CMSO concurrently by oral route once every day for six weeks. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg body weight of BPA. CG3 rats under control received 7.5 ml/kg of body weight of CMSO. TG1 rats were co-administered with 100 mg/kg body weight of BPA and 7.5 ml/kg body weight of CMSO. TG2 rats were co-administered with 100 mg/kg body weight of BPA and 5 ml/kg body weight of CMSO. TG3 rats were co-administered with 100 mg/kg body weight of BPA and 2.5 ml/kg body weight of CMSO.

Testicular tissue sampling

The animals were sacrificed under moderate sedation at the end of the sixth week. Their testes were harvested for the preparation of testicular homogenate, according to the method of Alboghobeish *et al.* (2019), used for the biochemical analysis.

Biochemical analysis

Determination of oxidative stress markers level in the testicular homogenate of BPA-exposed male rats

The oxidative stress status of the rats' testes was estimated in the testicular homogenate. Levels of Reactive oxygen species (ROS), reduced glutathione (GSH), Superoxides dismutase (SOD), Catalase activity, Glutathione peroxidase activity, and Lipid peroxidation expressed in terms of malondialdehyde (MDA) were determined by the methods of Resim *et al.* (2015), Benke *et al.* (1974), Flohe and Otting (1984), Aebi (1984), Paglia and Valentine (1967), and Ohkawa *et al*. (1979), respectively.

Determination of levels of inflammatory mediators in the testicular homogenate of BPA-exposed male rats

Testicular homogenate interleukins (IL-1β, IL-6), tumor necrosis factor-α (TNF-α), and nuclear factor-kappa B (NF-κB) levels were measured using enzyme-linked immunosorbent assay (ELISA) rat kits following the manufacturer's protocols (Tsai *et al.*, 2015). Briefly, each well of a 96-well plate was seeded with the capture antibody for TNF-, NF-κB, IL-1, or IL-6 overnight. The next day, the second set of biotinylated antibodies was added to the plate and incubated with the sample tissues or standard antigens before streptavidin was added. At 450 nm, the reaction's color changed from purple to yellow and was seen. Each sample's TNF-, NF-κB, IL-1, and IL-6 cytokine concentrations were reported as picograms per milligrams (pg/mg) of protein.

Determination of levels of caspase 3 activity in the testicular homogenate of BPA-exposed male rats

The level of caspase 3 was determined using the Caspase-3 Activity Assay Kit which is a fluorescent assay that detects the activity of caspase-3 in cell lysates (Campos *et al.*, 2017; Moraes *et al.*, 2013). Briefly, cells were plated in 24-well plates at 105 cells per mL, grown in 10% FBS-containing media, and exposed to the IC50 of the AECL or AECR for 24 hours. The cells were then centrifuged, cleaned, and fixed in 2% paraformaldehyde in PBS for 30 minutes following treatment. The cells were then rinsed in PBS containing 0.1 M glicin, permeabilized for 30 min with 0.01% saponin, then blocked for 30 min at room temperature in PBS containing 1% BSA. The cells were then exposed to an anti-active-caspase-3 monoclonal antibody conjugated with FITC for 40 minutes at room temperature and in the dark. After incubation, a FACScalibur Flow Cytometer was used to assess fluorescence.

Statistical Analysis

All results were analyzed using GraphPad Prism 5. Data were expressed as mean ± standard deviation. The means of the parameters were compared using one-way ANOVA and $P<0.05$ was considered a statistically significant level in the Turkey analysis.

RESULTS

As shown in Figures 1-6 after BPA administration, ROS and MDA concentrations in the testicular homogenates were significantly ($p < 0.05$) elevated. Besides, BPA administration significantly ($p < 0.05$) decreased activities of CAT, SOD, GPx, and the level of GSH in testicular homogenates. However, the levels of ROS and MDA in testicular homogenate were significantly ($p < 0.05$) lowered

when BPA and CMSO were co-administered to the rats as shown in Figures 1-2. Also, there was a significant ($p < 0.05$) increase in the level of GSH with co-administration of BPA and CMSO (7.5ml) (Figure 3). Activities of CAT, SOD, and GPx were significantly ($p < 0.05$) elevated when BPA and CMSO were co-administered in rats as shown in Figures 1-6. No significant difference ($p < 0.05$) was observed among the co-administered groups.

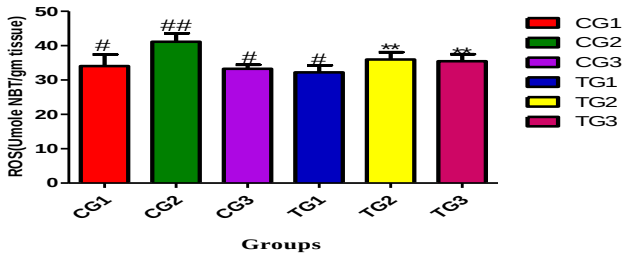

Figure 1. Effect of CMSO on Reactive Oxygen Species in BPA-induced testicular toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/Kg b.w. of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

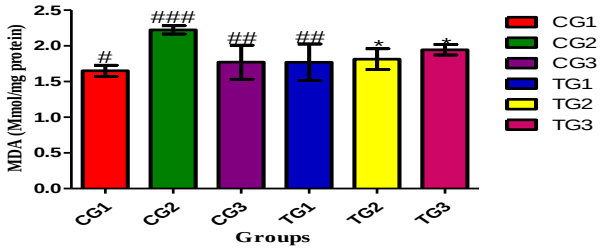

Figure 2. Effect of CMSO on Malondialdehyde level in testicular homogenates of BPA exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (C. mannii Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/Kg b.w. of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

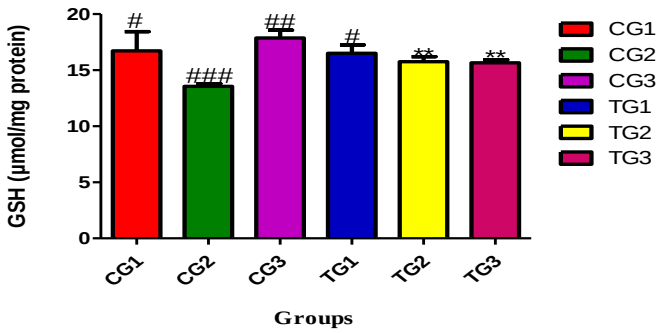

Figure 3. Effect of CMSO on Reduced glutathione level in testicular homogenates of BPA exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with different signs are significantly different at $P < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg b.w. of BPA. CG3 rats received 7.5 ml/kg of body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

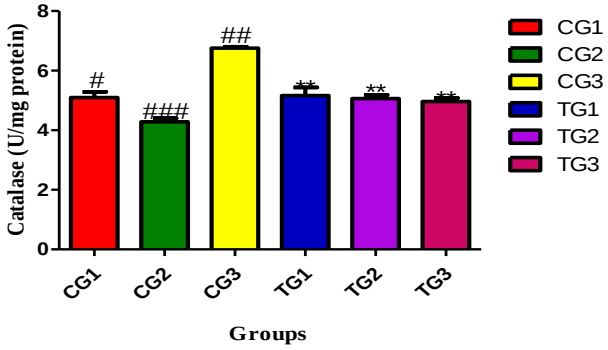

Figure 4. Effect of CMSO on Catalase activity in testicular homogenates of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg b.w. of BPA. CG3 rats received 7.5 ml/kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of

CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

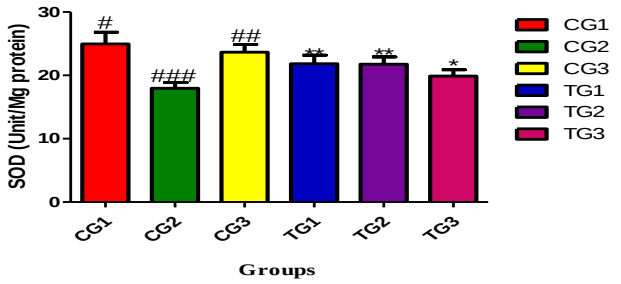

Figure 5. Effect of CMSO on Superoxide dismutase activity in testicular homogenates of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg b.w. of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

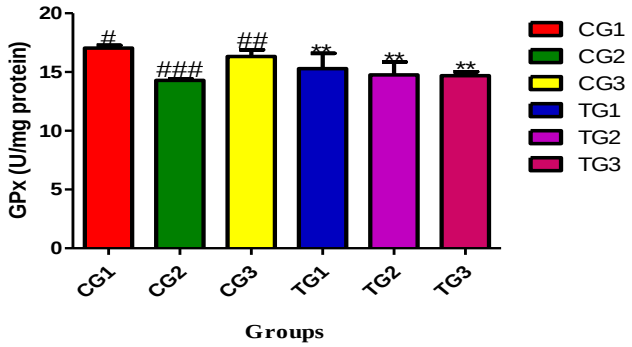

Figure 6. Effect of CMSO on Glutathione peroxidase activity in testicular homogenates of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/Kg b.w. of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

BPA administration in rats significantly ($p < 0.05$) upregulated the testicular expression of nuclear factor-kappa B (NF-κB) and significantly ($p < 0.05$) increased levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) but the co-administration of BPA and CMSO in rats significantly ($p < 0.05$) lowered the levels of these inflammatory markers in rat's testicular homogenate (Figures 7-10).

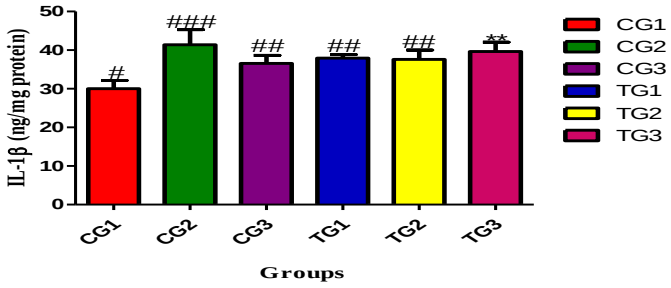

Figure 7. Effect of CMSO on Interleukin-1β level in testicular homogenates of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/Kg b.w of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

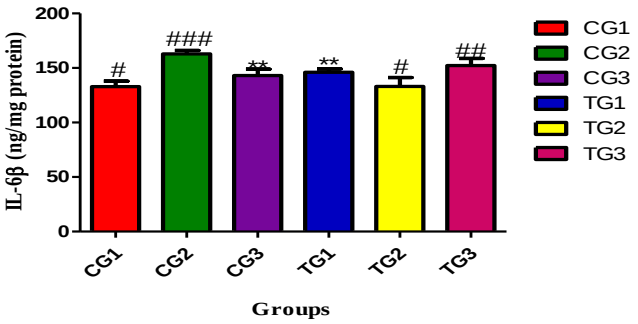

Figure 8. Effect of CMSO on Interleukin-6β level in the testicular homogenate of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/Kg b.w. of BPA. CG3 rats received 7.5 ml/kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of

CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

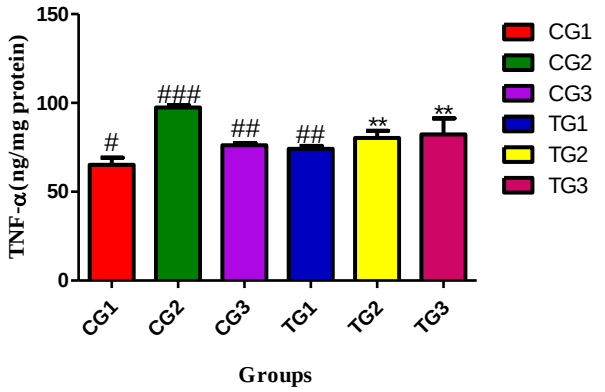

Figure 9: Effect of CMSO on Tumor necrosis factor-α level in the testicular homogenate of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at p < 0.05. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/Kg b.w of BPA. CG3 rats received 7.5 ml/Kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

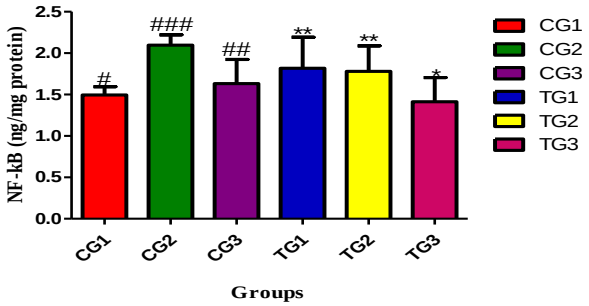

Figure 10: Effect of CMSO on Nuclear factor-kappa B level in the testicular homogenate of BPA exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at p < 0.05. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg b.w. of BPA. CG3 rats received 7.5 ml/kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

BPA administration in rats significantly ($p<0.05$) up-regulated caspase-3 activity whereas the co-administration of BPA and CMSO in rats significantly ($p<0.05$) lowered the caspase-3 activity in rats' testicular homogenate (Figure 11).

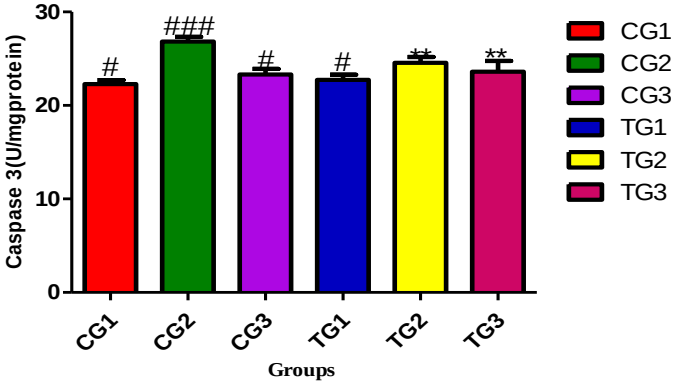

Figure 11: Effect of CMSO on Caspase-3 activity in the testicular homogenate of BPA-exposed male rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at p < 0.05. BPA (Bisphenol A), CMSO (*C. mannii* Seed Oil). Control groups were designated as CG1, CG2, and CG3, and 3 test groups were designated as TG1, TG2, and TG3 respectively. CG1 rats received 1 ml of olive oil. CG2 rats received 100 mg/kg b.w. of BPA. CG3 rats received 7.5 ml/kg body weight of CMSO. TG1 rats were co-administered 100 mg/kg b.w of BPA and 7.5 ml/kg b.w of CMSO. TG2 rats were co-administered 100 mg/kg b.w of BPA and 5 ml/kg b.w of CMSO. TG3 rats were co-administered 100 mg/kg b.w of BPA and 2.5 ml/kg b.w of CMSO.

DISCUSSION

Spermatogenesis and steroidogenesis are two critical and high-energy-demanding activities performed by the testes. Many intra-testicular and extra-testicular regulatory systems control normal spermatogenesis. Redox homeostasis may be a key focus for reducing testicular toxicity on multiple levels. In the present study, BPA administration significantly increased ROS and MDA levels in testicular homogenates but decreased GSH, CAT, SOD, and GPx levels. When BPA and CMSO were co-administered in rats, ROS and MDA levels decreased significantly while GSH, CAT, SOD, and GPx levels increased significantly (Figures 1-6). Previous studies in rat models have shown that antioxidants reduce BPA-induced oxidative stress in the testis by increasing MDA levels, decreasing GSH levels, and decreasing the activities of CAT, SOD, and GPx (Wu *et al*., 2013; Othman *et al*., 2016; Aydogan *et al*., 2009; Wang *et al*., 2016; Chitra *et al*., 2003; Tamilselvan *et al*., 2013). The current findings

show that BPA administration decreases the activities of enzymatic (SOD, CAT, and GPx) and non-enzymatic (GSH) antioxidants. As evidenced by a marked increase in MDA levels, the administration of BPA also increased ROS and lipid peroxidation levels, resulting in oxidative stress induction. In combination with BPA, on the other hand, CMSO reversed the oxidative stress signals, possibly by protecting the testicular cell. More so, this effect may be due to CMSO's high lipids and other exogenic antioxidants, all of which can scavenge free radicals and prevent phospholipid bilayer breakdown/oxidation. Previous investigations by Famurewa *et al*. (2018) in rats treated with methotrexate revealed the therapeutic function of essential oil against ROS-induced cytotoxicity.

Abundant evidence has shown that extracts from plants such as *Aloe vera* (Behmanesh *et al*., 2018), *Cordyceps militaris* (Cui, 2014), *Cistanche tubulosa* (Hu and Kitts, 2000), *Trigonella foenum-graecum*, and *Lespedeza cuneata* (Park *et al*., 2018) have antioxidant properties against BPA-induced toxicity. Therefore, CMSO modulated the redox homeostasis in the rat testis via protection of the testicular membrane to maintain decreased MDA and stabilize the GSH concentrations, thus lowering the concentrations of free oxygen radicals and halting lipid peroxidation in testicular tissues.

Similarly, Aja *et al*. (2020) reported the implication of increased oxidative stress in inflammation. BPA administration significantly increased the levels of inflammatory markers such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and nuclear factor-kappa B (NF-κB) in rats, whereas co-administration of BPA with CMSO significantly decreased the levels of these inflammatory markers at the highest dose of CMSO (7.5 ml/kg bw), except for interleukin-6 (IL-6) (Figure 7-10). These findings are consistent with several observations in the literature on the interaction between oxidative stress, proinflammation, and apoptosis (Jiao *et al*., 2019; Nna *et al*., 2017; Famurewa *et al*., 2019; Aja *et al*., 2020). Nuclear factor-kappa B (NF-κB) is a redox-sensitive transcription factor that controls several genes involved in inflammatory responses and is inducible in all cells (Jin et al., 2020). BPA-induced oxidative stress may have increased the DNA-binding affinity of NF-κB by triggering the production of an inhibitor of kappa B kinase (IKK) for degradation of the inhibitor of kappa B (IκB). Furthermore, NF-κB activated cytoplasm-to-nucleus translocation, which controls the production of several target genes like TNF-α, IL-2β, and IL-6β (Yan *et al*., 2018). The stimulation of the NF-κB signaling pathway, on the other hand, is crucial in testicular toxicity (Bashir *et al*., 2016). Excess IL-6β, TNF-α, IL-1β, and NO levels are thought to be blocked by inhibiting the NF-B signaling pathway, according to research (Rehman *et al*., 2020; Wang *et al*., 2018; da Silva *et al*., 2019; Kandemir *et al*., 2017).

Caspases are mediators of cell death that are programmed, named apoptosis. Caspase-3 is a commonly activated death protease that catalyzes the specific cleavage of some cellular proteins (Li *et al*., 2009). In the current work, BPA treatment raised caspase-3 activity in rats considerably but co-administration of BPA with CMSO significantly decreased caspase-3 expression in a dose-dependent way (Figure 11). This investigation suggests that BPA's activation of caspase-3 may be attributed to oxidative stress and cytokine overexpression. Consequently, the combination of CMSO and BPA reduced oxidative stress and NF-kB activation by suppressing the production of proinflammatory cytokines and lowering caspase-3 levels in Sertoli and Leydig cells. Other investigations have found that BPA causes apoptosis in mice (Li *et al*., 2009) and goat testis Sertoli cells by increasing caspase-3 (Zhang *et al*., 2017). CMSO, like fenugreek (Trigonella foenum-graecum), regulated caspase-3 in mice with BPA-induced testicular injury (Li *et al*., 2009; Kaur and Sadwal, 2019). Hence, inhibition of NF-κB/IL-2β/ IL-6β/ TNF-α and caspase-3 in BPA-induced testicular damage in male Wistar albino rats is due to the high concentration of nutraceuticals in CMSO.

CONCLUSION

The overall result of the present study shows that exposure to BPA mediates testicular toxicity by inducing oxidative stress. This increased oxidative stress mechanistically provokes inflammatory mediators and further initiate apoptosis. Concomitantly, this plethora of events disrupts the normal biochemistry and physiology of the testes, thereby impairing male reproductive health. This study shows that, with the co-administration of CMSO, the dysregulations caused by BPA were prevented. We report that CMSO has antioxidant, anti-inflammatory, and anti-apoptotic potential due to its potential to mitigate oxidative damage caused by BPA. Therefore, CMSO could be a promising therapeutic for inflammation and apoptosis prevention within the testicular milieu.

AUTHORS' CONTRIBUTIONS

Conceptualization: PMA., OGA, and PCA. Data curation: PCA., EUE, PMA and HAO Formal Analysis: JNA. and PCA Funding Acquisition: DCC, PCA, BAA, HAO. and PMA Investigation: PMA, DCC, PCA, HAO, FEN, AAA, JNA, FCN, EUE. and OUU. Methodoogy: PMA, OGA and PCA. Project Administration: PMA and PCA. Resources: DCC., PCA, HAO, FEN, EME, Ukachi, OUU, OUO., PCN,

FCN, EUE and GOE Supervision: PMA and UEE. Validation: IOI, EUA. Writing original Draft: PCA. and EKM. Writing – Review and Editing: PMA. and BAA. All the authors consent to be accountable to every aspect of the paper and approved the final version for publication.

FUNDING STATEMENT

The authors declare that they have no conflict of interest

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ACKNOWLEDGEMENT

This study received administrative support from the Department of Biochemistry, Faculty of Sciences, Ebonyi State University, Abakaliki, Nigeria.

REFERENCES

Aebi, H. (1984). Catalase *in vitro*. In: Packer L edition, Methods of Enzymology. *Academic Press*, New York, pp, 121–126.

Agu, P. C., Aja, P. M., Ekpono, Ugbala Ezebuilo, Ogwoni, H. A., Ezeh, E. M., Oscar-Amobi, P. C., Agbor Asuk Atamgba, Ani, O. G., Awoke, J. N., Nwite, F. E., Ukachi, O. U., Orji, O. U., Nweke, P. C., Ugbala, Ejike Ekpono, Ewa, G. O., Igwenyi, I. O., Egwu, C. O., Alum, E. U., Chukwu, D. C. and Famurewa, A. C. (2022). *Cucumeropsis mannii* seed oil (CMSO) attenuates alterations in testicular biochemistry and histology against Bisphenol A-induced toxicity in male Wister albino rats. *Heliyon*, 8: e09162

Aja, P. M., Izekwe, F. I., Famurewa, A. C., Ekpono, E. U., Nwite, F. E., Igwenyi, I. O., Awoke, J. N., Ani, O. G., Aloke, C., Obasi, N. A., Udeh, K. U. and Ale, B. A. (2020). Hesperidin protects against cadmium-induced pancreatitis by modulating insulin secretion, redox imbalance, and iNOS/NF-κB signaling in rats. *Life Sciences*, 259: 118268.

Alboghobeish, S., Mahdavinia, M., Zeidooni, L., Samimi, A., Oroojan, A. A., Alizadeh, S., Dehghani, M. A., Ahangarpour, A. and Khorsandi, L. (2019). Efficacy of naringin against reproductive toxicity and testicular damages induced by bisphenol A in rats. *Iranian Journal of Basic Medical Science*, 22: 315-323.

Anhwange, B. A., Ikyenge, B. A., Nyiatagher, D. T. and Ageh, J. T. (2010). Chemical analysis of *Citrullus lanatus* (Thunb.), *Cucumeropsis mannii* (Naud.), and *Telfairia occidentalis* (Hook F.) seeds oils. Journal *of Applied Sciences Research*, 6(3): 265–268.

Aydo˘gan, M., Korkmaz, A., Barlas, N. and Kolankaya, D. (2009). Pro-oxidant effect of vitamin C coadministration with bisphenol A, nonylphenol, and octylphenol on the reproductive tract of male rats. *Drug Chemistry and Toxicology*, 33: 193–203.

Bashir, N., Manoharan, V. and Miltonprabu, S. (2016). Grape seed proanthocyanidins protect against cadmium-induced oxidative pancreatitis in rats by attenuating oxidative stress, inflammation, and apoptosis via Nrf-2/HO-1 signaling. *Journal of Nutritional Biochemistry*, 32: 128–141.

Behmanesh, M. A., Najafzadehvarzi, H. and Poormoosavi, S. M. (2018). Protective effect of aloe vera extract against bisphenol A-induced testicular toxicity in Wistar rats. *Cell Journal*, 20(2): 278-283.

Benke, G. M., Cheever, K. L., Mirer, F. E., and Murphy, S. D. (1974). Comparative toxicity, anticholinesterase action, and metabolism of methyl parathion and parathion in sunfish and mice. *Toxicology & Applied Pharmacology*, 197: 2897–110.

Besong, S. A., Ezekwe, M. O., Fosung, C. N., and Senwo, Z. N. (2011). "Evaluation of nutrient composition of African melon oilseed (*Cucumeropsis mannii* Naudin) for human nutrition. *International Journal of Nutrition and Metabolism*, 3(8): 103–108.

Buchbauer, G. (2000). The detailed analysis of essential oils leads to acc n understanding of their properties. *Perfumer and flavourist*, 25: 64-67.

Campos, J. F., Espindola, P. P. dT., Torquato, H. F. V., Vital, W. D., Justo, G. Z., Silva, D. B., Carollo, C. A., de Picoli, Souza, K., Paredes-Gamero, E. J. and dos Santos, E. L. (2017). Leaf and Root Extracts from Campomanesia adamantium (Myrtaceae) Promote Apoptotic Death of Leukemic Cells via Activation of Intracellular Calcium and Caspase-3. Frontier in Pharmacology, 8: 466.

Chitra, K. C., Latchoumycandane, C. and Mathur, P. P. (2003). Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology*, 185: 119–127.

Cimmino, I., Fiory, F., Perruolo, G., Miele, C., Beguinot, F., Formisano, P. and Oriente, F. (2020). Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease. *International Journal of Molecular Sciences*, 21: 1561.

Cui, J. D. (2014). Biotechnological production and applications of Cordyceps militaris, a valued traditional Chinese medicine. *Critical Review in Biotechnology*, 35: 475–484.

da Silva, L. M., Pezzini, B. C. Somensi, L. B., Mariano, L. N., Mariott, M. and Boeing, T. (2019). Hesperidin, a citrus flavanone glycoside, accelerates the gastric healing process of acetic acid-induced ulcers in rats. *Chemistry and Biological Interactions*, 308: 45–50.

EL makawy, A., Eissa, F. I. and EI-Bamby, M. (2018). Flaxseed oil as a protective agent against bisphenol-A

deleterious effects in male mice. *Bulletin of the National Research Centre*, 42(1): 5-9.

Elhamalawy, O. S., Eissa, F. I., ELmakawy, A. and EI-Bamaby, M. M. (2018). Bisphenol-A Hepatotoxicity and the Protective Role of Sesame Oil in Male Mice. *Jordan Journal of Biological Sciences*. 11(4): 461-467.

Famurewa, A. C., Asogwa, N. T., Aja, P. M., Akunna, G. G., Awoke, J. N., Ekeleme-Egedigwe, C. A. Maduagwuna, E. K., Folawiyo, A. M., Besong, E. E., Ekpono, E. U. and Nwoha, P. A. (2019). Moringa oleifera seed oil modulates redox imbalance and iNOS/NF-κB/caspase-3 signaling pathway to exert antioxidant, anti-inflammatory, and antiapoptotic mechanisms against anticancer drugs 5-fluorouracil-induced nephrotoxicity in rats. *South African Journal of Botany*, 127: 96–103.

Flohe, L., and Otting, F. (1984). Superoxide dismutase assays. *Methods in Enzymology*, 105: 101-104.

Ho, Y. S., Magnenat, J. L., Gargano, M., and Cao, J. (1998). The nature of antioxidant defense mechanisms: a lesson from transgenic studies. *Environmental Health Perspective*, 106:1219-1225.

Hu, C. and Kitts, D. D. (2000). Studies on the antioxidant activity of Echinacea root extract. *Journal of Agricultural and Food Chemistry*, 48: 1466–1472.

Huang, Y. Q., Wong, C. K., and Zheng, J. S. (2012). Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. *Environment International*, 42: 91–99

Jacks, T. J., Hensarling, T. P. and Yatsu, L. Y. (1972). Cucurbit seeds: I. Characterizations and its uses of oils and proteins, a review. *Economics and Botany*, 26: 135-141.

Jiao, D., Jiang, Q., Liu, Y. and Ji, L. (2019). Nephroprotective effect of wogonin against cadmium-induced nephrotoxicity via inhibition of oxidative stress-induced MAPK and NF-kB pathway in Sprague Dawley rats, *Human and Experimental Toxicology*, 38: 1082–1109.

Jin, W., Xue, Y., Xue, Y., Han, X., Song, Q. and Zhang, J. (2020). Tannic acid ameliorates arsenic trioxide-induced nephrotoxicity, contribution of NF-κB and Nrf2 pathways, *Biomedicine and Pharmacotherapy*, 126: 110047.

Kandemir, F. M., Kucukler, S., Caglayan, C., Gur, C., Batil, A. A. and Gülçin, I. (2017). Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. *Journal of Food Biochemistry*, 41: 12398.

Kate, A. E., Lohani, U. C., Pandey, J. P., Shahi, N.C. and Sarkar, A. (2014). Traditional and mechanical method of the oil extraction from wild apricot kernel: a comparative study. *Research Journal of Chemistry and. Environmental Science*, 2(2): 54-60.

Kaur, S. and Sadwal, S. (2019). Studies on the phytomodulatory potential of fenugreek (Trigonella foenum-graecum) on bisphenol-A induced testicular damage in mice. *Andrology*, 52: e13492.

Li, Y-J., Song, T-B., Cai, Y-Y., Zhou, J-S., Song, X., Zhao, X. and Wu, X-L. (2009). Bisphenol A Exposure Induces Apoptosis and Upregulation of Fas/FasL and Caspase-3 Expression in the Testes of Mice. *Toxicological Sciences*, 108(2): 427–436.

Mathur, P. P, Saradha, B., and Vaithinathan, S. (2008). Impact of environmental toxicants on testicular function. *Immunology Endocrine Metabolic Agents of Medical Chemistry* 8: 79–90.

Moraes, V. W. R., Caires, A. C. F., Paredes-Gamero, E. J., and Rodrigues, T. (2013). Organopalladium compound 7b targets mitochondrial thiols and induces caspase-dependent apoptosis in human myeloid leukemia cells. *Cell Death Diseases*, 4: 1–8.

Nna, V. U., Ujah, G. A., Mohamed, M., Etim, K. B., Igba, B. O., Augustine, E. R. and Osima, E. E. (2017). Cadmium chloride-induced testicular toxicity in male Wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal, *Biomedical Pharmacotherapy*, 94: 109–123.

OECD. (2008). Acute Oral Toxicity Testing Procedures. *http://www.oecd.org/env/testguidelines*.

Ogunbusola, E. M., Fagbemi, T. N., and Osundahunsi, O. F. (2010). Amino acid composition and protein quality of white melon (*Cucumeropsis mannii*) flour, *Nigerian Food Journal*, 28(1): 14–21.

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. *Annals of Biochemistry*, 95: 351–358.

Ohore, O. E., and Songhe, Z. (2019). Endocrine-disrupting effects of bisphenol A exposure and recent advances on its removal by water treatment systems-A review. *Scientific African*, 5: e00135.

Othman, A. I., Edrees, G. M., El-Missiry, M. A. Ali, D. A., Aboel-Nour, M. and Dabdoub, B. R. (2016). Melatonin controlled apoptosis and protected the testes and sperm quality against bisphenol A-induced oxidative toxicity. *Toxicology and Industrial Health*, 32: 1537–1549.

Paglia, D. E., and Valentine, W. M. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory & Clinical Medicine*, 70: 158–69.

Park, B., Kwon, J. E., Cho, S. M., Kim, C. W., Lee, D. E., Koo, Y. T., Lee, S. H., Lee, H. M. and Kang, S. C.

(2018). Protective effect of Lespedeza cuneata ethanol extract on Bisphenol A-induced testicular dysfunction in vivo and in vitro. *Biomedicine and Pharmacotherapy*, 102: 76–85.

Rehman, K., Munawar, S. M., Akash, M. S., Buabeid, M. A., Chohan, T. A., and Tariq, M. (2020). Hesperidin improves insulin resistance via downregulation of inflammatory responses: biochemical analysis and in silico validation. *PLoS One*, 15: 0227637.

Resim, S., Kurutas, E. B., Gul, A. B., Eren, M., Benlioglu, C., Efe, E. and Atli. Y. (2015). The Levels of Oxidative Stress Biomarkers in Rats as a Response to Different Techniques of Testicular Biopsy. *Indian Journal of Surgery*, 77(2): 310-313.

Ribeiro, T. P., Fonseca, F. L., de Carvalho, M. D., Godinho, R. M., de Almeida, F. P., Saint'Pierre, T. D., Rey, N. A., Fernandes, C., Horn, A. and Pierre, M. D. (2017). Metal-based superoxide dismutase and catalase mimics reduce oxidative stress biomarkers and extend the life span of Saccharomyces cerevisiae. *Biochemical Journal*, 474(2): 301-315.

Tamilselvan, P., Bharathiraja, K., Vijayaprakash, S. and Balasubramanian, M. P. (2013). Protective role of lycopene on bisphenol A-induced changes in sperm characteristics, testicular damage, and oxidative stress in rats. *International Journal of Pharmacy and Biological Science*, 4: 131–143.

Tsai, D. S., Huang, M. H., Tsai, J. C., Chang, Y. S., Chiu, Y. J., Lin, Y. C., Wu, L.Y., Peng, W. H. (2015). Analgesic and Anti-Inflammatory Activities of *Rosa taiwanensis* Nakai in Mice. *Journal of Medicinal Food*, 18(5): 592-600.

Wang, H., Ding, Z., Shi, Q. M., Ge, X., Wang, H. X., Li, M. X., Chen, G., Wang, Q., Ju, Q. and Zhang, J. P. (2017). Anti-androgenic mechanisms of Bisphenol A involve androgen receptor signaling pathways. *Toxicology*, 387: 10–16.

Wang, J., Chen, C., Jiang, Z., Wang, M., Jiang, H. and Zhang, X. (2016). Protective effect of *Cordyceps militaris* extracts against bisphenol A-induced reproductive damage. *System Biology and Reproductive Medicine*, 62: 249–257.

Wang, L., He, T., Fu, A., Mao, Z., Yi, L., Tang, S. and Yang, J. (2018). Hesperidin enhances angiogenesis via modulating expression of growth and inflammatory factor in diabetic foot ulcers in rats. *European Journal of Inflammation*, 20: 1–13.

Wu, H.-J., Liu, C., Duan, W.-X., Xu, S.-C., He, M.-D., Chen, C.-H.; Wang, Y.; Zhou, Z.; Yu, Z.-P.; Zhang, L. (2013). Melatonin ameliorates bisphenol A-induced DNA damage in the germ cells of adult male rats. *Mutation Research in Toxicology and Environmental Mutagenesis*, 752: 57–67.

Yan, N., Wen, D.-S., Zhao, Y.-R. and Xu, S.-J. (2018). Epimedium sagittatum inhibits TLR4/MD-2 mediated NF-κB signaling pathway with anti-inflammatory activity, BMC Complementary and *Alternative Medicine*, 18: 303–311.

Zhang, Y., Han, L., Yang, H., Pang, J., Li, P., Zhang, G., Li, F., and Wang, F. (2017). Bisphenol A affects cell viability involved in autophagy and apoptosis in goat testis Sertoli cells. *Environmental Toxicology and Pharmacology*, 2832: 13-25.