J Bangladesh Agril Univ 19(2): 223–229, 2021 https://doi.org/10.5455/JBAU.68360

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Beneficial Effects of Black Seed Oil on Blood, Hormone Profile, Sperm Parameters and Histotexture of Testis of Male Mice Exposed to Bisphenol-A

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ARTICLE INFO

Article history
Received: 28 Mar 2021
Accepted: 16 Apr 2021
Published: 30 Jun 2021

Keywords
Bisphenol-A,
Black Seed Oil, Mice,
Hematology, T4,
Testosterone,
Sperm and Testis

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ABSTRACT

Bisphenol-A (BPA) is an endocrine disruptor that is widely used in the manufacture of polycarbonate plastics, epoxy resins and dental sealants. It has adverse effects on reproduction. Black seed oil is frequently used as folk medicine for the promotion of good health and treatment of many ailments. The study was carried out to investigate the effect of Bisphenol-A (BPA) and black seed oil (BSO) on hematological parameters, hormone, sperm parameters and patho-physiological changes of testis in mice. A total of 15 male Swiss Albino mice (*Mus musculus*), of 25-28 days were used and randomly divided into three groups (A, B, and C) consisting five mice in each group. Mice of groups B and C were administered orally with BPA (50 mg/kg/day) and BPA plus BSO (1 ml/kg/day) while group A served as vehicle control. Results revealed that BPA caused reduced hematological parameters (Hb, TEC, and PCV) in mice. Inclusion of BSO in BPA treated mice prevented the alterations of these values. Serum T_4 and testosterone concentration were significantly ($p<0.01$) decreased in the BPA-treated mice whereas BSO dramatically improved the levels of these two hormones. There was reduction in the sperm count and sperm motility along with increased head and tail abnormalities in BPA treated mice. BSO reduced the abnormal alterations of sperm parameters. Histopathological analysis of testis demonstrated that there were degenerative and necrotic changes in the seminiferous tubules in mice upon treatment with BPA. It can be concluded that BPA has harmful effects on hematology, hormonal assay, sperm parameters and testis. These harmful effects could be alleviated by ingestion of black seed oil.

Introduction

Endocrine disrupting chemicals (EDCs) become a public health concern due to increase the incidences of impaired reproductive functions in humans and animals (Diamanti-Kandarakis *et al.,* 2009). Bisphenol-A (BPA), is widely known as an EDC that can interfere with the endocrine system (Dang *et al.,* 2007; Choi *et al.,* 2003). It is used as chemicals in many products, such as dental sealants and thermal paper receipts, food packaging, epoxy resins, polycarbonate plastics, water and food plastic containers, baby bottles and feeders (Kim *et al.,* 2010; Singh and Li, 2012). Human and animal may be exposed to BPA by ingestion, inhalation and dermal exposure. Ingestion or oral intake of food and water are the main exposure route in human being. It is reported that a human body is normally exposed to 10 µg/ day of BPA (Geens *et al.,* 2012). BPA can be detected in urine, serum, placental tissue and even in the fetal liver (Kasper-Sonnenberg *et al.,* 2014) and its residues were found in surface water, in fish tissues and can transfer from cans to food and from polycarbonate baby bottles to milk (Wisniewski *et al.,* 2015). BPA reduces the epididymal weight, testicular weight, testicular and epididymal sperm counts, plasma testosterone levels and causes structural deformities of sperms in rodents (Al-Hiyasat *et al.,* 2002). BPA can suppress spermatogenesis and decrease semen quality by impairing meiosis, inducing apoptosis of spermatids, or depressing the function of hypothalamic-pituitary-gonadal axis (HPG axis) (Manfo *et al.,* 2014). Environmental exposure to BPA is associated with many disorders in humans including heart failure (Shankar *et al.,* 2012), kidney diseases (Li *et al.,* 2012) and immune system dysfunction (Holladay *et al.,* 2010). Medicinal plants and their constituents have therapeutic potentials. World Health Organization (WHO) reported that traditional medicine won the trust of 70–80% of people in primary health care (Bashandy, 2007) and draws about 30% of all modern medicines (WHO, 2002). The use of herbal medicine increases every day and

Cite This Article

Sujan, K.M., Miah, M.A., Mustari, A., Islam, M.K. 2021. Beneficial Effects of Black Seed Oil on Blood, Hormone Profile, Sperm Parameters and Histotexture of Testis of Male Mice Exposed to Bisphenol-A. *Journal of Bangladesh Agricultural University*, 19(2): 223–229. https://doi.org/10.5455/JBAU.68360

traditional herbs have more acceptance than prescribed drugs. The medicinal plant, *Nigella sativa* commonly known as black cumin, black seed, belonging to the family Ranunculaceae. It is native plant from the Mediterranean area and also found in other parts of the world used for culinary and medical purposes (Rifat-uz-Zaman and Khan, 2004; Kamal *et al.,* 2010). Alcoholic extract of *Nigella sativa* significantly increases the body weight gain, reproductive parameters (seminiferous tubules thickness and diameters, account of spermatogonia, primary and secondary spermatocytes, spermatids, account of sertoli and leydig cells, diameter of leydig cells and the height of epithelial cells entirely covered epididymal caudal and influences the testosterone and follicle stimulating hormone (Al-Sa'aidi *et al.,* 2009). The *Nigella sativa* seeds suspension caused significant increase in ejaculation volume, sperm activities and motility with enhancement of accessory glands secretion in rams (Al-Zamily, 2008) and in male rat, it causes enhancement of testes histological function with decrease in the sperm's abnormalities (Al-Mayali, 2007). Scientists have been searching for the appropriate measures to counter the hazardous effects of EDC. Few studies were performed in relation to BPA and BSO on blood and reproductive organs in mice and found that BPA is one of the potential risk factors for hyperlipidemia, obesity, anemia, hormonal homeostasis disturbances and granulosa cells degeneration and these harmful effects could be alleviated by the ingestion of black seed oil. The present research work was investigated the counteraction effects of BSO on physiological alteration in BPA treated male mice.

Materials and Methods

Experimental design

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh, from 1 February to 25 April 2018. In this study, total 15 male Swiss Albino mice (*Mus musculus*), aged 25-28 days with an average body weight of 27.4 g were used. Mice used were purchased from ICDDR'B, Dhaka. At first, mice were randomly divided into three groups viz., A, B and C consisting five mice in each group. Group A served as vehicle control (received only basal mouse pellet feed mixed with sunflower oil) whereas group B was administered with bisphenol-A (BPA) @ 50 mg/kg body weight daily, formulated in sunflower oil (as vehicle for BPA), while group C received BPA and black seed oil (BSO) @ 50 mg/kg bw/day and 1ml/kg bw/day, respectively. The experiment was carried out for a period of 12 weeks. Mice were reared in a compartmentalized square wooden cages wrapped with wire mesh under controlled conditions of temperature (26-30) °C and relative humidity of 70-80% with natural day light. BPA was purchased from Sigma-Aldrich Company, USA. BSO, i.e. pure oil extract of *Nigella sativa* L. seeds was bought from local market in Mymensingh.

Ethical approval

The present study and all experimental procedures were approved and performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh [AWEEC/BAU/2019(36)].

Blood collection and serum preparation

Blood samples were collected by cardiac puncture at 12th week by sacrificing the mice. About 1 to 1.5 ml blood was collected and transferred half of blood into anticoagulant containing eppendorf tube and the remaining half of blood was transferred to another tube without anticoagulant for serum preparation. Sera were separated by centrifugation and collected by using 200 µl pipettes. Serum samples were stored in capped tube at -20ºC for hormonal analysis.

Hematological parameters

Hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Erythrocyte indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured as per methods described by Ghai (2012).

Hormonal assay

The hormonal parameters: Serum Testosterone and Serum Thyroxine (T_4) were determined by using Testosterone Radioimmunoassay Kit and T_4 Radioimmunoassay Kit, respectively at the Institute of Nuclear Medicine & Allied Sciences (INMAS), Mymensingh Medical College.

Sperm physiological parameters

At the end of the experiment, all the mice were sacrificed after anesthesia and testicles were removed to collect epididymis for evaluation of sperm. Epididymal sperm motility was tested by transferring a small amount of the diluted suspension (PBS) on a pre-warmed slide and then applying a cover slip and observed at low power of the microscope. At least ten widely-spaced fields were examined to provide an estimate of the percentage of motile sperms. Sperm motility was recorded in percentage (%) (Saalu *et al.,* 2010). Cauda epididymis from mice was collected in petri-dish and it was minced with sharp scissors. Torned epididymis was transferred to a test tube containing 4 ml phosphate buffer saline at 37°C temperature. Then the sperms were allowed to disperse for 5-10 min and sperm counts were made in

the Neubauer's chamber using a pipette. Sperms were observed at high power of the microscope (Del Val and Robledano, 2013). Sperm head morphology evaluated by the process adopted by Luke *et al*. (2014). Briefly, 1 ml of sperm suspension was transferred to a marked test tube. Then 5-6 drops of 1% Eosin yellow were added to it and gently mixed it by simple finger tapping. 45 min incubation was done in room temperature to facilitate the staining. Then the suspension was agitated gently by pipette. Smear was prepared by simple push technique. The smears were air-dried and preserved by mounting with cover slips. About 100 sperms/mice from each experimental group were examined at high power.

Histopathology

The testis (n = 3) from each group of mice were collected after completely removal of blood by perfusion with phosphate buffered saline and kept in 10% neutral buffered formalin for 15 days. The well-fixed tissues were processed, sectioned and stained with Hematoxylin and Eosin (H & E) for histo-pathological as per standard procedure Banchroft *et al.* (1996) in collaboration with the Department of Pathology, Mymensingh Medical College, Mymensingh. The stained slides were observed under Optika Vision Lite 21 and photographs of these slides were taken in the Department of Physiology, Bangladesh Agricultural University.

Statistical analysis

Data were entered in Microsoft Excel- 2010 and exported to the software IBM SPSS Statistics 20 for analysis. One-way analysis of variance (ANOVA) was performed to determine the effect of different parameters. Descriptive statistics analysis was done to measure the mean, standard deviation and standard error and p value of different parameters. Because of using multiple comparisons, the corrected p value was calculated adjusted at 0.01 and 0.05 considered for level of significance.

Results

Effect of BPA and BSO on blood parameters in mice

The effects of BPA on hematology and countering action of BSO on those effects were evaluated in male Swiss Albino mice (Table 1). Data revealed that hematological parameters including Hb concentration, TEC and PCV values were significantly reduced ($p<0.05$) in BPA-treated mice without changes in MCV, MCH and MCHC values. Supplementation of BSO in BPA-treated mice restored those values comparable to values of control mice (Table 1).

Effect of BPA and BSO on hormonal assay in mice

Thyroxine (T_4) and testosterone concentration were assessed from the sera of different treatment groups and the mean values are presented in Table 2. Both T4 and testosterone values were found significantly ($p<0.01$) decreased in BPA treated mice and the addition of BSO along with BPA in food improved the concentration of these hormones to a normal level.

Effect of BPA and BSO on sperm physiology in mice

To know the role of BSO on BPA induced path-physiological alterations on sperm morphology and concentration, the sperm analyses were done according to standard methods. Results showed that (Fig. 1, Table 3) sperm count were reduced and percentage of sperm motility and head and tail abnormalities were increased ($p<0.01$) in BPA-treated mice. Upon treatment with BSO in BPA-treated mice, there were significant changes in sperm count, head and tail abnormality percentage, and sperm motility increased significantly ($p<0.05$).

Effect of BPA and BSO on Patho-physiological alterations in testis

To see the effects of BPA directly in the reproductive organ, histology of testis had been carried out. Section of testis of control mice showed normal tissue structures and no detectable changes found in seminiferous tubules but in case of BPA treated mice, there were severe degenerative and necrotic changes in the seminiferous tubules and loss of spermatogonial cells (Fig. 2). On the other hand, BPA treatment with BSO had mild degenerative changes in the seminiferous tubules.

Discussion

Reproductive disorders concerning male infertility are more common nowadays caused by environmental pollution. BPA is a chemical that ubiquitously infiltrates our environment because of continuous release via effluent discharge from municipal wastewater treatment plants, leaching from landfills, combustion of domestic waste and the natural breakdown of plastics in the environment (Flint *et al.,* 2012). The black seeds contain both fixed and essential oils, unsaturated fatty acids, proteins, tannins, resins alkaloids, steroids, saponin, vitamins, and minerals that play an important positive role in the reproductive function system (Ali and Blunden, 2003).The administration of 1ml/kg/day of *Nigella sativa* oil stimulated the secretion of the sexual hormones that led to improved protein synthesis, white blood cells count, and decreased serum cholesterol concentration in blood (Juma and Abdulrahman, 2011).

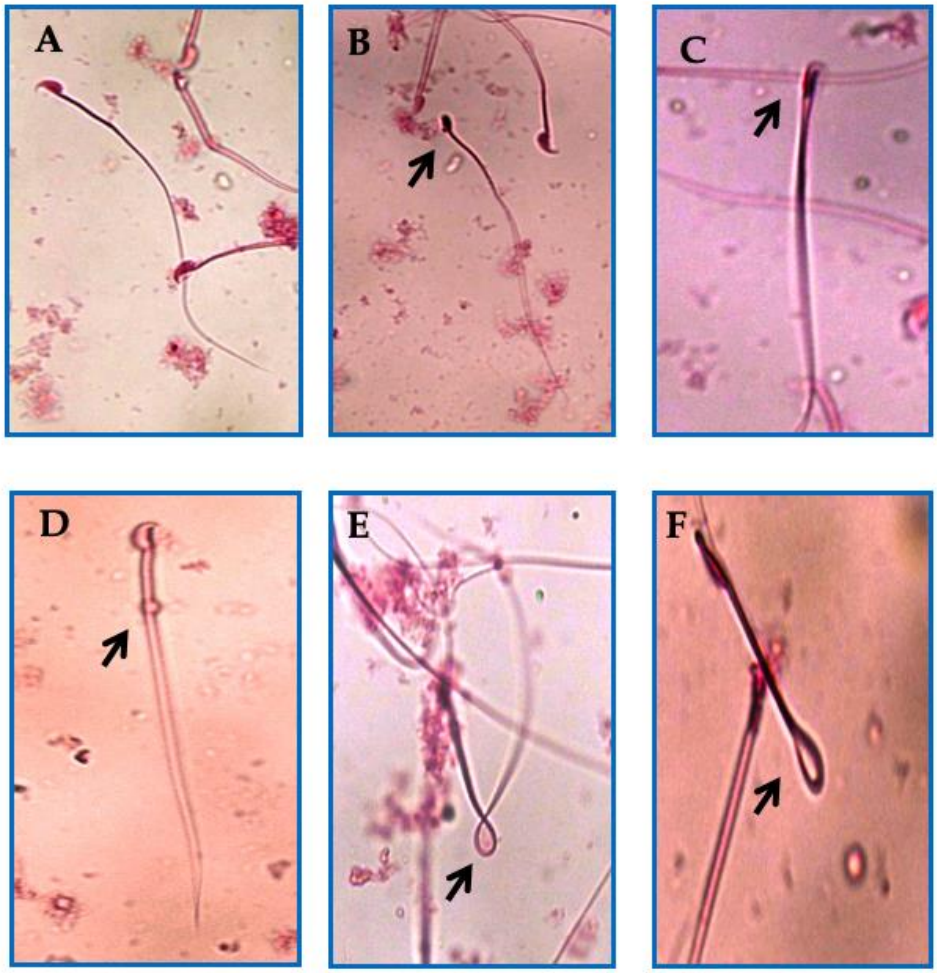

Figure 1. Effect of BPA on sperm morphology. (A) Normal sperm, (B) Sperm without hook, (C) Decapitated sperm, (D) Distal cytoplasmic droplet, E&F) Folded tail.

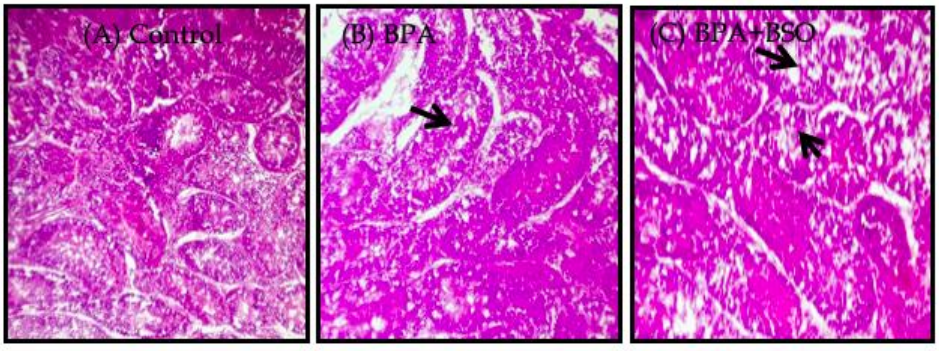

Figure 2. Photomicrograph (10X) of histostructures of testis of male mice. (A) control group, (B) treatment with BPA, (C) treatment with BPA and BSO

Table 1. Effect of BPA and BSO treatment on hematological parameters in male mice at 12th week

Parameters	Control	BPA	BPA & BSO
Hb (gm %)	9.20±0.14	8.48±0.10 **	8.92±0.19^{NS}
TEC (million/mm3)	8.57±0.04	8.27±0.04 **	8.47±0.03^{NS}
PCV (%)	29.60±1.08	26.20±0.97 *	27.40±1.08^{NS}
MCV (fl)	34.51±1.10	31.66±1.02^{NS}	32.32±1.16^{NS}
MCHC (%)	31.18±0.70	32.49±0.81^{NS}	32.66±0.65^{NS}
MCH (pg)	10.73±0.12	10.25±0.07 **	10.52±0.18^{NS}

**Significant at 1% level (p<0.01); * Significant at 5% level (p<0.05); NS= not significant

Table 2. Effect of BPA and BSO treatment on T_4 and Testosterone concentration in male mice at 12th week

Parameters	T_4 (nmol/L)	Testosterone (ng/mL)
Control	49.62±1.40	1.95±0.05
BPA	26.55±1.10 **	0.74±0.10 **
BPA & BSO	47.58±0.96^{NS}	1.87±0.06^{NS}

**Significant at 1% level (p<0.01); *Significant at 5% level (p<0.05); NS= not significant.

Table 3. Effect of BPA and BSO treatment on sperm motility, sperm concentration and sperm morphology in male mice at 12th week

Parameters	Control	BPA	BPA & BSO
Sperm motility (%)	63.4±1.21	45±1.97 **	58±1.70 *
Sperm concentration (million/ml)	31.2±1.56	24.2±1.39 **	28.4±1.78 ^{NS}
Sperm Head abnormality (%)	19.2±1.77	34.8±1.28 **	20±2.10 ^{NS}
Sperm Tail abnormality (%)	14.8±2.42	38.6±1.4 **	18.4±1.21 ^{NS}

**Significant at 1% level (p<0.01), *Significant at 5% level (p<0.05); NS= not significant

The present study showed that BSO in BPA treated male mice significantly prevented the reduction of the hematological parameters: erythrocytes number, Hb concentration and PCV. Ulta *et al.* (2011); Yamasaki and Okuda, (2012) also reported that BPA induced a significant decrease in TEC, Hb concentration and PCV. The decrease in the red blood cells may indicate a disruption of erythropoiesis. BPA might have interfered with the Hb synthesis or the lysis of RBC or binding with Hb forming a complex, which can alter Hb's secondary structure, which may affect the physiological functions of Hb (Fang *et al.,* 2011). BSO significantly elevated Hb, TEC and PCV and decreased MCH and MCHC (Mohamed and Awad, 2008). BSO may increasing the erythrocyte count and hemoglobin concentration by an increased number of cells in bone marrow that reached advanced developmental stages and the accelerating effect of it's on the cellular respiratory mechanism (Ebaid *et al.,* 2011). The present study showed that BPA has a negative impact on the reproductive hormone, whereas BSO improves those parameters. The current findings are similar to the findings of Zang *et al.* (2016) and Munir *et al.* (2017).

The level of testosterone decreased in plasma might have been resulted from decreased expressions of enzymes and protein and decreased plasma LH levels (Nakamura *et al.,* 2010). BPA directly affects thyroid follicular cells and leads to an altered expression of the genes involved in thyroid hormone synthesis. Other possible mechanisms for the association between BPA and thyroid hormones include inhibiting T3 pathways during metamorphosis and thyroid hormone receptor (TR) transcription suppression. (Sheng *et al.,* 2012; Gentilcore *et al.,* 2013). BSO contains alkaloids, phenols, amino acids like glutamic acid, methionine, and lysine, which stimulate FSH and Testosterone's secretion. (Al-Sa'aidi, 2009). The treatment with oral administration of BSO increased T4 levels in rabbits (Sharif *et al.,* 2012). *Nigella sativa* oil against hypothyroidism is mostly attributed to its antioxidant effects and its thymoquinone constituents modulated thyroid hormones and improved thyroid status (Panahi *et al.,* 2011; Bacak and Avci, 2013). BSO improves sperm physiological parameters because it contains essential vitamins, minerals, steroids and fixed oil. The findings of this study are following the findings of Karnam *et al.,* 2015. BPA might have altered the mitochondrion, producing a delay in motility and, in some cases, eventually leading to sperm death resulting in reduced total sperm count (Vilela *et al.,* 2014). The increased abnormalities of sperm due to BPA might lead to genotoxic damage in germ cells through mutagenic events, non-mutagenic events, cellular degeneration, interfering either with the integrity of the DNA itself or with the expression of this genetic material.

BPA produces a spermatotoxic effect through induced alteration in testicular DNA and sperm chromatin structure (Sangai *et al.,* 2014). BSO caused a significant increase in sperm motility due to its effects on oxidative phosphorylation enzymes (Mohammad, 2009). The

increase in sperm concentration due to BSO increase in testosterone and FSH levels in testicular tissue, since these two hormones are responsible for spermatocytogenesis and spermiogenesis in seminiferous tubules (Parandin *et al.,* 2012). This increase may be due to the black seeds constituents of proteins, vitamins like A, B, and C, and the presence of important minerals like zinc, copper and magnesium (Kanter *et al.* 2005). Tian *et al.* (2017) found that microscopic examination under light and transmission electron microscopes showed spermatogenesis disorders after BPA exposure, including rough basal lamina of seminiferous tubules and damage of tight junctions between Sertoli cells. The results indicated that impairment of the basal lamina of seminiferous tubules and tight junctions might contribute to BPA-induced cell injury. The apparent improvement in the testis is agreed with Al – Helali 2002 result who mentioned that the use of black seed causes a clear improvement of spermatogenesis in the animals treated with aqueous and alcoholic extracts of black seeds.

Conclusion

The research findings suggest that BPA caused hematological alterations and suppressed the sexual behavior of male mice, which might be attributed to a decrease in testosterone and T_4 levels. It affects the germ cells, leading to spermatogenesis and reduction in sperm count, sperm motility percentage, and increased head and tail abnormality percentage. BSO might play a vital role in preventing BSA-induced infertility and reproductive health hazards by regulating the hypothalamo-pituitary-gonadal hormone axis.

Acknowledgements

This research work was supported by The National Science and Technology (NST) fellowship from The Ministry of Science and Technology of Bangladesh for MS degree to the first author of this article (Grant No. 1217/359/2017-2018). The authors thank the authority of the Institute of Nuclear Medicine & Allied Sciences (INMAS) and Department of Pathology, Mymensingh Medical College for hormonal analysis and histo-pathological study of testis, respectively.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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