Ameliorating effects of black seed oil on bisphenol-A induced abnormality of blood, hormone profile and gonadal histology of female mice

Khaled Mahmud Sujan¹, Emdadul Hoque¹, Md. Iqramul Haque¹, Afrina Mustari¹, Mohammad Alam Miah¹ and Md. Kamrul Islam*¹

¹Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

*Corresponding author: Professor Dr. Md. Kamrul Islam, Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, email: k.physiol@bau.edu.bd

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ABSTRACT: Bisphenol- A (BPA) is a multi-functional chemical produced in large volumes that is extensively used primarily in the manufacture of polycarbonate plastics and epoxy resins. Black seed oil (BSO) has been employed for many century as one of the main sources of nutrition and healthcare both for humans and animals. The study was carried out to investigate the effects of BPA and BSO on blood, hormone profile, and histopathological changes of ovary in female mice. Total 15 female Swiss Albino mice, aged between 6-8 weeks with an average body weight of 27.4±1g were randomly divided into 3 groups (5 mice/group). Group A act as vehicle control. Group B was administered 50 mg BPA /kg bw daily, while group C received both BPA and BSO and dose rate was 50 mg BPA /kg/day and 1ml BSO/kg/day respectively. Results revealed that Hb concentration, TEC and PCV (p<0.01) were significantly reduced while such difference was absent in other hematological values in BPA treated group compared to control. Inclusion of BSO along with BPA prevented or improved the alterations of those values. Hormonal study showed that, serum thyroxine (T4) and Estradiol level were significantly (p<0.01) reduced by BPA and BSO counteracted the negative effects of BPA. Histopathological study of ovary demonstrated that there were degenerative changes in the lining of granulosa cells of ovarian follicles upon treatment with BPA. From the present study it could be concluded that subjection to BPA is accompanied with anemia, hormonal homeostasis disturbances and granulosa cells degeneration.

KEYWORDS: Bisphenol-A, Black Seed Oil, Hematology, Hormone profile, and Gonadal histopathology.

INTRODUCTION

There is growing concern about the impact of various endocrine disrupting chemicals (EDCs) on human and animal reproduction, that can affect the normal physiology of the endocrine and reproductive systems by inhibiting the endogenous hormone biosynthesis, metabolism, or modulating the action of hormones [1,2]. Bisphenol-A (BPA), is widely known as an EDC that has been produced on large quantity and extensively utilized industrial chemical for manufacturing many products such as thermal printer paper, electronic equipment, water pipes, sports safety equipment, medical devices, plastic containers, lining of cans for food and beverages [3]. Initially BPA was manufactured as a synthetic estrogen which was used to enhance the rapid growth of poultry and cattle [4]. Human and animal may be exposed to BPA through ingestion, inhalation and dermal exposure. It is reported that daily exposure to BPA to a human body about 10μg/ day [5]. BPA can be detected in urine, serum, placental tissue and even in the fetal liver [6]. Environmental exposure to BPA is associated with many clinical manifestations in humans including immune system dysfunction [7], heart failure and kidney diseases [8]. BPA can suppress the normal reproductive action by inhibiting the function of hypothalamic-pituitary-gonadal axis (HPG axis) [9].
BPA also causes early start of adolescence, birth defects, breast cancer, miscarriages and effects on ability to reproduce [10].

The use of traditional medicine increases every day and have more acceptance than prescribed drugs because it was mostly attributed to being safer than drugs, also patients believe that by using this type of medication there was no need for a physician [11]. One of these alternatives medicinal plant, *Nigella sativa* commonly known as black cumin, belonging to the family Ranunculaceae and it is native plant from the Mediterranean area used for culinary and medical purposes [12]. The black seed oil is reported to be beneficial for reproductive system as its seeds contain essential oils, unsaturated fatty acids, proteins, steroids, vitamins and minerals [13]. The use of 1ml/kg/day of BSO restored the sexual hormones secretion that led to increase the protein synthesis, white blood cells count and decrease the serum cholesterol concentration [14]. *Nigella sativa* seeds extract has role in the expulsion of fetal membrane and return of uterus to normal condition, without interfere with the progesterone hormone level during the first cycle in cows treated 100 mg/kg body weight. [15]. The black seed is considered as antidiabetic, anthelminthic, anti-hyperlipidemic, analgesic, immunomodulatory, hypotensive, anti-oxidative, anti-cancer and anti-bacterial effects [16]. So far, in Bangladesh few studies were performed in relation to BPA and black seed oil influence on blood and reproductive organs in mice. The present research work was done with a view to fulfilling the objectives, a) to assess some selected hematological parameters (Hb, PCV, TEC and Erythrocyte indices), b) to observe the action of BPA and BSO on Thyroxine and Estradiol level, and c) to evaluate ovarian histopathology.

**MATERIALS AND METHODS**

**Experimental animals**

The mice were purchased from ICDDR’B, Dhaka and housed 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.

**Experimental chemicals**

Bisphenol-A (BPA) was purchased from Sigma-Aldrich Company, USA. The pure black seed oil with high quality (BSO) 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)].

**Experimental design**

The experiment was conducted during the period from February to April 2018 and totals15 apparently healthy female Swiss albino mice (*Mus musculus*); aged between 6-8 weeks with an average body weight of 27.4±1g was used. At first, the mice were randomly divided into three groups, each consisted of five mice. Group A (control group) received only basal mouse pellet feed mixed with sunflower oil. Group B was administered Bisphenol-A (BPA) @ 50 mg / kg bw daily, formulated in sunflower oil (as vehicle for BPA), while group C received BPA and BSO @ 50 mg/kgbw/day and 1ml/kgbw/day, respectively.

**Management practices**

The diet was calculated on daily basis, diet and water were provided ad libitum in all groups. Feeds were kept in air tight poly packed sac to prevent spoilage. During the experimental period, effective hygienic and sanitary measures were followed and also mice cage were cleaned regularly.

**Collection of blood and serum preparation**

At the end of the experiment (12th week), blood samples were collected by sacrificing the mice. The mice were kept fasting overnight. Then the mice were placed one by one in an airtight container containing diethyl ether pre-soaked cotton. They were being looked over for insensibility and taken out from the airtight vessel and blood was collected directly from heart by a sterile syringe. About 1 to 1.5 ml blood was collected and transferred 0.5-0.75 ml blood into anticoagulant containing tube and the remaining half of blood was transferred to another tube without anticoagulant for serum preparation. As per conventional method serum was separated by centrifugation and stored at – 20 °C until tested.
Hematological parameters

Hemoglobin (Hb), packed cell volume (PCV) and Total Erythrocyte Count (TEC) were performed as per standard method [17]. The RBC indices measure the size, shape, and physical characteristics of the RBCs. It provides information about the hemoglobin content and size of erythrocytes that can be determined by calculating the values obtained from TC of RBC in million/cubic mm, Hb concentration in gm% and PCV in %.

Hormonal assay

The following hormonal parameters: Serum Estradiol and Serum thyroxine (T4) were determined by using Estradiol radioimmunoassay kit and T4 radioimmunoassay kit at the Institute of Nuclear Medicine & Allied Sciences (INMAS), Mymensingh Medical College.

Histopathology

The ovary from each group of mice were collected after complete 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 study [18]. The stained slides were observed under Optka Vision Lite 21 and photographs of the characteristic findings were put down. Histopathologically, the degenerative lesions of ovary in all groups of experimental mice were graded as mild (+), moderate (++) and severe (+++).

Statistical analysis

All data were placed and stored in Microsoft Excel-2007 and imported to the software IBM SPSS Statistics 20 for analysis by one-way ANOVA followed by post-hoc Turkey’s test. Because of using multiple comparisons, the corrected p value was calculated and adjusted at 0.01 and 0.05 considered for level of significance.

RESULTS

Effects of BPA and BSO on hematological parameters in mice

Effects of BPA and BSO on hematological parameters including Hb, TEC, PCV and Erythrocyte Indices (MCV, MCHC and MCH) in different groups of mice were presented in Table 1. Data revealed that Hb concentration, TEC and PCV (p<0.01) were significantly reduced while such difference was absent in MCV, MCHC and MCHC values in BPA treated group compared to control. Supplementation of BSO in BPA treated mice improved hematological parameters which was non-significant compare to control group.

Table 1: Effect of bisphenol-A (BPA) and black seed oil (BSO) treatment on hematological parameters in mice at 12th week.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>BPA</th>
<th>BPA&amp;BSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (gm %)</td>
<td>8.60±0.09</td>
<td>7.92±0.12 **</td>
<td>8.40±0.14NS</td>
</tr>
<tr>
<td>TEC</td>
<td>7.69±0.08</td>
<td>7.12±0.04 **</td>
<td>7.54±0.05NS</td>
</tr>
<tr>
<td>(million/mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>26.20±0.80</td>
<td>23.80±0.86**</td>
<td>25.60±0.66NS</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>34.05±0.73</td>
<td>33.42±1.03NS</td>
<td>33.91±1.20NS</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.91±0.68</td>
<td>33.39±0.80NS</td>
<td>32.97±1.01NS</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>11.19±0.04</td>
<td>11.13±0.10NS</td>
<td>11.14±0.13NS</td>
</tr>
</tbody>
</table>

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

Effects of BPA and BSO on hormonal assay in mice

Effects of BPA and BSO on serum thyroxine (T4) and estradiol concentration in different treated groups of mice were shown in Table 2. BPA treated mice showed significantly (p<0.01) decreased the level of T4 and estradiol concentration compared to the control group. Addition of BSO in BPA treated mice restored those level of T4 (p<0.01) and Estradiol (p<0.05) concentration near to normal level.

Effects of BPA and BSO on patho-physiological alterations in ovary

To see the toxic effects of BPA directly in the reproductive organs, histology of ovary had been carried out. Section of ovary of non-treated mice showed normal tissue structures and no remarkable changes were found. BPA treated group showed severe degenerative (+++ changes in the lining of granulosa.
cells of ovarian follicles. On the contrary, it was noticed that restoration of granulosa cells (++) after treated with BSO than the BPA treated group. (Figure 1.)

**Table 2.** Effect of bisphenol-A (BPA) and black seed oil (BSO) treatment on Thyroxine (T4) and Estradiol concentration in mice at 12th week.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T4 (nmol/L)</th>
<th>Estradiol (pg/mL)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>79.26±1.51</td>
<td>69.42±1.77</td>
</tr>
<tr>
<td>BPA</td>
<td>48.62±1.01  **</td>
<td>47.12±0.92  **</td>
</tr>
<tr>
<td>BPA &amp; BSO</td>
<td>64.79±1.53  **</td>
<td>63.70±1.77 *</td>
</tr>
</tbody>
</table>

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

**Figure 1.** Photomicrograph (10X) of ovarian histostructure of female mice treated with bisphenol-A (BPA) and black seed oil (BSO). A. Control; B. BPA and C. BPA & BSO. [Scale bar: 5 µm].

**DISCUSSION**

The study was conducted to assess the effect of BPA and BSO on hematological parameters, hormonal assay and histotexture of ovary in female Swiss albino mice. In hematological study, we observed that Hb concentration, TEC and PCV (p<0.01) were significantly reduced in BPA treated mice and the present findings are closely similar with the [19, 20]. They 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 may interfere with the Hb synthesis or the hemolysis of RBC or react with Hb making a complex, which may affect the physiological functions of Hb [21]. One study reported that, black seed oil significantly elevated Hb, TEC and PCV and decreased MCH and MCHC [22]. The other study found that, BSO restoration the Hb, TEC and PCV value to normal level in ribavirin treated female albino rats [23]. Black seed oil may increase the erythrocyte count and haemoglobin concentration by stimulating the bone marrow and accelerating the effect of it’s on the cellular respiratory mechanism [24]. In case of hormonal assay, BPA treated mice showed significantly (p<0.01) reduced the T4 and estradiol concentration and BSO in BPA treated mice restored those values. The findings of the study are partially consistent with [25-27] stated that BPA can interfere in estradiol level by agonism with estrogen nuclear receptors as well as steroid biosynthesis inhibition. BPA has a direct effect on thyroid follicle and inhibit thyroid hormones synthesis via T3 pathways arrest and suppression of thyroid hormone receptor (TR) transcription [28]. BSO has a positive increased effect on estrogen concentration due to it’s content thymoquinone and unsaturated fatty acids [29]. The treatment with oral administration of black seed oil increased T4 levels in rabbits [30]. Nigella sativa oil used against hypothyroidism due to having its antioxidant effects and thymoquinone constituent that improved thyroid profile [31].

Concerning histopathological study, degenerative changes were found in granulosa cells of BPA treated group and BSO restoration, these changes near to normal ovarian histotexture. One study reports that exposure of BPA reduced the granulosa cells of preantral follicles in cultured mouse [32]. Other experiment showed that BPA may be the causal factor for the death of granulosa cells of ovary and antral follicles via inhibiting cell cycle and disturbances of the apoptotic pathway [33].

**CONCLUSIONS**

The findings of the present study suggested that exposure to BPA is a potential threat for development of anemia by decreasing Hb concentration, TEC and PCV level. BPA can suppress the sexual behavior and risk factor for developing hypothyroidism, which might be attributed to decrease in thyroxine (T4) and Estradiol levels and granulosa cells degeneration. On the other hand, ingestion of black seed oil may have a positive impact on hematology and reproductive function in female mice by accelerating cellular respiratory mechanisms and regulating the hypothalamo-pituitary-gonadal hormone axis respectively which are connected with the chemical composition of oil. This study seems to suggest a prophylactic activity of black seed oil in the blood and reproductive system of female mice. Further extensive research on this aspect is required to know the possible potential sources and disorders of BPA exposure and to pick up the active ingredients of black seed oil which are actually responsible for combating the BPA induced harmful effects.
ACKNOWLEDGEMENT

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AUTHOR CONTRIBUTIONS

MKI designed the experiment. KMS performed the experiment and wrote the draft; EH and MIH analyzed the data; MKI, MAM and AM critically revised the manuscript.

CONFLICTS OF INTEREST

The author declares that no conflict of interest exists.

REFERENCES


