

ORIGINAL ARTICLE

**Effects of Bisphenol-A (BPA) and black seed oil on body weight, lipid profile and serum glucose in male and female mice**

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**Abstract**

**Background**

Bisphenol-A [BPA, 2, 2-bis (hydroxyphenyl) propane] is widely used in the manufacture of polycarbonate plastic, water bottles, feeders, baby bottles, epoxy resins and inside coating in metallic food cans. Black seed oil (BSO) (*Nigella sativa*) commonly known as black cumin, reported to be beneficial in function of various systems in the body. The study was carried out to investigate the effect of BPA and BSO on body weight, lipid profile and serum glucose in male and female mice.

**Methods**

A total of thirty (15 male and 15 female) Swiss Albino mice (*Mus musculus*), aged 25-28 days with an average body weight of 27.4±1g were randomly divided into 3 groups consisting 5 mice in each for each sex. Group A served as vehicle control. Group B was administered BPA @ 50 mg/kg bw daily, while group C received both BPA @ 50 mg/kg/day and BSO @ 1ml/kg/day respectively.

**Results**

Data revealed that BPA treated mice showed slight increase in body weight gain while BSO controlled the weight gain in BPA treated mice. Cholesterol and LDL values were significantly ( $p<0.01$ ) increased and Triglycerides value was significantly ( $p<0.01$ ) decreased in BPA-treated mice without significant alterations in HDL value. BPA & BSO treated female mice showed significant ( $p<0.01$ ) decreased in cholesterol, triglycerides and LDL values. BPA reduced the blood glucose level and addition of BSO had synergistic effects of glucose utilization.

**Conclusions**

It can be concluded that BPA is one of the potential risk factors for hyperlipidemia and obesity. These harmful effects could be alleviated by the ingestion of black seed oil.

**Key words:** Bisphenol-A, Black Seed Oil, Mice, Cholesterol, LDL, Glucose

## **Introduction**

Bisphenol-A (BPA), is widely known as an endocrine disrupting chemical (EDC) that imitates, changes, and interferences the endogenous hormonal activity and impaired reproductive functions both in humans and animals (Diamanti-Kandarakis *et al.* 2009). It extensively used in food packaging, epoxy resins, polycarbonate plastics, water and food plastic containers, baby bottles and feeders, and medical tubing (Kim *et al.* 2010). Human and animal may be exposed to BPA by various sources and routes, including leaching from plastic lining of food cans, polycarbonate baby bottles, and by ingestion, inhalation and dermal exposure. (Vandenberg *et al.* 2012). Environmental exposure to BPA is associated with many disorders in humans like heart failure, kidney diseases (Li *et al.* 2012) and immune system dysfunction (Holladay *et al.* 2010). BPA also causes early start of adolescence, birth defects, miscarriages, effects on ability to reproduce (Al-Hiyasat *et al.* 2004), breast cancer, prostate cancer (Ho *et al.* 2006), diabetes, cardiovascular disorders and disrupt the metabolic processes (Lang *et al.* 2008). Black seed oil (*Nigella sativa*) commonly known as black cumin, black carawa, belonging to the family Ranunculaceae ( Kamal *et al.* 2010). The black seed oil is reported to be beneficial as its seeds contain more than one hundred of components such as: essential oils, unsaturated fatty acids, proteins, tannins, resins, alkaloids, steroids, vitamins and minerals those play an important positive role in the body function (Ali and Blunden, 2003). The administration of 1ml/kg/day of *Nigella sativa* oil stimulated the sexual hormones secretion that led to improve protein synthesis, WBC count and decrease the serum cholesterol concentration in blood (Juma and Abdulrahman, 2011). The black seed is considered as antidiabetic, antihyperlipidemic, anti-inflammatory, immunomodulating, hypotensive, antioxidative, anti-bacterial and anti-fungal agent (Halamova *et al.* 2010 and Rogozhin *et al.* 2011). So far, the research works related to BPA and black seed oil are very limited in Bangladesh. The objectives of this study were to investigate the effect of BPA and BSO on body weight and to observe the

effect of BPA and BSO on serum glucose and lipid profile e.g. Cholesterol, HDL-c, LDL-c and Triglyceride in male and female mice.

## **Materials and Methods**

### **Experimental animals**

The mice used for this study were purchased from ICDDR'B, Dhaka. They 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.

### **Experimental chemicals**

Bisphenol-A (BPA) was purchased from Sigma-Aldrich Company, USA and was dissolved in sunflower oil (vehicle) as stock before administration. At first, we made a stock solution of BPA that contains 500mg BPA and 10 ml methanol. For each 50 gm feed, we took 3ml sunflower oil and mixed with feed properly. The oil extract of *Nigella sativa* L. seeds was purchased from local market in Mymensingh.

### **Experimental design**

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh, from 1 February to 25 April 2018 (12 weeks). In this study, total thirty (15 male and 15 female) Swiss Albino mice (*Mus musculus*), aged 25-28 days with an average body weight of 27.4±1g were used. At first, for each sex, the mice were randomly divided into 3 groups viz., A, B and C consisting 5 mice in each group. Before the start of the treatment, we supplied sunflower oil all the groups for 2 weeks. Group A served as vehicle control and Group B was administered Bisphenol-A (BPA) @ 50 mg/kg body weight daily, while group C received BPA and Black Seed Oil (BSO) @ 50 mg/kg/day and 1ml/kg/day respectively.

### **Management practices**

The diet was prepared on daily basis and diet and water were supplied *ad libitum* in all groups. Initial body weight of each mouse was measured with the help of a digital balance. Body weight was taken on the first day and then we recorded

body weight at 7 days intervals until the end of the experiment. Mice cage were cleaned regularly and proper hygienic and sanitary measures were adopted during the experimental period.

**Serum biochemical studies**

At the end of the experiment (12<sup>th</sup> week), blood samples were collected by cardiac puncture and blood samples were placed into clean dry tubes without using anticoagulant for serum preparation. The tubes were placed in upright slanting position at room temperature for 6 hours. Then they were incubated overnight in the refrigerator (4°C). The serum samples were separated by centrifugation and stored in capped tube at -20°C for biochemical analysis. The biochemical parameters of serum like Glucose and Lipid profile (Total cholesterol, Triglycerides, HDL cholesterol and LDL cholesterol) were performed at Professor Dr. Mohammad Hussain Central Laboratory in

Bangladesh Agricultural University, Mymensingh-2200.

**Statistical analysis**

All data were subjected to statistical analysis using SPSS program by one-way ANOVA followed by post-hoc Turkey’s test.

**Results and Discussion**

**Effect of BPA and BSO on body weight gain in mice**

Average body weight gain in both male and female mice upon treated with BPA and BPA with BSO is shown in Table 1 and Table 2. BPA treated male and female mice showed slight increase in body weight and addition of BSO revealed slight reduction in body weight in BPA treated male and female mice at 12<sup>th</sup> week of experiment.

Table 1. Comparison of average body weight gain in different treatment groups of male mice

| Parameters | Initial body weight (g) | Body Weight (g) (Mean ± SE) |                         |                         |                         |
|------------|-------------------------|-----------------------------|-------------------------|-------------------------|-------------------------|
|            |                         | 3 <sup>rd</sup> week        | 6 <sup>th</sup> week    | 9 <sup>th</sup> week    | 12 <sup>th</sup> week   |
| Control    | 43±1.14                 | 46.6±1.6                    | 49.6±1.44               | 51.8±1.62               | 54.2±1.83               |
| BPA        | 42.8±1.07               | 47.4±1.57 <sup>NS</sup>     | 50.2±1.28 <sup>NS</sup> | 53.2±1.02 <sup>NS</sup> | 56.2±0.86 <sup>*</sup>  |
| BPA & BSO  | 43±1.14                 | 45.8±1.39 <sup>NS</sup>     | 48.8±1.43 <sup>NS</sup> | 51.2±1.28 <sup>NS</sup> | 54.4±1.08 <sup>NS</sup> |

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

Table 2. Comparison of average body weight gain in different treatment groups of female mice

| Parameters | Initial body weight (g) | Body Weight (g) (Mean ± SE) |                         |                         |                         |
|------------|-------------------------|-----------------------------|-------------------------|-------------------------|-------------------------|
|            |                         | 3 <sup>rd</sup> week        | 6 <sup>th</sup> week    | 9 <sup>th</sup> week    | 12 <sup>th</sup> week   |
| Control    | 37.4±0.51               | 39.6±0.6                    | 42.2±0.58               | 44.6±0.68               | 47.2±0.66               |
| BPA        | 38±0.71                 | 41.2±0.73 <sup>NS</sup>     | 44.2±0.37 <sup>*</sup>  | 46.4±0.60 <sup>NS</sup> | 49.8±0.73 <sup>*</sup>  |
| BPA & BSO  | 37.8±0.66               | 39.8±0.58 <sup>NS</sup>     | 41.6±0.51 <sup>NS</sup> | 43.8±0.37 <sup>NS</sup> | 46.2±0.37 <sup>NS</sup> |

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

The present findings are agreement with findings of Wada *et al.* 2007; Hugo *et al.* 2008; Rubin and Suto, 2009. They suggested that BPA has a potential influenced on body weight by adipocyte differentiation, lipid accumulation, glucose transport and adiponectin secretion. Bano *et al.* (2009) suggested that black seed oil decrease body weight by lowers blood glucose levels, decrease serum cholesterol and triglyceride levels, hypophagial action and inhibits gluconeogenesis in the liver.

**Effect of BPA and BSO on serum biochemistry in mice**

Effect of BPA and BSO on serum glucose and lipid profile e.g. cholesterol, HDL-c, LDL-c and Triglyceride in different groups of mice both male and female are presented in Table 3 and Table 4 respectively. Serum cholesterol and LDL-c values were significantly (p<0.01) increased and TG value was significantly (p<0.01) decreased in BPA-treated mice without alteration in HDL-c value compared to the values of control group of both male and female mice.

### Effects of Bisphenol-A and black seed oil in male and female mice

Supplementation of BSO in BPA treated female mice showed significantly ( $p < 0.01$ ) decreased in cholesterol, LDL-c and TG values compared to the control female mice. On the other hand, hypoglycemia was observed in BPA treated both male and female mice compared to control mice and BSO enhanced the BPA induced hypoglycemic activity in mice.

Table 3. Effect of BPA and BSO treatment on lipid profile and glucose in male mice at 12<sup>th</sup> week

| Parameters         | Control     | BPA                      | BPA & BSO                 |
|--------------------|-------------|--------------------------|---------------------------|
| Cholesterol(mg/dL) | 225.79±1.17 | 258.49±1.56 **           | 221.88±1.46 <sup>NS</sup> |
| HDL(mg/dL)         | 40.21±0.44  | 39.43±0.62 <sup>NS</sup> | 41.12±0.52 <sup>NS</sup>  |
| LDL(mg/dL)         | 160.81±0.56 | 196.37±0.67 **           | 159.75±0.75 <sup>NS</sup> |
| TG (mg/dL)         | 123.84±1.15 | 113.44±1.55 **           | 105.05±1.29 **            |
| Glucose(mg/dL)     | 189.93±1.32 | 169.14±1.70 **           | 143.09±1.26 **            |

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

Table 4. Effect of BPA and BSO treatment on lipid profile and glucose in female mice at 12<sup>th</sup> week

| Parameters         | Control     | BPA                      | BPA & BSO                |
|--------------------|-------------|--------------------------|--------------------------|
| Cholesterol(mg/dL) | 242.94±0.94 | 265.66±1.46 **           | 235.30±2.87 **           |
| HDL(mg/dL)         | 42.13±0.99  | 40.59±0.56 <sup>NS</sup> | 41.65±1.46 <sup>NS</sup> |
| LDL(mg/dL)         | 172.64±0.31 | 199.49±0.65 **           | 169.21±1.26 **           |
| TG (mg/dL)         | 140.71±1.10 | 127.07±0.94 **           | 121.79±1.98 **           |
| Glucose(mg/dL)     | 189.25±1.74 | 151.96±1.48 **           | 143.34±3.34**            |

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

The present study is closely related to previous findings of Abbasnezhad *et al.* 2015; Moghaddam *et al.* 2015; Oguazu and Ezeonu 2017. They reported that BPA stimulates adipogenesis, this lead to hyperlipidemia due to having the estrogenic activity of BPA that have a significant effect on the lipoproteins associated with cholesterol in the circulation. Kersten, 2001 found that insulin is known to increase lipogenesis by both post-translational protein modifications and transcriptional mechanisms. Nadal *et al.* (2009) and Marmugi *et al.* (2012) stated that low doses of BPA increases the pancreatic insulin content that causes rapid raised in plasma insulin level and therefore lower glucose level. The decreasing tendency of TG recorded in this study goes in parallel with earlier report made with BPA related compound @100 and 300 mg/kg bodyweight (Yamasaki and Okuda 2012). The reduction in the lipid concentration by black seed oil may result from the hypolipidemic effects of oleic (Alman-Farinelli *et al.* 2005) and linoleic acids (Wendel and Belury, 2006) major unsaturated fatty acids

of the oil or from its effect on lipoprotein. The possible mechanism of lowered glucose level by black seed oil due to amelioration of oxidative stress, elevation of insulin, attenuation of insulin resistance and hepatic gluconeogenesis and direct insulin-like effects at the cellular and molecular levels (Benhaddou-Andaloussi *et al.* 2008; Abdelmeguid *et al.* 2010).

### Conclusions

The research findings suggest that exposure to BPA is a risk factor for cardiac diseases due to increase in body weight, cholesterol and LDL level. While BSO may have a potential effect in the prevention and treatment of patient with hyperlipidemia, diabetes and obesity owing to lower cholesterol, triglyceride, LDL, glucose and increased HDL level. Data obtained from this work may act as a research tract for filling information gaps in regarding to BPA and BSO, primarily in developing countries. However, further study is required on black seed oil to lower the health hazards associated with BPA

exposure and to find out exact mechanism of black seed oil against it.

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