



# Black Seed Oil Improves Beef Fat-induced Blood-biochemical Abnormalities in Swiss Albino Mice

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## ABSTRACT

**Background:** The high saturated fatty acid content of beef has been linked to obesity and cardiovascular diseases. The study investigated the impact of black seed oil (BSO) on beef fat-altered hemato-biochemical and pathophysiological alterations in albino mice.

**Methods:** Twenty four Swiss Albino mice (*Mus musculus*) of 25-28 days old were equally divided into three groups (n=8) namely A, B and C. Mice of group A was designated as the untreated control group. Groups B and C were fed pellets containing 10% beef fat and 10% BSO, respectively for 9 weeks.

**Result:** The results revealed that beef fat-fed mice gained weight and BSO normalized the weight gain. The hematological values of beef fat supplemented mice improved after being supplemented with 10% BSO. LDL-c, Triglycerides and total cholesterol levels were all significantly lower in mice fed BSO plus beef fat. HDL-c levels were significantly higher in BSO plus beef fat-fed mice. Spleens in BSO plus beef fat-fed mice were slightly enlarged without gross abnormalities. 10% beef fat caused minor changes in the histostructures of the kidney, heart and liver. There were significant changes in cardiac muscle and massive fatty changes in kidney tissue. This study concluded that BSO has beneficial effects on the body and can prevent beef fat-induced abnormalities.

**Key words:** Beef fat, Black seed oil, Blood-biochemical parameters, Mice.

## INTRODUCTION

Beef fat contains a larger amount of saturated fatty acid (SFAs), trans fatty acid and a minimal amount of polyunsaturated fatty acid (PUFAs). Consumptions of this fat are unhealthy for the body and may increase the risk of heart disease by raising cholesterol levels, especially low-density lipoprotein cholesterol (Li *et al.*, 2019; Lichtenstein *et al.*, 2006). The lipid profile determines the risk of cardiac diseases. It includes total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) or 'bad cholesterol'. According to the National Institutes of Health, saturated fat is the primary dietary cause of high LDL cholesterol. LDL can contribute to plaque in the artery, increasing the risk of cardiovascular diseases and atherosclerosis. It also causes fatty liver disease (Tamura and Shimomura, 2005), diabetes and cancers. Though PUFAs are essential to decrease blood cholesterol levels, the amount is very low in beef fat to minimize heart diseases. Replacing SFAs with PUFA lowers the risk of coronary heart disease. Over the last 20 years, complementary and alternative medicine has grown in popularity around the world. One of these alternatives medicinal plants, *Nigella sativa*, also known as black cumin/black seed, is a member of the family Ranunculaceae. It is used in both cooking and medicine (Kamal *et al.*, 2010). The BSO is useful as its seeds contain a number of components such as polyunsaturated fatty acids (PUFA), essential oils, amino acids, tannins, resins, alkaloids, steroids, saponin, vitamins-minerals that help to proper functioning of the reproductive system (Hannan *et al.*, 2021)

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and prevention of obesity. The black cumin seed is reported to have anti-diabetic, anthelmintic, antihyperlipidemic, laxative, carminative, diuretic, bronchodilator, analgesic, immunomodulation, hypotensive, histamine release inhibitor and antioxidative effects and is used in the treatment of mild cases of puerperal fever (Mashhadian and Rakhshadeh, 2005) and also has anti-fungal (Rogozhin *et al.*, 2011), anti-bacterial (Halamova *et al.*, 2010), anti-cancer and anti-inflammatory activities (Ayed and Talal, 2011). The use of 1 ml/kg/day of BSO triggered the secretion of the reproductive hormone, resulting in increased synthesis of protein, white blood cell count and a decrease in total cholesterol levels in blood (Juma and Abdulrahman, 2011). BSO enhances the body weight gain, reproductive parameters, male sex hormone and follicle-stimulating hormone (FSH) (Sujan *et al.*, 2021; Al-Sa'aidi *et al.*, 2009).

Several studies have reported favorable effects on BSO on lipid profile. Oral treatment with BSO decreased serum cholesterol and TG levels in normal mice by 15.5% and 22%, respectively (Zaoui *et al.*, 2002). Since numerous studies on BSO have been conducted and documented, but precise information on beef fat-related abnormalities are needed to be elucidated. In connection with that, the current research work was designed to explore the consequences of BSO in a beef fat-fed mouse models especially on their body weight, blood-biochemical parameters.

## MATERIALS AND METHODS

### Experimental animals

The experiment was conducted at the Department of Physiology, Bangladesh Agricultural University (BAU), Mymensingh. Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh, Bangladesh approved all experimental protocols and methodologies. In this study, a total of twenty-four female Swiss Albino mice (*Mus musculus*), aged 25-28 days with an average body weight of 28-30 g were used. The mice were divided into three groups at random: A, B and C, with eight mice. Group A served as non-treated control and was given mice pellets; group B was administered 10% beef fat oil with mice pellets daily, while group C received 10% BSO and 10% beef fat mixed with mice pellets, respectively for mice. The experiment was carried out over a nine-week period starting from 14 February, 2021-30 April 2021. The beef fat and the black seed oil (BSO) were obtained at the local market in Mymensingh.

### Management practices

The mouse cages were kept in a clear, dry and well-ventilated room. Throughout the duration of the experiment, the cage was cleaned frequently and strict hygienic and sanitary procedures were followed.

### Body weight

With the aid of an electric balance, the starting body weight of each mouse was determined prior to grouping and then at intervals of three weeks until the conclusion of the experiments. Body weight growth (g) was computed by formula:

Weight gain (g) = Mean final body weight (g) - Mean initial weight was used to compute body weight growth (g).

Per cent weight gain (g) =

$$\frac{\text{Mean final weight (g)} - \text{Mean initial weight (g)}}{100}$$

### Blood analysis

Blood analysis were done according to the method described (Sarker *et al.*, 2019). Briefly, the mice were kept fasting overnight. Blood was collected directly from the heart of each diethyl ether made unconscious mice by a sterile syringe. Half of the collected blood was transferred into an eppendorf tube containing anticoagulant and the remaining blood was moved to another tube for serum preparation. Total

erythrocyte count (TEC) and hemoglobin concentration (Hb conc.) and packed cell volume (PCV) were determined as per the standard procedure.

### Biochemical analysis

The lipid profile includes total serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were performed colorimetrically using Humalyzer 2000 (Human type, Germany) as per standard procedures (Rakib *et al.*, 2021).

### Histopathology

The liver, heart and kidneys of each group of mice were collected after completely removing the blood by perfusion with phosphate-buffered saline and stored at least for 15 days in 10% formalin. Then tissues were processed, sectioned and stained according to standard procedure described (Mustari *et al.*, 2022) in cooperation with the Department of Pathology, Bangladesh Agricultural University.

### Statistical analysis

The data obtained in the laboratory were stored in Microsoft Excel-2010 and imported to GraphPad Prism 8 software for statistical analysis. One-way analysis of variance (ANOVA) with posthoc Tukey's test was used to compare the parameters of different groups. For each parameter, at least six blood samples from eight mice in each group were used (n=8). Mean values, standard deviations and standard error means were calculated and compared. P<0.05 was taken into account as statistically significant.

## RESULTS AND DISCUSSION

### Body weight

Body weights of treated and non-treated healthy mice were recorded at 3 weeks intervals and the final body weight after nine weeks are shown in Table 1. Body weights of all mice were increasing during the experiment and highest weight gain was observed in mice of beef fat fed group. All beef fat supplemented mice gained more weight compared to the non-treatment and BSO treated group (Table 1).

Fat-rich diets induce obesity in both human and animals. High-fat diet consumption is strongly correlated with overweight and obesity in both rats and mice (Sarker *et al.*, 2019; Cho *et al.*, 2012). Black seed oil (BSO), a natural available safe supplement, can be used to prevent excess weight and obesity (El-Magd *et al.*, 2021). All these findings are in line with our current findings that 10% BSO significantly normalized the extra body weight induced by the beef fat diet (Table 1).

### Blood parameters

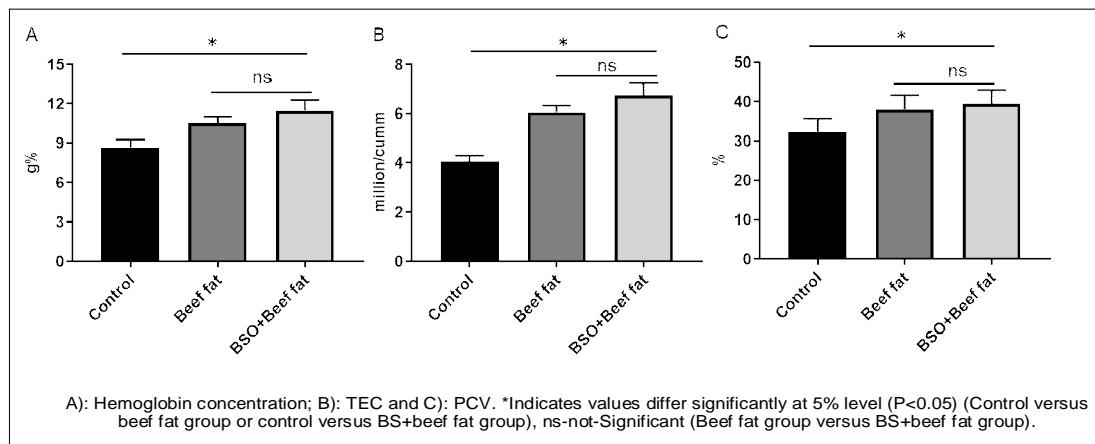
After 9 weeks of experiments, TEC, Hb and PCV of different groups of mice were analyzed and results are presented in Fig 1. Mice of with beef fat group had significantly (p<0.01) increased blood parameters: Hb conc. (10.60±0.62 g%), TEC (6.10±1.65 million/μL) and PCV (37.00±4.50%) compared with those parameters (Hb conc. 8.80±0.25 g%, 3.75±1.05 million/μL and PCV 33.50±3.23%) of control mice (Fig 1). However, use of BSO along with beef fat further

improved the hematological values (Hb conc.  $11.20 \pm 0.75$  g%, TEC  $6.80 \pm 2.05$  million/ $\mu$ L and PCV  $38.28 \pm 3.12\%$ ). BSO along with beef fat synergistically improved the hemoglobin concentration, red cell counts and hematocrit values. Sarker *et al.*, 2019 and Ekanem and Yusuf 2008 showed similar results that hemoglobin concentration and TEC were significantly increased in butter-fat fed mice.

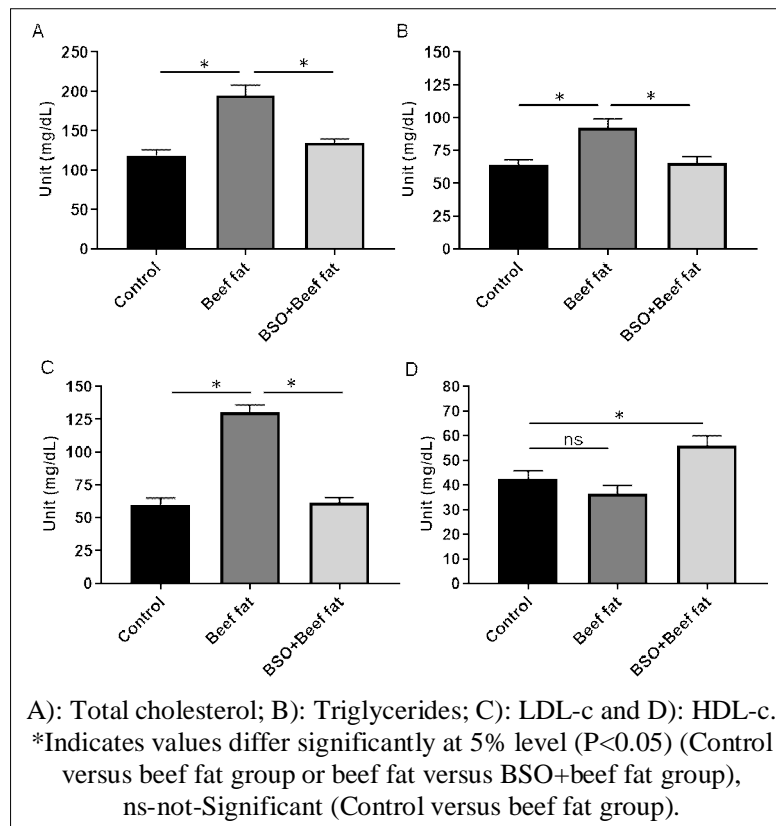
### Lipid profile

The effect of BSO and beef fat on lipid profile is shown in Fig 2. It is known that beef fat induced the serum lipid profile

such as serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c). BSO decreased the lipid profile. The results of the present study also proved that BSO with beef fat significantly decreased the total cholesterol values ( $130.50 \pm 3.11$  mg/dL), triglycerides ( $61.50 \pm 2.08$  mg/dL), LDL-c ( $57.50 \pm 1.73$  mg/dL) except HDL ( $56.75 \pm 4.65$  mg/dL). Dietary lipids rich in saturated fats actively develop atherosclerosis, which is characterized by elevated total serum cholesterol, especially LDL-c (Akter *et al.*, 2013). Butter was observed to lower HDL-C while increasing VLDL



**Fig 1:** Effect of BSO on selected hematological parameters in beef fed treated mice.

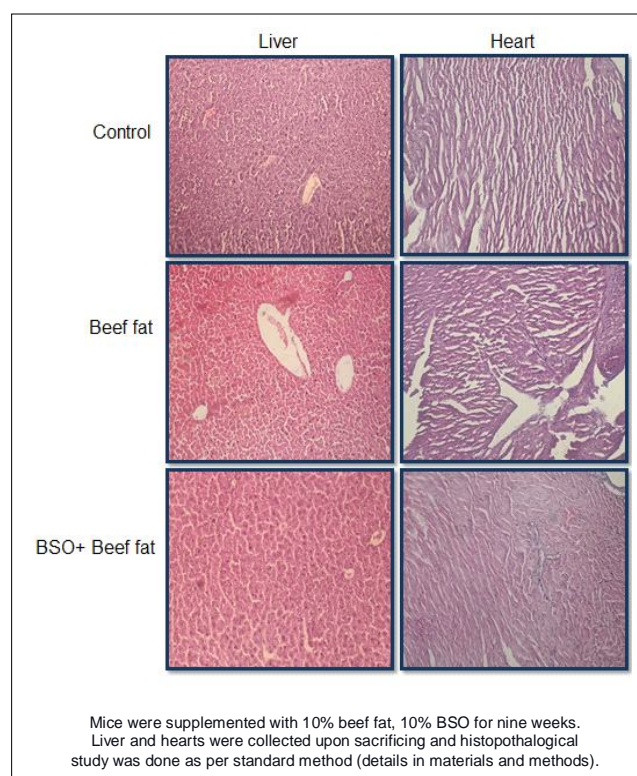


**Fig 2:** Effect of BSO on lipid profile in beef fat fed mice.

**Table 1:** Comparison of body weight and body weight gain in different treatment groups during the course of experiment.

Treatment groups	Body weight (g) (mean±SD)				Weight gain (%)
	Initial	3 <sup>rd</sup> week	6 <sup>th</sup> week	9 <sup>th</sup> week	9 <sup>th</sup> week
Control	29.38±2.20	33.75±2.9	35.88±3.58 <sup>a</sup>	42.12±3.80 <sup>a</sup>	43.36 <sup>a</sup>
Beef fat	29.80±1.50	35.7±2.67	38.97±4.19 <sup>b</sup>	49.86±2.18 <sup>b</sup>	67.31 <sup>b</sup>
BSO+beef fat	30.25±2.40	34.90±3.29	36.07±5.10 <sup>a</sup>	44.60±4.58 <sup>a</sup>	47.48 <sup>a</sup>

<sup>a, b</sup>Values with different superscript letters in a column differs significantly (p<0.05).

**Fig 3:** Photomicrograph (10X) of histopathological sections of liver and heart tissues of different group of mice.

and decreasing LDL-C in atherosclerosis-prone mice. It has been reported that the proportion of saturated and polyunsaturated fatty acids (PUFA) in the diet influences plasma cholesterol levels and high linoleic acid diets impact lipid metabolism in adult rat offsprings (Shrestha *et al.*, 2021). Polyunsaturated Fatty acids (PUFA) have anti-inflammatory and insulin-sensitizing properties and develop hypolipidemia (Tishinsky, 2013). The current study showed similar results that beef fat containing saturated fatty acids had a higher TC and LDL-c in blood. These findings may be explained by the high SFA and low PUFA contents of butter and beef fats, which are important factors in raising serum cholesterol levels. The detrimental effects of the beef fats diets were observed in mice with increased body weight and lipid profile. Our findings are very similar to the previous findings of increased TC and TG levels in beef fat fed animals (Sarker *et al.*, 2019, Khairy *et al.*, 2014 and Alaam *et al.*, 2012). However, adding 10% BSO to the beef fat fed mice normalized the TC and LDL-c values significantly compared to the beef fat fed mice (Fig 2). In addition, HDL levels were

significant increased in BSO-treated groups. The present findings are partially consistent with those of Talukder *et al.*, 2017 who reported that ginger extracts reduced the LDL-c, triglycerides and total cholesterol level induced by butter fat in mice. BSO represents a natural therapy that can prevent and treat HFD-induced obesity in rats, which may be mediated through the Nrf2/HO-1 pathway's activation and insulin receptor expression increase (El-Magd *et al.*, 2021).

#### Effects on BSO and beef fat on patho-physiological alterations in liver and heart

Excess lipids, such as TC and TGs are accumulated in the different organs leading to cardiovascular diseases, fatty liver syndrome, kidneys diseases. TC, TG, LDL-c values were found increased in beef fat-fed mice except for HDL-c and these values were decreased or prevented upon the addition of black seed oil on beef fat (Fig 2). Grossly, there were no remarkable changes found in the spleen, liver, kidney and thymus of beef fat and BSO group (data not shown). However, the size and weight of the spleen in those mice were slightly increased compared to similar body weight and age of the other two groups. Histological sections of the liver showed that there was an insignificant fatty change found in the liver hepatocytes of beef fat group. Again upon BSO treatment, the fat depositions were minimal (Fig 3). Huge muscle fibrosis and pale color muscle were observed in the beef fat group. In the BSO group, fat tissue depositions were not detected in heart tissues (Fig 3). The present findings are partially identical to the result of Sujana *et al.*, (2021) who studied that BSO restored the degenerative changes in ovarian granulosa cells of BPA treated mice.

#### CONCLUSION

10% of beef fats are detrimental to weight gain, lipid profile and organ structure. Such negative effects may be eliminated by using black seed oil. To fill the gaps and laps in the current research, more concise studies with a longer time span and involving more animals are required.

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#### Author contributions

MAM designed the experiment, analyzed the data and wrote the final draft of the manuscript; D. Roy and KMJ carried



out the experiment, analyzed the data and wrote the first draft of the manuscript. The manuscript was revised critically by KKIK and AM.

# Conflict of interest

The author declares no conflict of interest.

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