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EFFECTS OF DIETARY SUPPLEMENTATION WITH SOYBEAN AND CANOLA OIL ON LIPID PROFILES AND HEART IN SWISS ALBINO MICE

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Abstract

Soybean and canola oil are two of the most widely used cooking oils on the planet. The experiment was conducted to study the dose dependent toxicity of dietary supplementation of soybean and canola oil on heart of Swiss albino mice. In the study, 30 male Swiss albino mice aged 6 weeks were randomly assigned to one of five equivalent groups: Group A was considered as control by feeding rat pellet only, group B1 and B2 were supplemented with 25 and 35 ml soybean oil/kg pellet and group C1 and C2 were provided with 25 and 35 ml canola oil/kg pellet, respectively for 60 days, adopting established research procedure. After conducting the experimental period, blood and organ (heart) samples were collected from control and oil supplemented mice through deep anesthesia. The biochemical study of blood revealed that maximum total cholesterol, triglycerides and high density lipoprotein values (153.85 ± 0.44, 88.82 ± 0.37 and 105.50 ± 0.33 mg/dl) were found statistically significant ($P<0.05$) in soybean oil supplemented group (B2) as compared to other groups (B1, C2, C1 and A). Gross study revealed that highest mean weight of heart (0.41 ± 0.01 g) was recorded in soybean oil supplemented group (B2) significantly ($P<0.05$) in comparison to other experimental groups B1, C2, C1 and A. Histopathological study revealed that marked lymphocytic infiltration was found in the ventricular wall of the heart of high dose supplemented groups. So, it may conclude that dietary supplementation of soybean and canola oil have adverse effects on blood lipid profiles and heart of mice.

Keywords: Soybean oil, canola oil, mice, heart, biochemical, histopathology.

Introduction

Edible oils like soybean oil and canola oil are vegetable oils that are widely used for the purpose of cooking all around the world. Fats and oils are esters formed by the condensation of glycerol and three carboxylic acids known as fatty acids. They can be saturated, monounsaturated, or polyunsaturated. Fat or oil provide almost double energy compared to protein or carbohydrate. Vegetable oils contain such as phytosterols, isoflavonoids, tocopherols and fat-soluble vitamins (Sauerwald *et al.,* 2000). Fat-soluble vitamins are transported as well as stored in our body through fats and oils. Excess consumption of fats and oils in the long run may lead to several health problems. The consequence of such consumption of diet containing high amount of fats and oils can be diseases

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like atherosclerosis, obesity, hypertension, diabetes, hyperlipidemia etc. in human and animal body (Jakobsen *et al.,* 2009; Xenoulis and Steiner, 2010). Lipid profile as like total cholesterol, triglycerides, high density lipoprotein are gradually increased in mice when increased the dose of soybean oil concentration in the diet (Salahuddin *et al.,* 2013). For both human and animal bodies heart is considered to be one of the most vital organs. Dietary consumption of fats and oils are harmful for heart of a rat (Ahmad *et al.,* 2007). Dietary supplementation with palm and soybean oil thickens the blood vessel wall of the rat heart. On the basis of lipid profile and histopathology, mustard oil had minor noxious effect on rat than the other oils as like soybean oil (Hoque *et al.,* 2018). Canola oil supplementation resulted in focal infiltrations of mononuclear cells between cardiac muscles in the heart of a rat (El-Reffaei *et al.,* 2015). Dietary supplementation with rapeseed oil high in erucic acid raises enzyme biomarkers in the heart tissue, which leads to inflammation, necrosis, and hemorrhage (Hasan *et al.,* 2018). So undoubtedly, soybean and canola oil play a significant role on the heart of both human as well as animal bodies. Worldwide, there is a scarcity of data on the effects of dietary supplementation of soybean oil and canola oil in humans and laboratory animals. As it is difficult to continue experimental research on humans for some reasons, the laboratory animal (mice) was chosen as the experimental animal for this study. Hence, the present research experiment was structured to study the effects of dietary supplementation of soybean and canola oil on lipid profiles and heart in Swiss albino mice.

Materials and Methods

Experimental animals and diet

Male Swiss albino mice were purchased from the Department of Pharmacy at Jahangirnagar University, Savar, Dhaka. At the time of collection, the mice were 6 weeks old and weighed about 25-30g. Mice appeared to be healthy and free of external injury. Mice were carefully observed after acquisition for one week to allow them to adjust to their new environment before beginning the experiment. Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, approved the experimental protocols. Ethical approval No. [AWEEC/BAU/2018(13)]. Following a one-week acclimatization period, thirty male Swiss albino mice were randomly divided into five equal groups, each of which contained six mice and was labeled A, B1, B2, C1 and C2. Group A was considered to be a control group fed only rat pellets, group B1 and B2 were fed rat pellets with soybean oil, the dosing rate was (25 and 35 ml soybean oil: 1000 gm rat pellet, respectively), and Group C1 and C2 were provided rat pellets containing canola oil and the dosing rate was (25 and 35 ml canola oil: 1000 gm rat pellet) for 60 days, following previous studies (Nafis *et al.,* 2018 and Sharif *et al.,* 2019). Canola and soybean oil were purchased from the local market of Mymensingh and rat pellet was purchased from Jahangirnagar university laboratory animal residence. The experimental diet was prepared on a regular basis and served at a rate of 5gm/mice/day, with water available ad libitum (Sharif *et al.,* 2019). All grouped mice were housed in a mice cage, which was placed in a well-ventilated room with a temperature of 27.8°C and a relative humidity of 71-81 percent, as well as a 12 hours light/dark cycle.

Biochemical studies

Following the execution of the experiment, each Swiss albino mouse was sacrificed with chloroform. Then 2 ml of blood was acquired in a 5 ml disposable syringe via cardiac puncture for estimation of various blood biochemical values such as total cholesterol (TC), triglycerides (TG), and high density lipoprotein (HDL). Then autoclaves glass test tube was filled with 2 ml of blood. Test tubes bearing blood were fixed in a slanting position at room temperature for six hours. Then incubated overnight in the refrigerator at 4°C. The serum from the blood samples was separated and centrifuged at 3000 rpm to remove any extraneous blood cells. Serum samples were stored at -20°C for biochemical analysis (Sharif *et al.,* 2019). From serum sample, TC, TG and HDL were estimated as instructions provided by Trinder (1969).

Gross and histopathological studies

Following mouse sacrifice, the heart sample of each group of mice were collected and inspected for obvious changes. The color and weight of the heart were considered during the gross examination. The weight was measured in grams using an electronic balance. Following gross examination, heart samples were preserved in 10% formalin. Following proper fixation, samples were processed for histopathological examination. For histopathology, the Hematoxylin and Eosin staining protocol was performed according to Jannat *et al.* (2018).

Photo microscopic studies

Photo Microscopic studies were taken in accordance with our previous research (Sharif *et al*., 2019). Photomicrographs were taken with an Olympus BX 51 photographic light microscope as needed and placed for better illustration of the result.

Statistical analysis

Experimental research data were saved in Microsoft Excel 2013 and analysis was done by using software Graph Pad Prism 7. Experimental research data were presented as mean standard error, and variation among mouse groups was compared using the one-way ANOVA followed by Duncan's multiple range test. When the p values were less than 0.05, the variation was considered statistically significant.

Results and Discussion

In the present study, Swiss Albino mice were used to observe the changes of blood lipid profiles and also morphological alterations (both gross and microscopic) of the heart after dietary administration of Soybean and Canola oil.

Biochemical findings

Results showed that in control group A, group B1, group B2, group C1 and group C2 the mean TC values were 122.02 ±0.32, 141.6 ± 0.37, 153.85 ± 0.44, 131.30 ± 0.37 and 137.02± 0.30 mg/dl, respectively (Fig. 1), the mean TG values were 47.37 ± 0.32, 73.29 ± 0.36, 88.22 ± 0.37, 52.97± 0.26 and 57.92 ± 0.46 mg/dl, respectively (Fig. 2) and the mean HDL values were 86.80 ± 0.14, 101.26 ± 0.41, 105.50 ± 0.33, 96.94 ± 0.40 and 98.62 ± 0.12 mg/dl, respectively (Fig. 3). The TC, TG and HDL values were increased significantly in a dose depended aspect in both the Soybean (B1, B2) and Canola (C1, C2) oil supplemented groups comparing to the control group (A). The maximum TC, TG and HDL values were found statistically significant in soybean oil supplemented group (B2) as

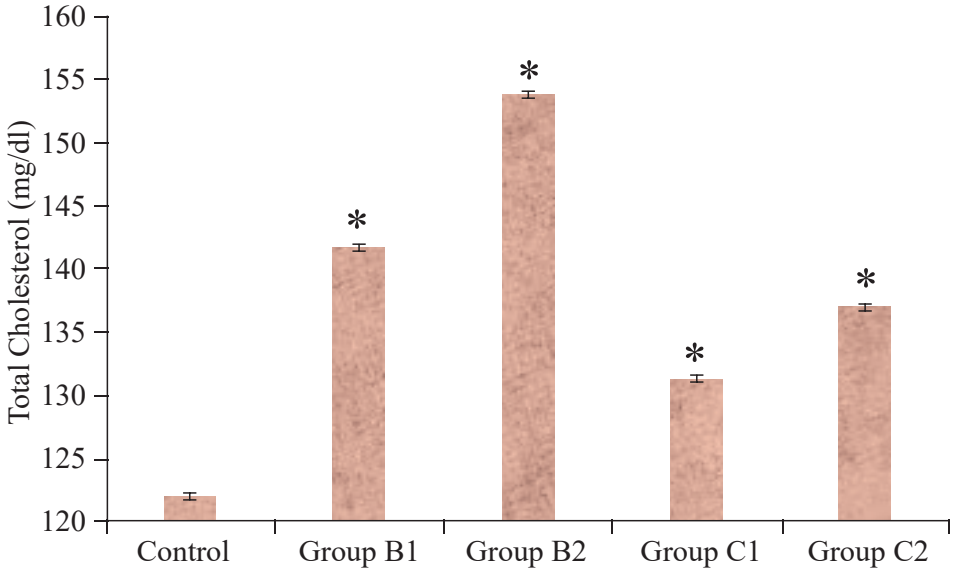

Fig. 1. Total cholesterol level in control, Soybean (B1, B2) and Canola (C1, C2) oil supplemented groups. Data are represented as mean ± SEM, *indicates significant difference (P<0.05) with control.

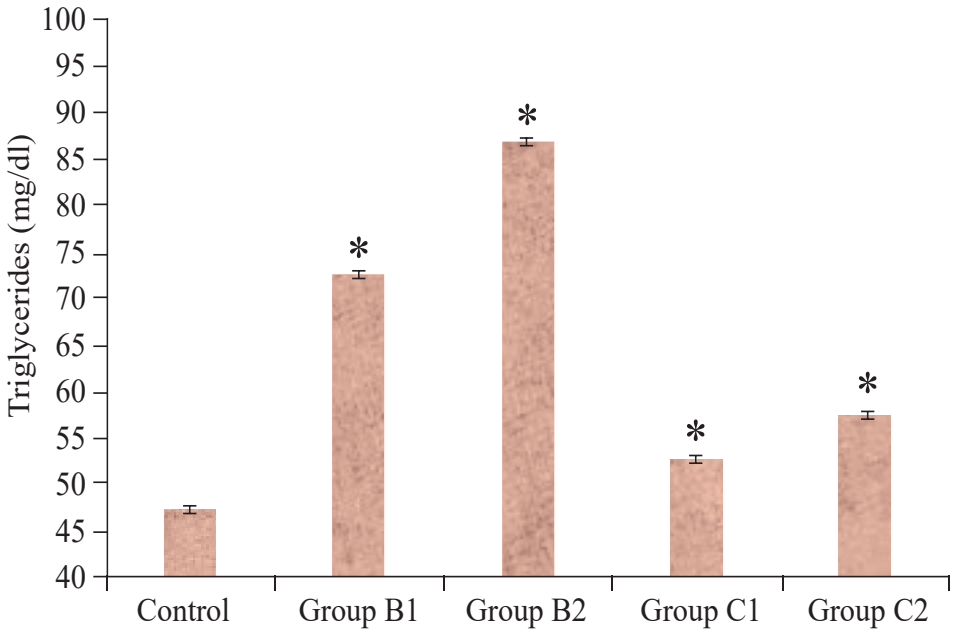

Fig. 2. Triglycerides level in control, Soybean (B1, B2) and Canola (C1, C2) oil supplemented groups. Data are represented as mean ± SEM, *indicates significant difference (P<0.05) with control.

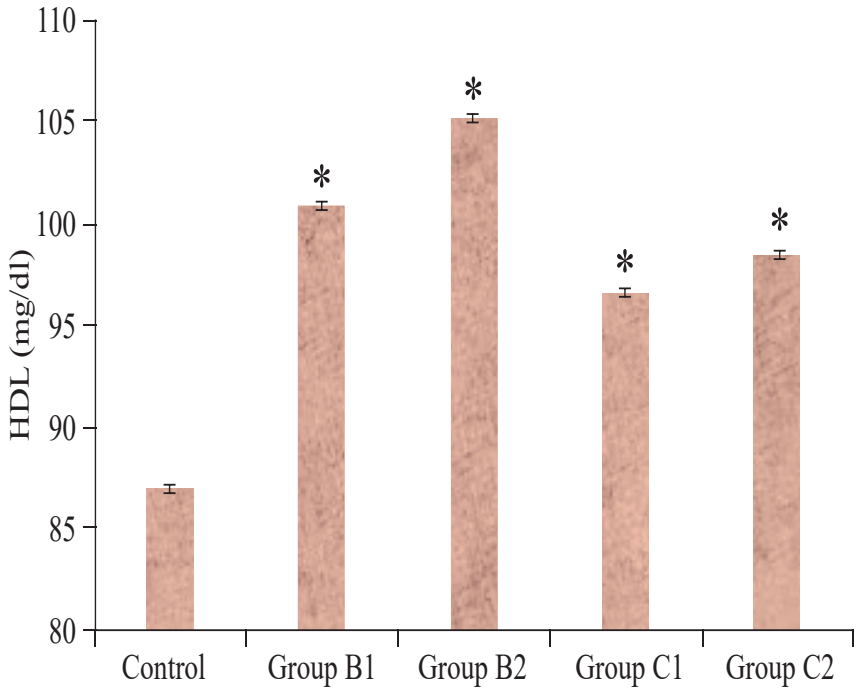

Fig. 3. HDL values in control, Soybean (B1, B2) and Canola (C1, C2) oil supplemented groups. Data are represented as mean ± SEM, *indicates significant difference (P<0.05) with control.

compared to other groups (B1, C2, C1 and A). Salahuddin *et al.* (2013) stated that The TC, TG and HDL values were increased gradually in a dose depended manner and maximum values were recorded in highest soybean oil supplemented group in Swiss albino mice. The same research finding was observed in the present research. Fouladi *et al.* (2008) stated that dietary supplementation of canola oil in a dose dependent manner in broiler chicks decrease TC and TG significantly and increase HDL significantly comparing to the control but in the present research found that dietary supplementation of canola oil in a dose dependent manner increased TC, TG and HDL values significantly comparison to control group. So present research finding in canola oil supplemented group is not similar with Fouladi *et al.* (2008). The present research finding in TC value is slightly differ with Papazzo *et al.* (2011) who stated that Canola oil ingestion significantly reduced the concentration of TC compared with soybean oil in rat. However, linolenic acid presents in Soybean and Canola oil may increase the biochemical parameters (TC, TG, HDL) value in Soybean and Canola oil treated groups of mice than control group. So further research should be needed to find out specific reason.

Gross observation

Grossly, the heart of the control, soybean oil and canola oil supplemented groups of mice were reddish in color and oval in shape. There were no gross morphological changes observed in control and treated groups of mice. The mean

weight of heart in group A, B1, B2, C1 and C2 were 0.12±0.01, 0.32±0.01, 0.41±0.01, 0.21±0.01 and 0.26±0.01g, respectively (Fig. 4). The mean weight of the heart was increased significantly in a dose-depended manner in both the Soybean and Canola oil supplemented groups compared to the control group. The highest mean weight of heart was recorded in soybean oil supplemented group (B2) significantly in comparison to other groups B1, C2, C1 and A. The present finding in soybean oil supplemented group (B2) is not consistent with Ahmed *et al.* (2007) who stated that heart weight of rats of soybean oil supplemented groups increased insignificantly as compared to control group. The mean weight of heart were maximum in soybean oil supplemented group than canola oil supplemented group comparison to control but Innis and Dyer (1999) stated that no deference of heart weight was found in canola and soybean oil supplemented group in piglet that is not consistent with present research, it is inconsistent due to species variation, so there further research should be needed.

Histopathological observation

In the present investigation, heart was found with normal histological architecture in control group (A), Soybean oil supplemented group B1 and Canola oil supplemented group C1 (Fig. 5: Group A, B1 and C1). Marked lymphocytic infiltration was found in the cardiac muscle of the ventricular wall of the heart of Soybean (B2) and Canola (C2) oil supplemented groups of mice, (Fig. 5: Group B2 and C2). The present research finding in soybean oil treated group (B1) is not similar with Farber *et al.* (1976) who stated that marked diffuse fatty degeneration and necrosis of small groups of cardiac muscle fibers were seen in the group fed brominated soybean oil. The present finding in Canola oil treated group (C2) is partially similar

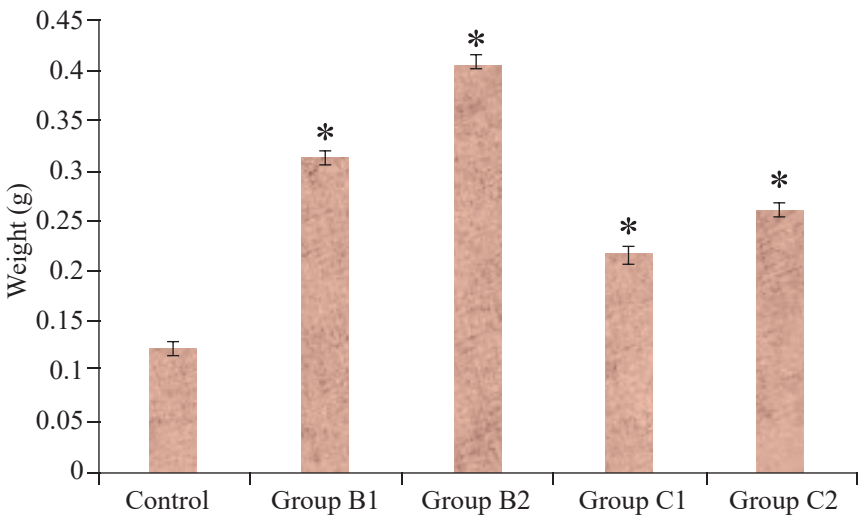

Fig. 4. The mean weight of Heart in control, Soybean (B1 and B2) and Canola oil (C1 and C2) supplemented groups. Data are represented as mean ± SEM, *indicates significant difference (P<0.05) with control.

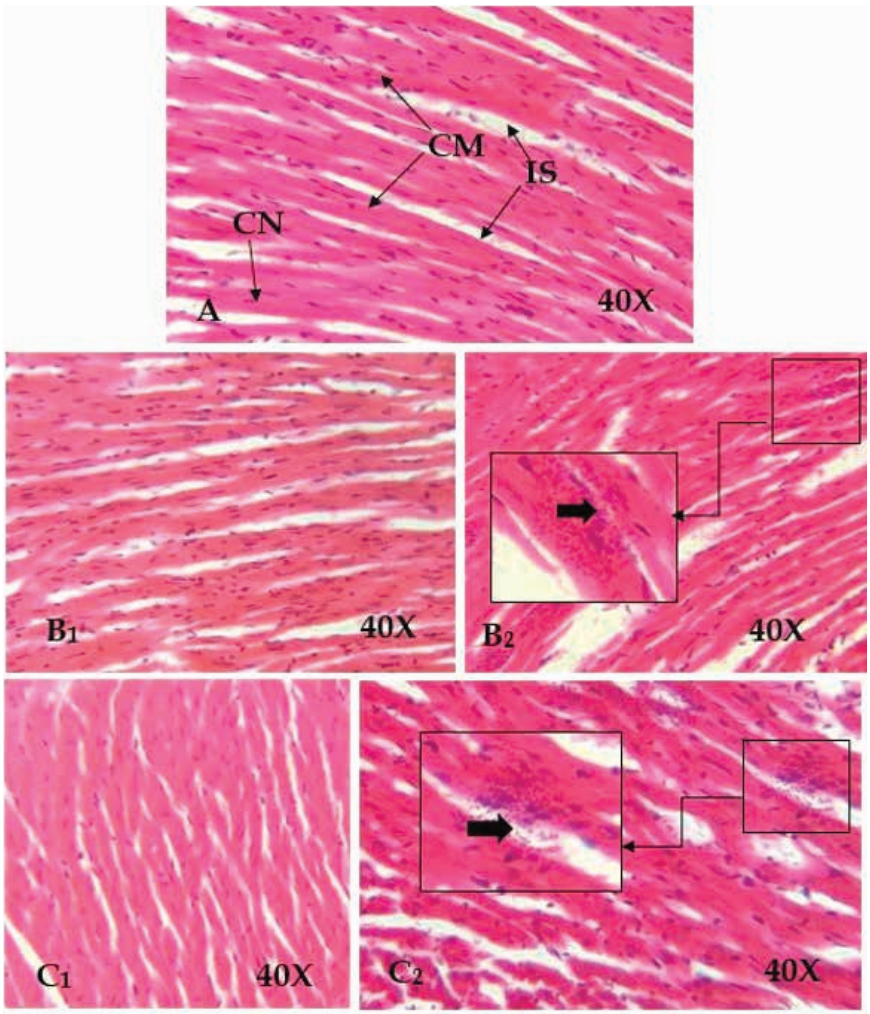

Fig. 5. Histomorphological observation of the heart in control (A) and treated (B1, B2, C1 and C2) groups of mice. No change was observed in the control, B1 and C1 groups of mice but in B2 and C2 groups of mice showed marked infiltration of lymphocytic cells (black arrow) in the cardiac muscle of the ventricular wall of the heart. CM= Cardiac myofibers, IS= Interstitial space, CN= Central Nuclei. Hematoxylin and Eosin stain (40X).

with El-Reffaei *et al.* (2015) who stated that focal area of mononuclear cells infiltration in between cardiac muscle were found in the heart of canola oil supplemented group rat. Dunnick *et al.* (2004) stated that myocardial cells react to toxic agents in a limited number of ways, and the basic pattern of lesion development is comparable to that seen in spontaneous inflammatory cell infiltration in rat. In the present research, inflammatory lymphocytic cell was found in both canola and soybean oil supplemented groups when the oil supplementation dose rate was increased, so in that dose Soybean and Canola

oil supplementation may be harmful for Swiss albino mice. Charlton *et al.* (1975) Stated that transient myocardial lipidosis was found in cardiac myofiber eating of high erucic acid content rapseed oil in rat. Canola oil is also a rapeseed oil but erucic acid content is less in canola oil. In the present research there was no myocardial lipidosis was found in canola oil supplemented group because of lower erucic acid content canola oil. So further study should be needed for advancement of research.

Conclusion

Instead of having some negative impacts, edible oils are essential for calorie requirements for their high-energy component of meals. Soybean oil and canola oil are edible vegetable oils which are extensively used for cooking purpose all over the world. Cholesterol, saturated fatty acids and trans- fatty acids in fats and oils raise the risk of coronary heart disease and atherosclerosis. In the present research work, we found that by feeding of rat pellet with different doses of soybean oil and canola oil, the biochemical parameters like TC, TG and HDL were increased significantly in oil treated groups comparing to that of control group and the highest values were observed in the soybean oil treated groups. Significantly higher mean weight of heart was observed in soybean oil supplemented groups than other groups grossly. The histomorphological study revealed marked lymphocytic infiltration found in the cardiac muscle of the ventricular wall of the heart of Soybean and Canola oil supplemented groups of mice in higher doses. The findings of the current study indicate the harmful effects of supplementation of soybean and canola oil in lipid profile and heart of mice. However, more detailed studies are required to make final conclusion about the effects of soybean and canola oil supplementation in mice.

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