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Original research

Effects of sunflower oils and beef tallow on serum parameters and liver histopathology in experimental rats

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ABSTRACT

Background: The effects of dietary fat composed of different vegetable and animal fat sources on haematological and biochemical parameters in humans or animals remain conflicted. Therefore, this study aimed to investigate the effects of sunflower oil and beef tallow diets on haematological and biochemical parameters and liver histopathology in rats.

Methods: Thirty female rats were fed either a standard diet or a dietary fat composed of sunflower oil or beef tallow for four weeks. The haematological, biochemical and histopathological analyses were performed.

Results: Beef tallow diet resulted in a significant increase in body weight and white blood cells count, while red blood cells count, haemoglobin and haematocrit were significantly reduced compared with those fed standard and sunflower oil diets. Also, beef tallow diet caused a significant increase in the levels of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Serum cholesterol, triacylglycerol, low-density lipoprotein-cholesterol, very low-density lipoprotein were markedly increased, while high-density lipoprotein was significantly reduced in rats fed beef tallow diet than in those fed standard and sunflower oil diets. Moreover, beef tallow diet caused several histopathological changes in rat liver tissues compared with standard and sunflower oil diets.

Conclusion: This study suggests that diet rich in beef tallow may increase the risk of developing a number of obesity-related disorders due to its influence on body weight gain as compared with a diet rich in sunflower oil. Also, the beef tallow diet has negative effects on metabolic parameters indicating its hyperlipidemic activity.

1. Introduction

Over the past decades, there are growing health concerns about the effect of dietary fat composition on human body (Matsuo et al., 2002, 1995; Shimomura et al., 1990). This is due to the fact that consumption of dietary fat has been associated with several health problems such as obesity, fatty liver, cardiovascular diseases, mortality and cholestasis (Chinchu et al., 2020; Singh and Rai, 2019; Vijaimohan et al., 2006). Importantly, the quantity and quality of fat in the diet play a vital role in the development of insulin resistance and associated metabolic disturbances, such as glucose tolerance, hepatic steatosis and dyslipidaemia (Donaldson et al., 2017; Vessby et al., 2001). Also, different degrees of saturation in fat (monounsaturated, polyunsaturated or saturated fat) may exert different effects on insulin action and secretion (Xiao et al., 2006) and glycaemic response (Lau et al., 2016). In this context, vegetable oil has been demonstrated to show hypolipidemic effect due to its low content of saturated fatty acids and high content of polyunsaturated fatty acids. It was reported that the polyunsaturated fatty acids such as linoleic acid can reduce the alterations in liver metabolism and prevent heart diseases (Berrougui et al., 2003; Chandrashekar et al., 2010; Fu et al., 2012). Edible oil is a fatty liquid that is extracted from several plants and some animal tissues used in food preparation, frying and baking (Go et al., 2015). Sunflowers have large amount of oil content and protein therefore they were used to produce edible oil. Sunflower oil is the non-volatile oil derived from sunflower (*Helianthus annuus*) seeds and composed of high content of natural antioxidant such as tocopherols and large amounts of unsaturated fatty acids such as linoleic acid and oleic acid (Akkaya, 2018; Rai et al., 2016). Sunflower oil possess different health benefits therefore its consumption has been increased in Western and Asian countries and it has become the most common cooking oil (Rai et al., 2016). A series of experiments have shown the benefits effects of the vegetable oil compare with animal tallow. Lower body fat accumulation was observed in rats fed a diet with safflower oil compared with those fed

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with beef tallow diet (Matsuo et al., 2002). Also, the triacylglycerol and serum insulin levels were lower in the former (Matsuo and Suzuki, 1994a). Moreover, sympathetic nerve activities in heart (Young and Walgren, 1994) and liver (Matsuo and Suzuki, 1994a) were found to be higher in rats fed a vegetable oil diet rich in n-6 polyunsaturated fatty acid than in rats fed a beef tallow diet rich in saturated and monounsaturated fatty acid for two months (Matsuo et al., 2002, 1995; Matsuo and Suzuki, 1994a, 1994b). Interestingly, it was observed that animal tallow intake inhibits sympathetic activity in brown adipose tissue resulting in lower diet-induced thermogenesis and then greater body fat accumulation. The sympathetic nervous system plays an important role in the regulation of cardiovascular homeostasis and metabolic. Low sympathetic nervous system activity is considered to be a main risk factor for obesity development and weight gain (Davy and Orr, 2010). Although the effects of dietary fat that contain different types of lipids are evaluating, the comparison effects between sunflower oil diet and beef tallow diet on haematological, biochemical and histopathological analyses remain uncertain with studies suggesting conflicting findings. Therefore, this study was performed to investigate the effects of sunflower oil diet (rich in n-6 polyunsaturated fatty acid) and beef tallow diet (rich in saturated and monounsaturated fatty acid) on haematological indices, biochemical parameters and liver tissues of female Wister rats for 30 continuous days.

2. Material and methods

2.1. Animal care

Thirty healthy female Wister albino Kyoto rats (four weeks old) weighing 30 g–38 g were purchased from animal centre in College of Veterinary/University of Basrah (Basrah, Iraq). The animals were housed in animal facility at College of Pharmacy, University of Basrah. Rats were kept in a 12 h light/dark environment at a constant temperature of 25 ± 1 °C with a relative humidity of 55 ± 5%. All animals were used for *in vivo* experiments and were approved by the Animal Research Ethical Committee of Basrah University.

2.2. Diet preparation

Animals were randomly assigned into three groups with ten animals in each group. The control group was given a standard purified diet (AIN-76) which was reported by the American Institute of Nutrition and well accepted as a basic nutrition for experimental mice and rats in the scientific laboratory. Standard diet prepared by using following components: sucrose, casein, cornstarch, cellulose, corn oil, mineral mix, vitamin mix, DL-Methionine and choline bitartrate (Anonymous, 1977). The experimental groups included: the sunflower oil group was given a sunflower oil-enriched diet and the beef tallow group was given a beef tallow-enriched diet. Sunflower oil and beef tallow diets were prepared by adding 20% of fat (sunflower oil or beef tallow) to the same ingredients of the standard diet. Each diet was freshly made every day throughout the experimental time. Sunflower oil was purchased from a super market and the beef tallow was extracted from adipose tissues of animals that obtained from local slaughter houses. All animal groups were free access to drinking water and they were meal-fed the diet for 30 continuous days.

2.3. Analysis of body weight and relative liver weight

The body weights of rats were measured before the onset of the experiment and prior to the sacrifice of the animals. After euthanasia, the whole livers were carefully removed and weighed on analytical balance for relative weight analysis, using the formula: organ weight (g)/animal weight (g) X 100. The data were tabulated and statistically analysed.

2.4. Haematological analysis

At the end of four weeks, animals were weighed and anesthetized using chloroform. Blood samples were collected from the rats by cardiac puncture then they were transferred into a lavender top collection tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and used for haematological analysis. Analysis of complete blood count (CBC) was performed through automated blood cell analyser. The parameters analysed were total white blood cell (WBC) count, lymphocyte and monocyte counts, red blood cells (RBC) count, haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW).

2.5. Biochemical analysis

Blood was collected from the rats by cardiac puncture and transferred into tubes without anticoagulant and left at room temperature for 30 min for clot retraction. Serum was obtained from the blood samples by centrifugation for 20 min (Genex, Florida,USA). After centrifugation, the serum was collected for further biochemical analysis. The alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol were investigated to determine liver function using commercial kits (JOURILABS, Ethiopia) following the manufacturer's instructions. Low-density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) were measured using formula given by friedewald (Friedewald et al., 1972).

2.6. Liver histopathological analysis

After euthanasia, the whole livers were dissected and carefully removed. Livers were then preserved in 10% neutral buffered formaldehyde at room temperature. Tissue samples were embedded in paraffin and 5 μm sections were cut by using a rotary microtome and the samples were then stained with haematoxylin and eosin (H&E) for microscopic examination.

2.7. Statistical analysis

All results such as changes in animal body weight, haematology and biochemistry studies were analysed with the software GraphPad Prism 5 for windows (San Diego, CA, USA). Findings were reported as Mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) was performed followed by Bonferroni's multiple comparison tests (MCT). The results were considered significant when P-values < 0.05.

3. Results

3.1. Effect of dietary fat on body weight and relative liver weight

The body weight was measured before the experiment and prior to sacrifice. The present results showed that there is a significant increase in the final weights of beef tallow group ($P < 0.05$) compared with the control group. Whereas, sunflower oil groups did not observed any significant change in rat body weights compared with the control group (Fig. 1A). However, no statistically significant difference was noted in relative liver weight of rats fed with experimental diets (beef tallow or sunflower oil diets) as compared to those that fed standard diet (Fig. 1B).

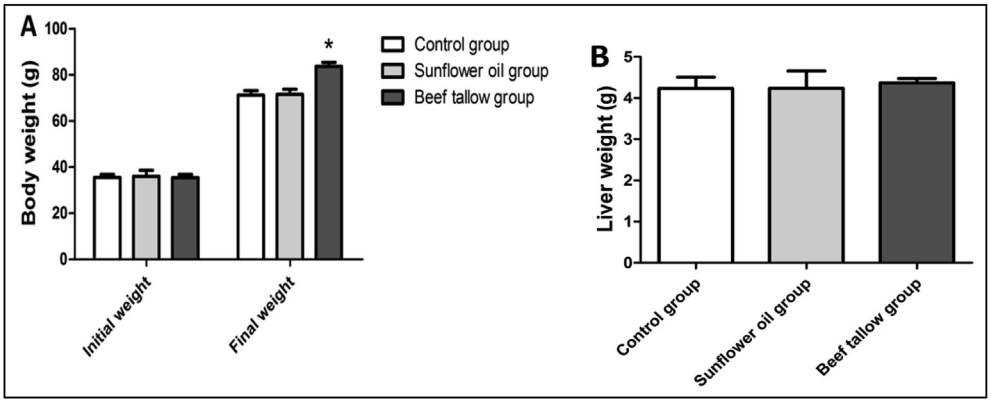

Fig. 1. Effect of dietary fat on body weight and relative liver weight. (A) Bar chart showing the initial and final body weight of experimental rats and (B) bar chart representing the relative liver weight across all groups; *P < 0.05 versus control group; one-way ANOVA, Bonferroni's post-test with error bars representing SEM.

3.2. Haematological evaluation

Haematological investigation was performed to study the different effects of sunflower oil and beef tallow on haematological parameters in rats. The findings of the CBC evaluation showed a significant increase the total leukocytes count in rat fed beef tallow diet compared with rats fed standard and sunflower oil diets (Fig. 2A). While, the levels of RBC, HGB and HCT in beef tallow group were significantly lower than in control and sunflower oil groups (Fig. 2B and C). In contrast, the present findings did not observed significant differences in levels of MCV, MCH, MCHC, RDW, PLT, MPV, PCT and PDW (data not shown).

3.3. Biochemical evaluation

3.3.1. Effect of dietary fat on plasma biomarkers of liver injury

The specific liver enzymes including ALP, ALT and AST that increase in hepatic diseases and toxic damage of liver cells were assessed in this study. The present results found that serum levels of ALP, ALT and AST were significantly increased in rats fed beef tallow diet as in comparison with those fed with normal diet (Fig. 3A–C). No significant differences in these lipid components were found between control and sunflower oil groups (Fig. 3A–C).

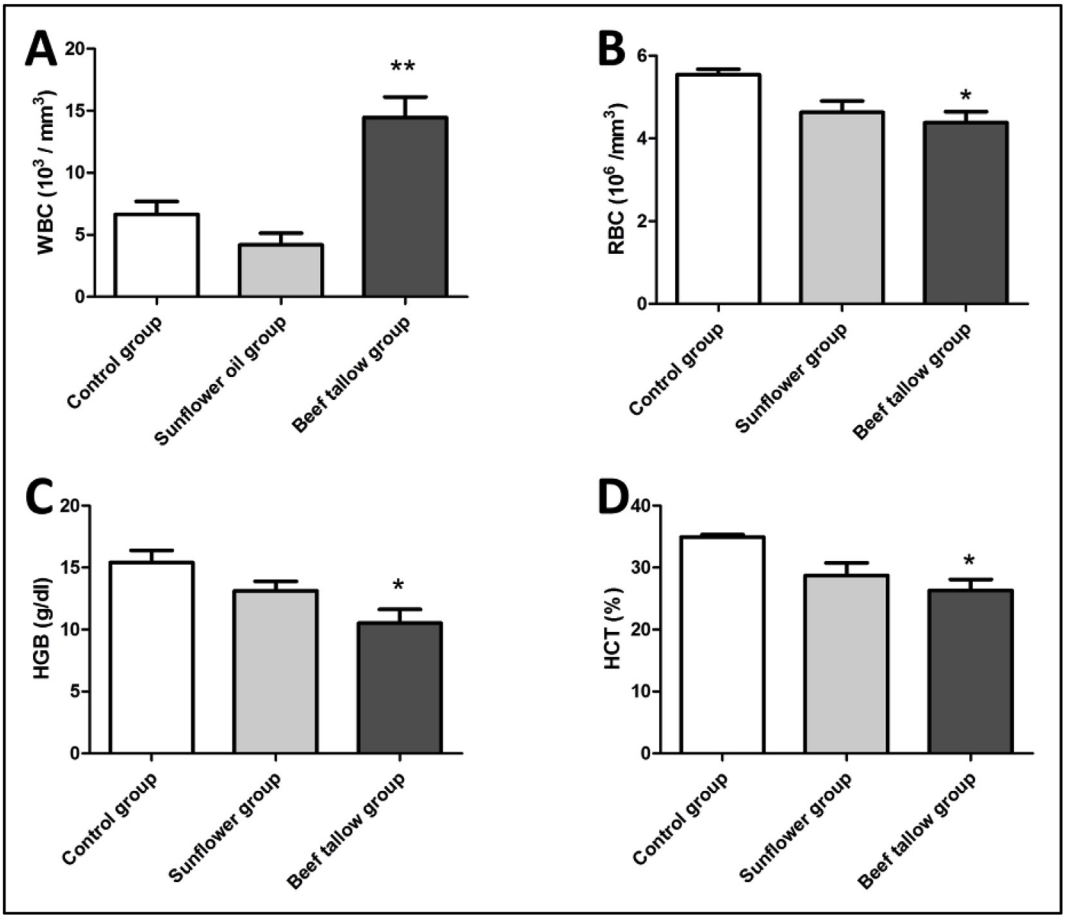

Fig. 2. Effects of dietary fat on some haematological parameters. The graphs represent the, (A) counts of white blood cells (WBC), (B) counts of red blood cells (RBC), (C) concentration of haemoglobin (HGB) and (D) percentage of haematocrit (HCT). One-way ANOVA, Bonferroni's post-test with error bars representing SEM, *P < 0.05, **P < 0.01 versus control group.

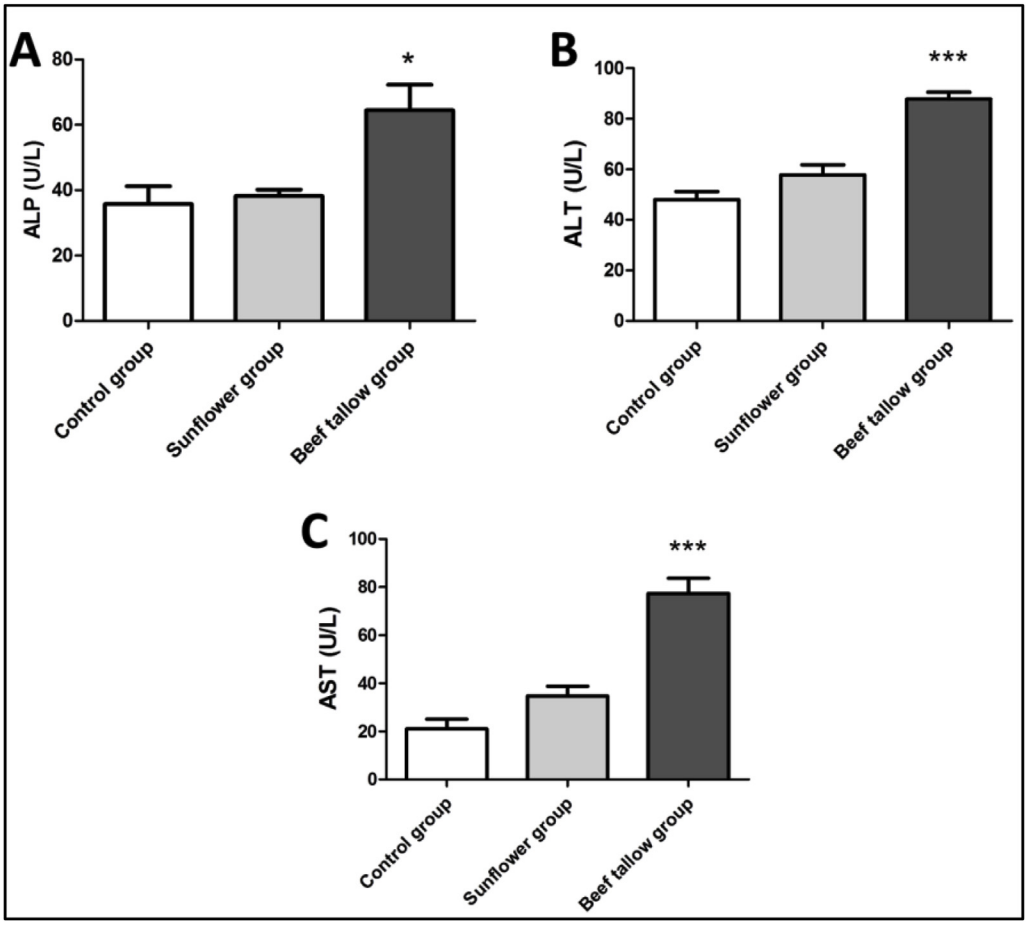

Fig. 3. Effect of dietary fat on plasma biomarkers of liver injury. The graphs show the, (A) the alkaline phosphatase (ALP), (B) alanine aminotransferase activity (ALT) and (C) aspartate aminotransferase activity (AST). One-way ANOVA, Bonferroni's post-test with error bars representing SEM, *P < 0.05, ***P < 0.001 versus control group.

3.3.2. Effect of dietary fat on serum lipid profiles

Serum lipid concentrations including TC, TG, LDL cholesterol, HDL cholesterol and VLDL were investigated to determine the effects of dietary fat in experimental rats. The present results showed that beef tallow-enriched diet resulted in a significant increased levels of TC and TG ($P < 0.01$) (Fig. 4A and B). However, the level of HDL cholesterol was significantly decreased ($P < 0.05$) in rats fed beef tallow-enriched diet (Fig. 4C) with a significant increase in the levels of LDL cholesterol ($P < 0.05$) (Fig. 4D) and in VLDL ($P < 0.01$) (Fig. 4E) in comparison with rats fed with standard diet and sunflower oil enriched diet.

3.4. Liver histopathological evaluation

Histopathological examination of the liver in control group (Fig. 5A) showed intact hepatocytes architecture that were also evident in rats fed sunflower oil enriched diet (Fig. 5B). While several morphological alterations were observed in rats fed beef tallow enriched diet. These alterations were manifested by marked empty space or fat vacillation, the hepatic vein was markedly dilated and congested with blood. Also, the hepatic lobe depicted irregular localization of hepatocyte which may be due to fatty changes in liver tissue indicating fatty liver (Fig. 5C).

4. Discussion

It is well known that dietary fat is considered as a main factor for the development of several diseases such as cardiovascular diseases and obesity due to its role in developing abnormal lipid metabolism, hyperlipidemia and atherosclerosis (Chinchu et al., 2020; Feoli et al., 2003; Ónody et al., 2003). Therefore, the current study aims to examine the effects of two types of dietary fat on haematological and biochemical parameters and hepatic tissue in rats fed either sunflower oil-enriched diet or beef tallow-enriched diet. In the present study, the body weight was significantly increased, while liver relative weight did not show any significant changes in rats fed with beef tallow-enriched diet in comparison with those fed with standard diet and sunflower oil-enriched diet. Similar findings were observed in previous studies, it was found that the high fat diet caused significant increase in rats body weights, resulting in clinical secondary problems (Goyal et al., 2019; Jayasooriya et al., 2000; Shimomura et al., 1990). The possible explanation for this result is due to saturated and monounsaturated fatty acids found in beef tallow which are responsible for hyperlipidemic effects and body weight gain in rats. It was demonstrated that overweight and obese individuals are at higher risk for metabolic and heart diseases compared with non-obese individuals (Lee et al., 2006). Also, the development of atherosclerotic plaques is found to be associated with alterations of lipid profile such as LDL cholesterol, TC and TG. Further, they were observed that several haematological indices such as HGB, RBCs and PLT are associated with cardiorespiratory problems (Ferreira et al., 2013). Therefore, due to the relationship between lipid and haematological parameters with body weight gain, the haematological analysis was performed in this study. The heamatological analysis showed a significant increase the total WBC count and a reduction in RBC count, HGB and HCT in rat fed beef tallow diet compared with rats fed standard and sunflower oil diets. The body weight gain and obesity are chronic inflammatory states, and increased WBC counts have largely known associations with inflammatory conditions due to increased cytokine production by adipose tissue (Mbbs and Brien, 2006; Womack et al., 2008). Also, several previous studies have been demonstrated the relationship between WBC counts and dyslipidaemia (dyslipidaemia characterized by high level of triglycerides and LDL cholesterol and low level of HDL cholesterol) (Heydari and Galassetti,

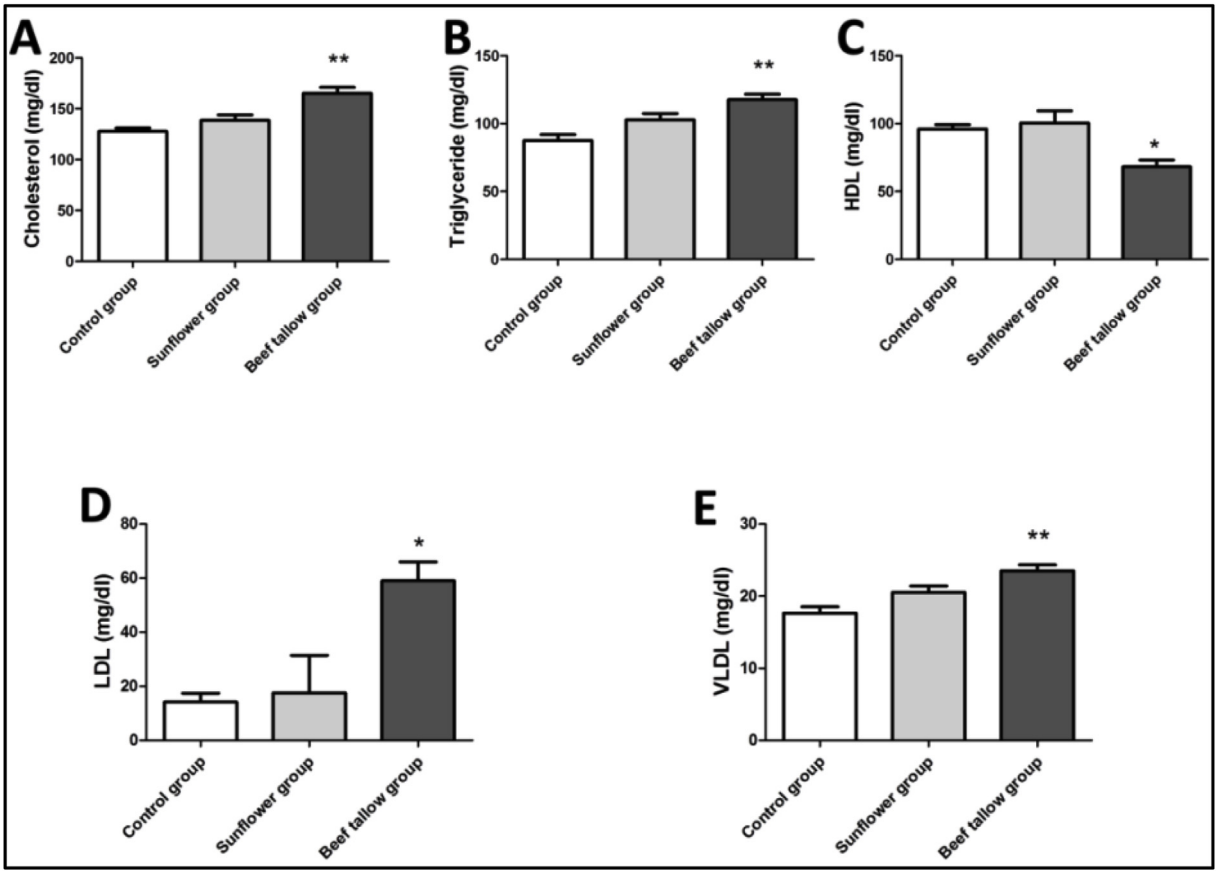

Fig. 4. Effect of dietary fat on serum lipid profiles. The graphs show the, (A) the total cholesterol (TC), (B) triglycerides (TG), (C) high-density lipoprotein (HDL) cholesterol, (D) low-density lipoprotein (LDL) cholesterol and (E) very low-density lipoprotein (VLDL). One-way ANOVA, Bonferroni's post-test with error bars representing SEM, *P < 0.05, **P < 0.01 versus control group.

2011; Kim et al., 2008). Interestingly, the distribution of fat has been described as a vital marker of inflammation and is also associated with alterations in cytokine levels (Ferreira et al., 2013). However, the current study is dissimilar with previous research; they were reported that sunflower oil diets impact several haematological parameters such as RBC count and haemoglobin concentration in mice (Basak et al., 2017). This discrepancy may be due to use different age of animals and different time of treatment. The high levels of liver enzymes ALP, ALT and AST are major factors for the development of fatty liver disease (Nanji et al., 1986). The present study showed an increase in the ALP, ALT and AST levels in rats fed beef tallow diet for 30 continuous days. These results strongly suggest that the continuous feeding with beef tallow diet may lead to cause hepatic diseases and liver cells damage compared to those fed sunflower oil diet. Also, the present findings found that the serum lipid concentrations of cholesterol was increased in rats fed beef tallow diet compared with standard and sunflower oil diets. The high levels of cholesterol in rats fed beef tallow diet may be ascribed to the presence of saturated and monounsaturated fatty acid in beef tallow. On the contrary, it was demonstrated that intake of polyunsaturated fatty acids which are found in vegetable oils can reduce incidence of cardiac diseases by lowering the levels of TC and TG (Simopoulos, 1999). Similar to cholesterol, TG levels also was found to be increased in rats fed beef tallow diet compared to those that fed standard and sunflower oil diets. The increase of serum TG as a result of beef tallow intake has been noted previously (Shimomura et al., 1990). The high levels of TG can effect on the endothelial cells, causing cardiovascular diseases (Suprijana et al., 1997). It was demonstrated that the dietary fat caused an increase in the levels of TG due to lipoprotein lipase triacylglycerol hydrolysis and then resulting in increased TG concentration in the liver (Feoli et al., 2003). Furthermore, they were reported that the dietary fat can increase the biosynthesis of phospholipids by reducing the phospholipase activity or increasing phospholipid turnover because of starting the inflammatory process. High fat diet increases free fatty acids levels in plasma and tissue resulting in an

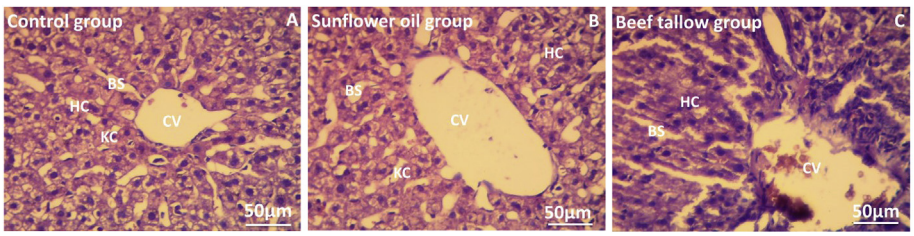

Fig. 5. Effects of dietary fat on Liver histopathology. (A) Photomicrograph of control group showing normal hepatocytes architecture. (B) Photomicrograph of rats fed with sunflower oil-enriched diet depicting normal hepatocytes architecture and absence of any pathologic alterations with normal central vein in liver tissue. (C) Photomicrograph of rats fed with beef tallow-enriched diet showed irregular localization of hepatocyte with dilated and congested hepatic vein. Sections were stained with H&E, central vein (CV), hepatocytes (HC), Kupffer cells (KC), blood sinusoids (BS) magnifications of images: ×200.

increase in the production of phospholipids and cholesterol esters in fed rats (Whereat and Rabinowitz, 1975). Further, the present results showed an increase in the LDL cholesterol and VLDL cholesterol levels and a reduction in HDL cholesterol. LDL cholesterol transfer cholesterol from the liver to the arteries, therefore an elevation of LDL levels can cause cholesterol deposition in the arteries and aorta and thereby leads to reducing the artery diameter. Hence LDL cholesterol is associated with a direct risk of cardiovascular diseases therefore this type of lipoprotein is often called bad cholesterol. In contrast, HDL cholesterol is known as good cholesterol because it can help to remove and recycle cholesterol by transporting it to the liver. Therefore, HDL cholesterol keeps the walls of inner blood vessels healthy (Go et al., 2015). The liver has a vital role in the control of cholesterol synthesis and excretion (Choi et al., 2001). The high intake of exogenous cholesterol causes an increase in cholesterol levels in liver and plasma resulting in lipid deposition and reducing cholesterol catabolism (Jagannathan et al., 1974). It was reported that the free and ester cholesterol metabolism was impaired in hyperlipidemic animals tissues (Feoli et al., 2003). Histological analysis of liver tissue revealed several histopathological changes in beef tallow diet fed group compared with sunflower oil and control groups. The rats fed with sunflower oil diet for four weeks did not exhibit any alterations in their liver tissues. These results confirmed the protective effect of sunflower oil group against the liver histopathological changes in beef tallow group. The present findings suggested that the continuous feeding with beef tallow diet may be caused several health problems such as obesity, hepatic and cardiovascular diseases, mortality and cholestasis.

5. Conclusions

Taken together, the present findings indicate that beef tallow, a highly saturated fatty acid, can affect serum lipid levels and induce histopathological changes in liver. However, sunflower oil, a highly unsaturated fatty acid, was found to be well metabolized in liver. Therefore, we can conclude that the type of fatty acid impacts the lipid profile and liver metabolism. Further studies, including human researches are needed to clarify the mechanisms and relative effects of the compositions of sunflower oil and beef tallow on cholesterol and lipoprotein levels.

CRediT authorship contribution statement

Manal Nasser Al-Hayder: Conceptualization. **Rawaa S. Al-Mayyahi:** Formal analysis, Writing - review & editing. **Abrar S. Abdul-Razak:** Data curation.

Declaration of competing interest

The authors report no conflicts of interest.

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Abbreviations

EDTA	Ethylenediaminetetraacetic acid
CBC	complete blood count
WBC	white blood cell count
RBC	red blood cells count
HGB	haemoglobin
HCT	haematocrit
MCV	mean corpuscular volume
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
RDW	red cell distribution width
PLT	platelets
MPV	mean platelet volume
PCT	plateletcrit
PDW	platelet distribution width
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
TC	total cholesterol
TG	triglycerides
HDL	high-density lipoprotein cholesterol
LDL	low-density lipoprotein cholesterol
VLDL	very low-density lipoprotein
SEM	Standard Error of Mean
ANOVA	analysis of variance

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