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The effects of ghee administration in comparison to sunflower seeds oil on liver tissue and some biochemical parameters in rats

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Abstract

Ghee is well known clarified butter of animal origin (namely derived from sheep milk), it has been used for decades in Iraq for preparing deserts, but nowadays, its nutritional value has been reviewed and is an area of controversy because of its high content of saturated fatty acids; however, the sunflower seeds oil available in the market may not be of that good quality. Comparing the effect of ghee to the effect of sunflower seeds oil may help better understand this controversy, the purpose of this study was to compare the effects of using animal ghee and sunflower seed oil on the liver's histopathology and related to various biochemical alterations in rats. We used 36 animals divided into three groups to accomplish this goal. The first group, which received a typical conventional diet, was regarded as a control group. The second group was given a diet that included 5% animal ghee. And the final group had a diet that included 5% sunflower oil. Blood samples were taken at intervals of 0, 2, and 4 consecutive weeks. There are an increase in the weights of animals in the sunflower oil-fed group with an increase in cholesterol and liver function enzymes ALT and AST in the blood compared with the group treated with ghee, which showed no change in animal weights and low cholesterol with decreased liver function enzymes The histopathological changes of the rat's liver revealed mild to moderate lesions in the Ghee fed group representing by vacuolar degeneration of hepatocytes and focal infiltration of inflammatory cells after four weeks of treatment. In the Sunflower seeds oil-fed group, the liver revealed more severe lesions than the rat treated in the ghee group, as severe vacuolar degeneration and necrosis of hepatocytes with fatty change, generalized congestion of blood vessels, infiltration of inflammatory cells in the portal area and hyperplasia of bile canaliculi. According to the study's findings, animal ghee has advantages over sunflower seed oil regarding hepatic histological changes and concomitant biochemical changes in rats.

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Introduction

Lipids are one of the types of big biological molecules; they make up 5% of the organic material in a cell's structure and can be divided into simple lipids and complex lipids, which are further subdivided into phosphorous lipids, non-phosphorous lipids, derived lipids, and compound lipids, according to katan et al (1). The definition of fatty acid is a hydrocarbon chain containing one or more double bonds with a carboxyl group (COOH) at one end and a methyl group (CH_3) at the other end (2). PUFAs include corn oil, sunflower oil, omega-6 and omega-3 fish oils, as well as unsaturated to monounsaturated fatty acids (MUFAs) like omega-9 olive oil and PUFAs like corn oil and sunflower oil

(3). According to the location of the double bond, polyunsaturated fatty acids (PUFA) are divided into two groups, omega-3, and omega-6; linolic acid (LA) is a source of omega-6, and alpha-linolenic acid (ALA) is a source of omega-3 (4). Because they can be produced by the body, non-essential fatty acids go by this name. If the diet contains a lot of non-essential fatty acids, metabolic processes will be hampered, which will impair the function of essential fatty acids and result in a variety of health issues (5), because they are exposed to more non-essential fatty acids in the diet, cells change in composition and become more solid and viscous. as it exposes them to inflammation and facilitates their exposure to infection (6). Ghee is a substance produced using traditional techniques in various nations across the world, primarily in Asia. A product made only from milk, cream, or butter using procedures that almost entirely remove water and non-fat, flavorful particles is what is meant by the term "ghee" in modern usage. Physical structure and evolution (7). Because animal ghee poses a risk to the heart and arteries, agencies in charge of food and nutrition have advised substituting hydrogenated vegetable oils for it since the 1950s. The products' oxidative stability and texture are both enhanced by hydrogenation. Unfortunately, some double bonds can switch from a cis to a trans-state during the hydrogenation process. Saturated fatty acids are known to have harmful effects on health and contribute to a number of disorders (8). However, Kadhum and Shamma (9) found that experimental Wistar rats' antioxidant status and anti-atherosclerotic efficacy were increased by milk fat (ghee) because of their greater conjugated linoleic acid concentration. Additionally, ghee enhances blood lipid profiles by raising HDL levels and lowering lipid peroxidation, according to Chinnadurai *et al.* (10).

The aim of the current study was to compare the effects of using sunflower oil and animal ghee on some biochemical and hepatic parameters in rats in order to demonstrate the differences between the two.

Materials and methods

Animals

White healthy albino adult rats maintained in the College of Veterinary Medicine; Animal House were utilized. The rats were housed under the standard hygienic conditions in separate plastic cages made specifically for this study with a room of temperature 25±2ºC in the animal house. Light will be maintained on a 12-h light / dark cycle, given the required water and food, and given the proper lighting, temperature, and hygienic conditions (11).

Ethical approval

Any measures taken to treat animals humanely, such as drawing their blood or sacrificing them for their organs, received ethical approval from the University of Mosul at College of Medicine.

Materials

Locally produced natural animal ghee is made from sheep's milk and ordinary sunflower oil from Mosul's markets. Using a kit from Biomagreb, the plasma glucose level was determined using a peroxidase oxidase technique (12) that is highly specific for D-glucose (Tunisia). Using a kit from Elabscience, to measure the activity of S.GOT and S.GPT by the colorimetric method according to Reitman - Frankel (13). A kit from Biocon (Germany) was used to test total cholesterol using the enzymatic approach (14). A kit from Biomerieux was used to separate HDL-C and determine the amount of cholesterol bound to this fraction (15).

Collection of samples

The animals were given ether anesthesia so that 7 ml of blood could be drawn from their eye sockets using capillary tubes, and then the blood was divided into two separate laboratory tubes, one with an anticoagulant to separate the plasma from the blood and the other without an anticoagulant. Two ml of whole blood was centrifuged at 3000 rpm for 10 minutes, and plasma was used for glucose determination. For the measurement of other biochemical parameters, the remaining 5ml of blood was transferred into disposable plain tube, allowed to clot for 15-30 minutes in water bath at 37ºC, then serum was separated by centrifugation at 3000 rpm. Sera were used for measurement of the following biochemical parameters included total cholesterol, HDL- cholesterol, GOT and GPT. The animals were sacrificed after the blood was obtained under anesthesia by an intramuscular injection of 50 mg /kg ketamine HCl (ether) in order to perform the autopsy and remove the liver. The liver was then cleaned with tap water, dried on absorbent paper, and then preserved in diluted formalin with a 10% concentration until tissue cutting was done on it. Hematoxylin and eosin were used to color the tissue slices.

Experience design

We utilized male albino rats that were 2 months old and weighed between 150 and 242 grams. Three groups of 12 animals each were formed from the 36 total animals. Three times at time zero, at time two weeks, and at time four weeks blood samples were taken. Rats were sacrificed at the end of the 4-week treatment period in order to collect liver samples for histopathological analysis under a light microscope.

Analytical statistics

The statistical program ANOVA employed the Duncan one-way test, and the level of significance was set at $P \leq 0.05$. The samples were reported as the mean ± standard error.

Results

The findings demonstrated a substantial difference between the animal weight increases at 2- and 4-weeks

intervals in the sunflower group in comparison to the animal ghee group (Table 1). The concentration of sugar in all groups up until the control group significantly increased in the second week compared to the remainder of the study's time periods, and there was no significant difference in concentration of sugar across the groups (Table 2). When compared to the control group and the sunflower oil group, the cholesterol concentration in the sunflower oil group dramatically increased in the second and fourth weeks while significantly decreasing in the animal ghee group in the fourth week (Table 3). The second week's HDL measurements revealed a significant difference in all groups, and the Ghee-fed group 's HDL level increased although not significantly (Table 4). When compared to the other groups, the levels of ALT and AST significantly increased in the fourth week in sunflower seeds oil group, while they significantly decreased in the group that received animal ghee treatment for the same time period (Tables 5 and 6).

Table 1: comparison of the rat's body weights (gram) among the three groups and the periods

Periods	Control group	Sunflower fed group	Ghee fed group	P-value
0 day	213.3 ± 9.6 Aa	217.1 ± 8.1 Aa	201.7 ± 5.3 Aa	0.205
2 weeks	217.1 ± 9.2 Aa	230.6 ± 8.0 Aa	211.5 ± 5.6 Aa	0.117
4 weeks	217.1 ± 6.6 Aa	242.0 ± 6.6 Bb	216.4 ± 7.2 Aa	0.03
P-value	0.402	0.04	0.87	

Data expressed as Mean ± Stander error (N. Total rats = 12/group). The Capital letters mean there are significant differences between groups at P≤0.05. The Small letters mean there are significant differences between periods at P≤0.05.

Table 2: comparison of the levels of sugar (Mg/dl) among the three groups of rats and the periods

Periods	Control group	Sunflower fed group	Ghee fed group	P-value
0 day	61.1 ± 3.0 Aa	71.2 ± 5.3 Aa	66.0 ± 2.7 Aa	0.206
2 weeks	118.6 ± 9.2 Ab	142.6 ± 8.0 Ab	133.0 ± 5.6 Ab	0.147
4 weeks	66.0 ± 2.7 Aa	74.5 ± 4.0 Ab	74.0 ± 4.2 Aa	0.213
P-value	0.00	0.00	0.00	

Data expressed as Mean ± Stander error (N. Total rats = 12/group). The Capital letters mean there are significant differences between groups at P≤0.05. The Small letters mean there are significant differences between periods at P≤0.05.

Table 3: comparison of the levels of cholesterol (Mg/dl) among the three groups of rats and the periods

Periods	Control group	Sunflower fed group	Ghee fed group	P-value
0 day	100.5 ± 8.3 Aa	99.1 ± 4.9 Aa	89.1 ± 5.9 Aa	0.356
2 weeks	148.0 ± 9.2 Ab	180.5 ± 8.0 Ab	163.8 ± 5.6 Ab	0.126
4 weeks	116.6 ± 7.5 Aa	155.6 ± 11.4 Bb	129.0 ± 10.5 Ac	0.069
P-value	0.00	0.00	0.00	

Data expressed as Mean ± Stander error (N. Total rats = 12/group). The Capital letters mean there are significant differences between groups at P≤0.05. The Small letters mean there are significant differences between periods at P≤0.05.

Table 4: comparison of the levels of HDL cholesterol (Mg/dl) among the three groups of rats and the periods

Periods	Control group	Sunflower fed group	Ghee fed group	P-value
0 day	48.5 ± 4.5 Aa	47.1 ± 3.3 Aa	43.9 ± 4.7 Aa	0.756
2 weeks	65.8 ± 10.0 Ab	74.9 ± 6.7 Ab	68.3 ± 6.8 Ab	0.693
4 weeks	48.3 ± 11.2 Aa	49.4 ± 4.0 Aa	55.5 ± 6.7 Aa	0.705
P-value	0.00	0.00	0.031	

Data expressed as Mean ± Stander error (N. Total rats = 12/group). The Capital letters mean there are significant differences between groups at P≤0.05. The Small letters mean there are significant differences between periods at P≤0.05.

The histopathological changes of the rat's liver tissue revealed the normal architecture in the control group (Figure 1) with mild to moderate lesions in the ghee fed group represented by vacuolar degeneration of hepatocytes, congestion of central vein and mild focal infiltration of inflammatory cells after 4 weeks of treatment (Figures 2-5). In the Sunflower seeds oil fed group, the liver sections of the rats revealed more severe histopathological changes than the

rat treated with ghee group. It is characterized by severe vacuolar degeneration and necrosis of hepatocytes with fatty change, generalized congestion of blood vessels, infiltration of inflammatory cells in the portal area, deposition of fibrin in the portal area around the blood vessels, hemorrhage in liver parenchyma, hyperplasia of bile canaliculi and presence of thrombus in the portal vein (Figures 6-9).

Table 5: comparison of the levels of the ALT (IU) among the three groups of rats and the periods

Periods	Control group	Sunflower fed group	Ghee fed group	P-value
0 day	205.8 ± 15.9 Aa	208.0 ± 12.0 Aa	180.1 ± 6.0 Aa	0.123
2 weeks	210.7 ± 18.6 Aa	228.7 ± 8.6 Aa	201.6 ± 12.0 Aa	0.233
4 weeks	235.5 ± 11.2 Aa	197.1 ± 4.0 Aa	118.6 ± 6.7 Bb	0.014
P-value	0.634	0.399	0.031	

Data expressed as Mean ± Stander error (N. Total rats = 12/group). The Capital letters mean there are significant differences between groups at P≤0.05. The Small letters mean there are significant differences between periods at P≤0.05.

Table 6: comparison of the levels of AST (IU) among the three groups of rats and the periods

Periods	Control group	Sunflower fed group	Ghee fed group	P-value
0 day	46.1 ± 8.9 Aa	49.0 ± 3.0 Aa	39.7 ± 3.9 Aa	0.292
2 weeks	38.6 ± 5.7 Aa	42.0 ± 2.3 Aa	43.3 ± 4.5 Aa	0.768
4 weeks	62 ± 9.8 Aa	49.2 ± 2.5 Aa	45.7 ± 3.7 Ba	0.05
P-value	0.18	0.301	0.324	

Data expressed as Mean ± Stander error (N. Total rats = 12/group). The Capital letters mean there are significant differences between groups at P≤0.05. The Small letters mean there are significant differences between periods at P≤0.05.

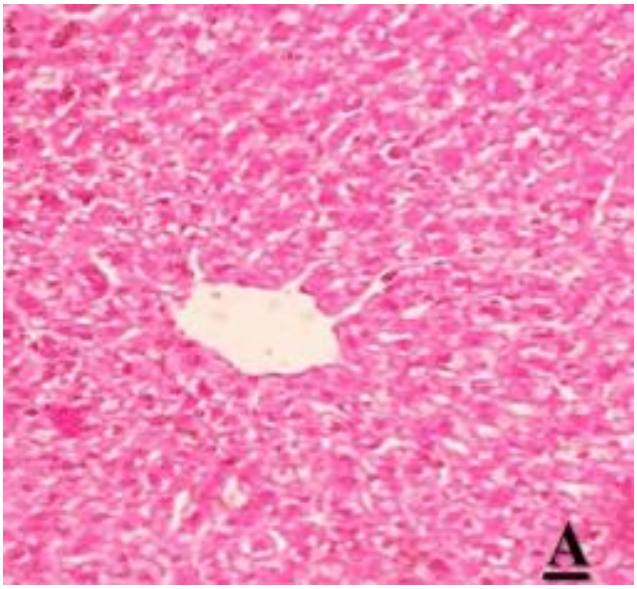

Figure 1: Control group, shows normal architecture. H&E, 100x.

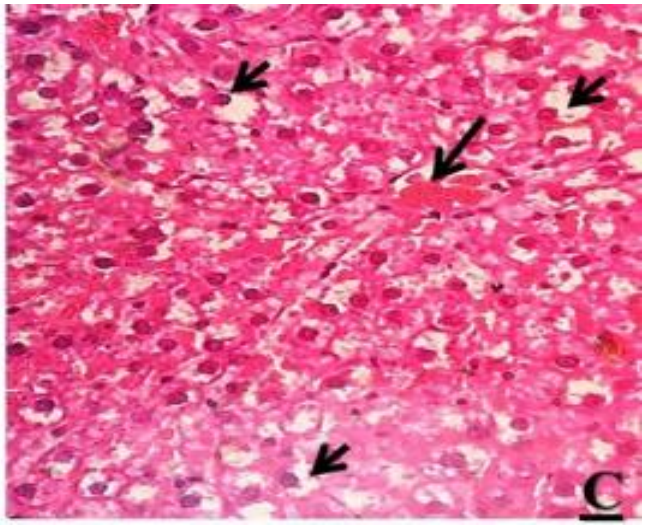

Figure 2: Ghee fed group, shows vacuolar degeneration of hepatocytes (arrow head) and congestion of central vein (arrow). H&E, 400x.

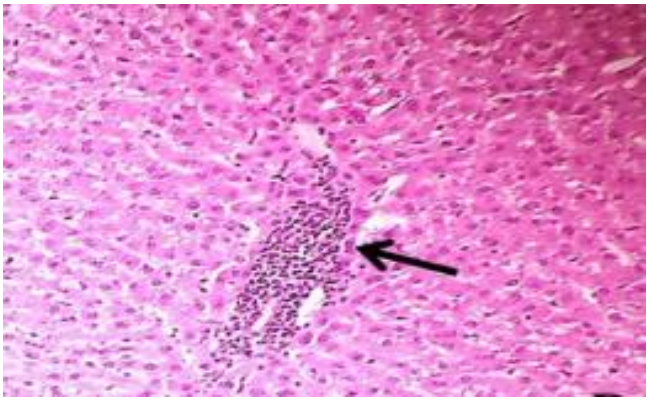

Figure 3: Ghee fed group, showed mild focal infiltration of inflammatory cells (arrow). H&E, 100x.

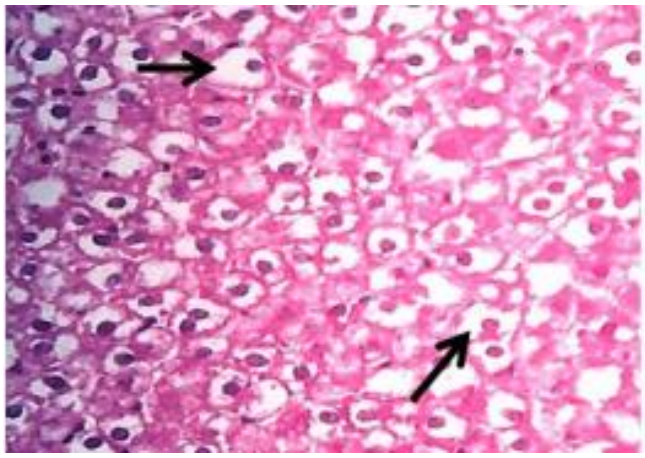

Figure 4: Ghee fed group, shows severe vacuolar degeneration of hepatocytes with fatty change (arrows). H&E, 400x.

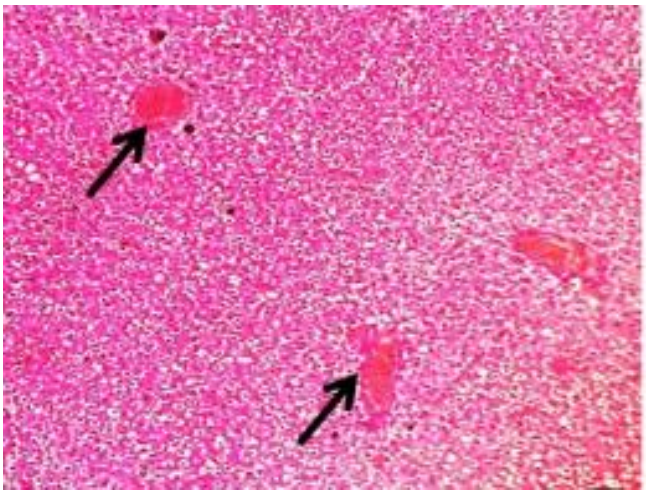

Figure 5: Ghee fed group, shows generalized congestion of blood vessels (arrows). H&E, 100x.

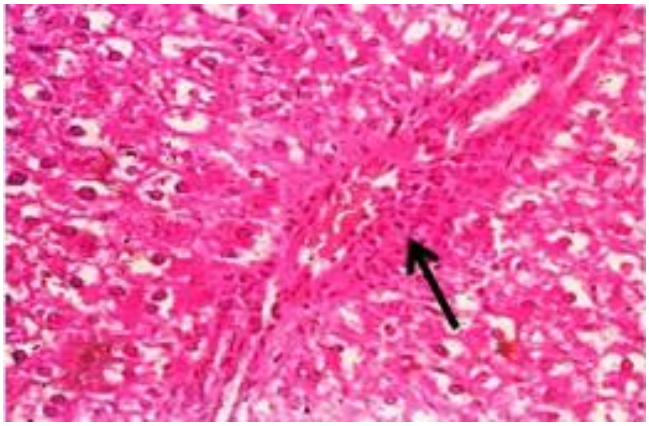

Figure 6: Sunflower seeds oil fed group, shows infiltration of inflammatory cells in the portal area (arrow). H&E, 400x.

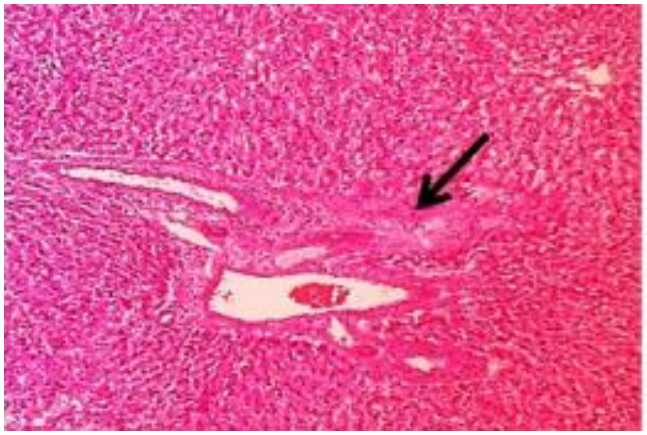

Figure 7: Sunflower seeds oil fed group, hows deposition of fibrin in the portal area around the blood vessels (arrow). H&E, 100x.

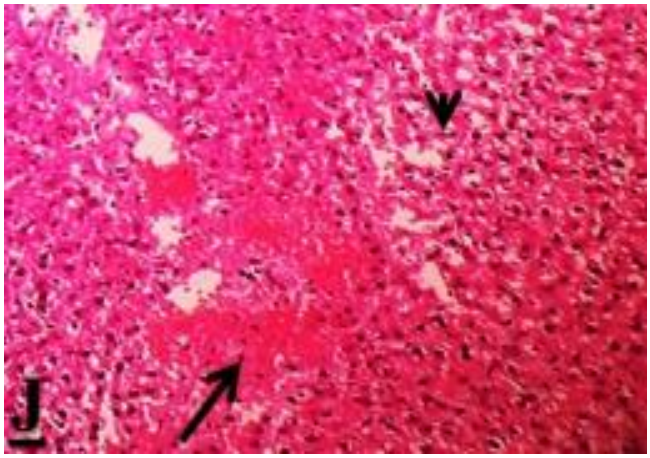

Figure 8: Sunflower seeds oil fed group, shows hemorrhage in liver parenchyma (arrow) with necrosis of hepatocytes (arrow head). H&E, 400x.

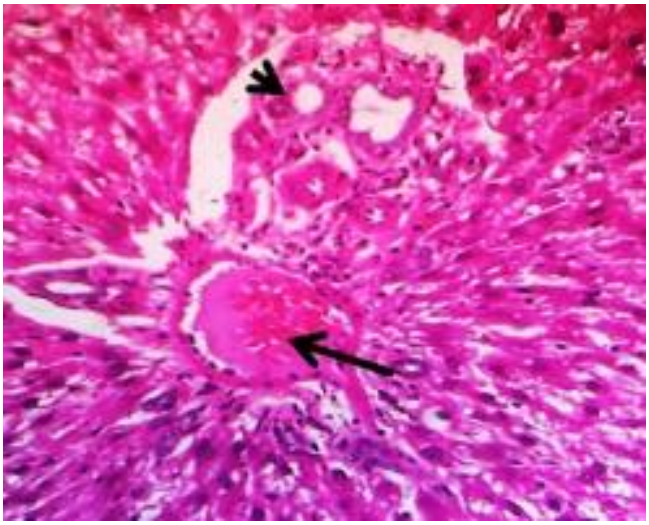

Figure 9: Sunflower seeds oil fed group, shows hyperplasia of bile canaliculi (arrow head) with presence of thrombus in the portal vein (arrow). H&E, 400x.

Discussion

According to the current study's findings, after 4 weeks of feeding white male rats a high-fat diet including sunflower oil, the rats' weight increased significantly in comparison to the control group and animal ghee group. During a one-month period, rat's weight significantly increased because of the high nutritional content of the food they consumed, and may be explained by the favorable ghee taste for rats, which resulted in an excess of calories that were converted into visceral fat, which caused obesity and a rise in the weight of the rats and other animals (16).

The current study's findings are in line with previous studies that have shown that eating foods high in fat increases blood fat levels and the rate at which food is converted to fats. This results in weight gain because eating foods high in fat also causes an increase in the number of fat tissue cells and their size, which results in abnormal fat distribution and weight gain (17,18).

Regarding the consumption of ghee, there was no discernible weight difference when compared to the other groups. This is in line with several studies that found short-chain fatty acids to be protective against obesity (19). However, Malinska *et al* in 2015 also discovered that mice given margarine had less visceral and ectopic fat buildup (20). According to Lei *et al.* (21), short- and medium-chain fatty acids can be handled by the liver by burning them as energy, preventing them from accumulating in adipose tissue or causing weight gain. While to Blankson *et al.* (22) linoleic acid may help people have less body fat.

As opposed to the control group and the sunflower oil group, cholesterol dropped in the animal ghee group in the fourth week. This is a result of the high-sunflower oil diet the rats consumed throughout the study period, and it is consistent with many other studies, as described by Schulze *et al.* (23). The high-fat diet in rats generates oxidative stress, which results in the production of significant numbers of active forms of oxygen species, which causes an increase in blood levels of total cholesterol and triglycerides (24).

High-density lipoprotein (HDL) concentration was found to be non-significantly higher in the sunflower oil group and non-significantly lower in the animal ghee group, which supports (25) Kong's conclusion that a high-fat diet raises levels of dangerous cholesterol (low-density lipoprotein). High-density lipoprotein is the advantageous (cholesterol) ratio in the body, whose molecules are high in fat and low in protein.

The serum lipid profile results utilizing animal ghee are in agreement with Sakr et al (26) who showed significant reductions in total cholesterol, LDL-c, and VLDL-c levels as well as significant improvements in HDL-c and triglycerides when mice were fed at a 10 percent concentration. Compared to control, raw ghee was consumed for 8 weeks, according to chinnadurai (10), raising HDL levels and lowering lipid peroxidation, improves lipid profiles. There are several research done on animal models ,Al othman (27) that show ghee has the complete opposite effect on cholesterol levels. A balanced meal including 2.5 percent to 10 percent ghee was administered to Wistar rats for eight weeks, and the results showed a negative correlation between ghee consumption and cholesterol (25).

The group that received sunflower oil treatment had lesions that included severe vacuolar degeneration and necrosis of hepatocytes, central venous congestion and fatty degeneration of hepatocytes with the presence of fat droplets in hepatocyte cytoplasm. The impact of food on the livers of rats, resulted in a variety of histological abnormalities including fatty change, degeneration and necrosis of hepatocytes, lymphocyte infiltration, and thickening of the central venous wall with fibrin. These outcomes all concur with our existing findings.

The high-fat diet alters the normal liver function, which is why it has these catastrophic consequences on the livers of rats. These imbalances may be caused by changes in the integrity of the hepatocyte membrane, increased production of free radicals and lipid peroxidation, or both (26). Fat metabolism results in the inability of hepatocytes to get rid of excess fat through Their association with proteins to form lipoproteins, which leads to the accumulation of fat in hepatocytes in the form of fat droplets in the cytoplasm (28).

This finding is supported by the high levels of serum ALT and AST found in the blood of rats given sunflower oil, which points to hepatotoxicity and cellular structure destruction. hepatocytes; ALT is a superior marker for analysis of liver injury since it is more specific for liver damage. High AST values suggest cellular extravasation as well as diminished hepatocyte activity (29). The current findings are congruent with those made by Setorki (30), who

noticed a rise in ALT concentration when feeding oxidized lipids to rabbits. According to Rani *et al.* (31), liver function markers decreased after using regular milk ghee. compared to sunflower oil after two months, the levels of ALT, AST, and ALP in serum plasma are consistent with our findings. The toxic effect of high fat diet is evitable (32) like toxicity of some plants extracts and other toxin (33)(34). It is however the matter of ratio of fat peroxidation to antioxidants that determine its toxicity (35).It worthwhile to mention some other studies that show the antihyperlipidemic effect of some plants(36) the effect of many food preservative may affect other organs (37) The bad effects of oils used nowadays may be attributed to the way of cooking(38,39) As fish oils are cardioprotective (40)and sesame oil which shows promise in decreasing high levels of cholesterol and inflammation, lowering risks of atherosclerosis(41).

Conclusion

In contrast to sunflower seeds oil, which had adverse effects on rats' levels of liver and other biochemical indicators, the study found that ghee had a number of advantages.

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Interest conflict

There is none.

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تأثيرات تناول السمن البلدي مقارنة بزيت بذور دوار الشمس على نسيج الكبد وبعض المتغيرات الكيميائية الحياتية في الجرذان

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الخلاصة

يقصد بالسمن الحيواني الدهن المستخلص من فرز حليب الأغنام ولقد استعمل لعشرات السنين في العراق لصناعة الحلويات توافرت دراسات عديدة حول مضار السمن الحيواني لاحتوائه على شحوم مشبعة إضافة لتوفر بديل تجاري وهو زيت بذور دوار الشمس. هدفت هذه الدراسة لإيجاد الفرق بين استخدام السمن الحيواني (البلدي) وزيت بذور دوار الشمس من حيث التأثيرات المرضية النسيجية في الكبد وعلاقتها ببعض المتغيرات الكيميائية الحيوية في الجرذان ولتحقيق هذا الهدف استخدمنا ٣٦ حيوان مقسم الى ثلاثة مجاميع تم اعتبار المجموعة الأولى مجموعة سيطرة أعطيت عليقة قياسية اعتيادية والمجموعة الثانية غذيت على عليقة فيها نسبة ٥% من السمن الحيواني والمجموعة الأخيرة غذيت على عليقة فيها نسبة ٥% من زيت دوار الشمس ولمدة ٤ أسابيع متواصلة وتم اخذ عينات الدم في الأوقات صفر و٢ أسبوع و٤ أسابيع متوالية. كان هناك زيادة في أوزان الحيوانات في مجموعة زيت دوار الشمس مع زيادة بنسبة الكولسترول ومستوى أنزيمات وظائف الكبد في الدم مقارنة مع المجموعة المعاملة بالسمن البلدي التي أظهرت عدم وجود تغيير في أوزان الحيوانات وانخفاض الكولسترول مع انخفاض أنزيمات وظائف الكبد، كما أظهرت التغييرات النسيجية المرضية للمقاطع الكبدية بعد ٤ أسابيع من التغذية بالسمن البلدي آفات نسيجية طفيفة الى متوسطة وتمثلت بالتنكس الفجوي للخلايا الكبدية وارتشاح بؤري للخلايا الالتهابية مقارن مع مجموعة زيت عباد الشمس التي أظهرت فات نسيجية أكثر شدة وتمثلت بالتنكس الفجوي الشديد والنخر للخلايا الكبدية مع التغير الدهني وارتشاح الخلايا الالتهابية في المنطقة البوابية والنزف والفرط التنسج بالقنوات الصفراوية. خلصت الدراسة أن السمن الحيواني (البلدي) له تأثيرات مفيدة مقارنة بالتأثيرات الضارة لزيت بذور دوار الشمس وعلى مستوى التغييرات النسيجية الكبدية والتغييرات الكيميائية الحيوية المرافقة في الجرذان.

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