

Effect of Washed Sunflower Oil on Lipid Profile in the Experimental Hypercholesterolemia in the Adult Male Rats

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الخلاصة

تعد الزيوت النباتية مثل زيت زهرة الشمس مواداً فعالة في تنظيم مستويات دهون الدم والوقاية من أمراض الجهاز القلبي الوعائي. لكن قد يحتوي زيت زهرة الشمس على بعض المواد ذات الخواص القطبية التي من شأنها التداخل مع تأثير الزيت الايجابي لذا كان من المفيد تعريض زيت زهرة الشمس لعمليات غسل بالماء الحار بغية تخليصه من تلك المواد القطبية. استخدم في البحث الحالي ثلاثون من ذكور الجرذان البالغة البيضاء قسمت إلى خمسة مجاميع وغذيت على غذاء عالي المحتوى من الدهون الحيوانية (١٦% من مجموع السعرات الحرارية) وقدم لها ماء شرب يحتوي على بيروكسيد الهيدروجين بتركيز ٠.٥% لمدة ثلاثين يوماً لغرض إحداث فرط كوليستيرول الدم فضلاً عن خمسة عشر يوماً التي تمثل فترة التجربة. تم إعطاء زيت زهرة الشمس عن طريق التغذية الأنبوبية في الفم بشكله الخام والمغسول وبجرعتي ٣٠٠، ٦٠٠ ملغم/كغم من وزن الجسم لكل من شكلي الزيت. أشارت نتائج تحليل البيانات إحصائياً إلى عدم وجود فرق معنوي لتأثير الزيت المغسول على النواحي التغذوية للجرذان لكن لوحظ انخفاض معنوي لمسوى الكوليستيرول الكلي بنسبة ٥٠%، الدهون الفوسفاتية بنسبة ٤٢%، البروتينات الدهنية واطئة الكثافة بنسبة ٧٤% هذا فضلاً عن ارتفاع مستوى البروتينات الدهنية عالية الكثافة بنسبة ٧٠% نتيجة المعاملة بزيت زهرة الشمس المغسول بجرعة ٦٠٠ ملغم/كغم وانخفاض مستوى المألوندايألديهايد في الكبد. يستنتج من البحث الحالي أن عمليات الغسل لزيت زهرة الشمس من شأنها تحسين قابليته لخفض دهون الدم وخواصه المضادة للأكسدة.

Abstract

Vegetable oils are considered to be active in modulation of the blood lipid profile and profilacting from cardiovascular diseases, oils extracted from plants (sunflower seeds) may also contains some polar substances which can interferes with their action, if these oils are exposed to some washing processes with worm water, these substances will be removed. A thirty adult albino male rats were divided into five groups and exposed to a hyperlipidemic and hypercholesterolemic diet (animal fats 16% of total calories) and H₂O₂ (0.5%) in the water for a 30 days for induction of hypercholesterolemia in addition to 15 days in which. sunflower oil are administered orally as a crude and washed oils at a doses of 300 and 600 mg/kg body weight. There was no significant effect of the washing process on the nutritional status but there were a decrease in total cholesterol TC (50 %), phospholipids PL (42%), low density lipoproteins LDL-c (74%) and elevation in high density lipoproteins HDL-c (70%) resulted from the treatment with washed oil (600 mg/ kg body weight) in addition to decrease in liver malondialdehyde (MDA) content. In conclusion, the washing process of sunflower oil will improve it's hypocholesterolemic and antioxidant effects.

Key words: Sunflower oil, Rats, Cholesterol, Triglycerides, Phospholipids, Malondialdehyde.

Introduction

Much has been studied about the benefits of replacing animal fats with vegetable oils in the diet, "Vegetable oil" is the name usually given to any oil product derived from plants of any description (fruit, vegetable) (1).

Sunflower (*Helianthus annus* seeds) oil considered to be an important vegetable oil used in human nutrition. It is light in density, yellow color appearance, and low in saturated fatty acids (SFAs) (2). The ratio of polyunsaturated fatty acids (PUFAs) to Monounsaturated fatty acids (MUFAs) is about 3:1 (3). The main (PUFA) contained in Sunflower oil is linoleic acid (C18:2), while the (MUFA) is Oleic acid (C18:1) (about 25 %) whereas the palmitic acid (C16:0) is the major SFA (6%) (3).

The consumption of (PUFAs) and (MUFAs) in place of SFAs may lower the risk of atherosclerosis and lowering the mortality due to heart disease by reducing cholesterol levels in the blood (4,5), in addition to the sunflower oil fat soluble vitamins content especially E and A which they are considered a powerful antioxidants (6), Phytosterols are also found in sunflower oil and they are effective because of their cholesterol lowering and antioxidant capacity (4), Sunflower oil has an iodine value about 130 and peroxide value about 0.5 meq/ kg and specific gravity at 25 °C is about 0.92 according to some national normal values (3).

Although the beneficial effect of the vegetable oils on the lipid metabolism and lipid peroxidation (4,7), but there is an evidence that mechanical washing processes could be applied on these oils, It will enhances their well known effect on the health by removing some of polar substances which interferes with the action of the vegetable oils active ingredients (8). The aim of this study is to evaluate the effects of crude and washed sunflower oils on the serum lipid profile, liver cholesterol, tissue's lipid peroxidation (liver, heart and kidney) of the male rats in cases of experimental hyperlipidemia and hypercholesterolemia induced in the male rats by using of saturated fats and hydrogen peroxide.

Materials and methods

Hyperlipidemic and hypercholesterolemic diet: diet ingredients were supplied from local market in order to meet the rats nutritional and physiological requirements according to the AOAC (9). 0.26% of cholesterol powder (Merck Co., Germany) and 4.69% saturated alcohol dissolved sheep tail fat were added to the other ingredients, diet was moistened with distilled water and formed in the shape of pellets dried in electrical oven at 55 C° then maintained in polyethylene bags in cool and dry place, moisture, ash and carbohydrates were determined according to Pearson (10), protein and crude lipid (ether extract) were determined according to American Nutrient Research Council (11) while the crude fibers were calculated from the difference between the sum of protein, lipid, carbohydrates, ash percentages and 100% (table 1).

Table (1) : dietary mixture and their ratios according to (11) and the chemical analysis based on dry weight.

<i>Diet composition</i>		<i>Chemical analysis</i>	
Ingredients%		Nutrients%	
Yellow corn	47.04	Crude fat	7.13
Wheat flower meal	18.91	Carbohydrates	68.53
Barley meal	16.40	Protein	12.16
Cake	7.40	Crude fibers	6.58
Sodium chloride	1.40	Ash	5.58
Calcium carbonate	1.0	Metabolic energy Kcal / 100 g**	380.98
Sodium monophosphate	1.40		
Inorganic elements	1.50		
Animal fat + cholesterol*	4.95		

*Dietary total cholesterol was 0.67%

**Metabolic energy 9 Kcal / g fat , 4 Kcal / g carbohydrates and protein .

Crude sunflower oil extraction: sunflower seed's husks were removed manually, dehusk seeds milled with an electrical blender.

Moisture was determined according to Pearson (10), diethyl ether solvent was examined for the presence of peroxide (9), crude sunflower oil was extracted by using of soxhlet by using a mixture of diethylether and ethanol as 1:1 (9), solvents were evaporated on water bath then completed on hot plate (45- 50 C°)

Sunflower crude oil ratio was calculated as following:

% of Sunflower crude oil =

$$\text{Weight of extracted oil} / \text{Weight of seed powder on dry matter} \times 100 \\ = 29.73 \%$$

Washing of extracted sunflower oil: the extracted oil was vigorously washed with warm water and benzene in a separatory funnel with continuous shaking in a water bath at 37 C° for 24 hours. The funnel settled overnight untill complete separation of the mixture for down watery and upper benzene layers which contains the distributed washed oil. Benzene layer was collected, benzene evaporated by using the rotary evaporator under vacuum. Dried oil weighted and maintained in a dark bottle at 7 C°.

% of washed sunflower oil =

$$\text{Weight of washed oil} / \text{Weight of crude oil} \times 100 \\ = 89.38 \%$$

Chemical and physical properties of the crude and washed sunflower oils: peroxide value (meq/kg), iodine number, percentage of steroides and pH value were determined according to Pearson (10) in addition to certain chemical constituents of the both oils (total cholesterol and triglycerides) and physical property (specific gravity) for both oils were determined (9).

Hydrogen peroxide in drinking water: daily prepared fresh water containing 0.5% hydrogen peroxide (H₂O₂) was used in drinking water of hypercholesterolemic rats (*Ad libitum*) (4).

Experimental design: thirty of adult albino male rats were divided into five groups and treated with hypercholesterolemic diet, in addition to hydrogen peroxide in drinking water for 30 days for induction of hypercholesterolemia as well as 15 days the period of treatment with sunflower oils as the following:

First group: control (hypercholesterolemia without treatment).

Second and third groups: treated with crude sunflower oil in a doses of 300 and 600 mg/ kg body weight respectively by gavage.

Fourth and fifth groups: treated with washed sunflower oil in a doses of 300 and 600 mg/ kg body weight respectively by gavage.

Quantitative determination of consumed oil fat and lipid absorption:

Consumed fat estimated as following:

Consumed fat (g) = consumed diet (g) × diet's fat percentage

Intestinal lipid absorption can be calculated as following:

Intestinal lipid absorption (g) = consumed dietary fat (g) – eliminated feces lipid (g).

Blood specimens collection: blood specimens were collected from retroocular vein by using capillary tubes. After coagulation blood samples were centrifuged (3000 RPM) for 15 min, then serum was collected and maintained in deep freezing.

Lipid profile determination: total lipid (TL mg/ 100 ml) was determined according to Toro and Ackermann (12) by oxidizing the lipid content in the sample then adding phosphovanilline reagent whereas total cholesterol (TC) and triglycerides (TG) were determined (mg/100 ml) by using determination kits from Syrbio reagents Co. Depending on enzymatic method, Low density lipoproteins (LDL-c) and high density lipoproteins (HDL-c) (mg/ 100 ml) determined by specific kits from Syrbio reagents Co. which depends on the precipitation of them and then estimation was done by using of cholesterol kit .

Calculation of very low density lipoproteins and phospholipids: very low density lipoproteins (VLDL-c mg/100 ml) calculated according to Tietz (13)
$$\text{VLDL-c (mg/ 100 ml)} = \text{Triglycerides} / 5$$

Phospholipids (PL mg/ 100 ml) = (Total cholesterol × 0.89) + 68 (13).

Tissue sampling: rats were sacrificed at the 15th day and the organs (liver, kidney and heart) were separated, washed with cold normal saline and maintained in deep freezing.

Determination of malondialdehyde in the tissues: malondialdehyde was determined according to Gilbert (14) by reacting the MDA content in the sample with thiobarbituric acid which gives a colored complex.

Determination of total cholesterol in the liver: liver total cholesterol determined according to Folk, Morita (15,16) by dissolving the cholesterol in the sample with chloroform and ether then determined by the specific kit .

Statistic: all data were analyzed for the mean characters with the analysis of variance and Duncan multiple test range for the standard error differences according to Steel and Torrie (17).

Results

The washing process led to a decrease in the iodine number, peroxide value, specific gravity and the percentage of steroids, pH was elevated in the

washed oil and the highly oil contents of total cholesterol and triglycerides was slightly affected by the process of washing (Table 2).

Table (2): Chemical and physical properties of crude and washed sunflower oils.
TC= Total cholesterol, TG= Triglycerides.

Sunflower extract	Iodine number	Peroxide value mg/kg	Specific gravity	Steroides %	pH	TC g/100g	TG g/100g
Crude sunflower oil	122.40	7.10	1.23	41.60	5.30	50.71	182.0
Washed sunflower oil	102.0	5.50	0.93	39.41	7.30	53.91	180.61

Table (3) showed that there was a significant decrease in the rat body weight gain, diet intake and dietary fat consumption in the groups treated with the dose 600 mg/ kg body weight of both oils while the weight gain was increased in the group treated with the dose 300 mg/ kg body weight of crude oil which was higher than that value of control group, diet intake and dietary fat consumption were less than that values of the control especially with the treatment with crude oil 300 mg/ kg body weight but there was no significant difference between the control and the treatment with washed oil 300 mg/ kg body weight.

The weight of feces was the same in the control and group treated with sunflower oils 300 mg/ kg body weight but it was elevated by the treatment with 600 mg/ kg body weight of both two trails of oils, also there was no significant difference in the fecal fat between all groups except the group treated with the washed oil 600 mg/ kg body weight.

There was no significant difference ($p > 0.05$) in the intestinal fat absorption between the groups treated with the washed oil of both doses and control group, the least significant ($p \leq 0.05$) value of the intestinal fat absorption was seen in the group treated with the crude oil 600 mg/ kg body weight which was not significant with the group treated with the crude oil 300 mg/ kg body weight.

Table (3): Effect of crude and washed sunflower oil on the nutritional status in the male rats with induced hypercholesterolemia by saturated animal fats, cholesterol and hydrogen peroxide (mean±S.E.).

Rat groups	Body weight (g)		Ingested diet		Eliminated feces		Fat absorption
	Primary weight	Gain	weight(g)	Fat content mg	weight (g)	Fat content mg	
Control	204.27±4.94a	22.59±1.41b	153.70±5.45a	10.96±0.38a	46.20±1.55b	6.56±0.16a	4.42±0.42a
Crude sunflower oil 300mg/kg	209.09±3.12a	43.56±4.18a	116.70±1.87bc	8.32±1.33bc	45.05±1.27b	6.57±0.11a	1.75±0.17bc
Crude sunflower oil 600mg/kg	213.58±5.14a	30.47±0.86b	98.50±1.79c	7.02±0.12c	51.95±0.99a	6.25±0.19a	0.81±0.25c
Washed sunflower oil 300mg/kg	207.00±2.79a	42.90±6.24a	135.62±12.06ab	9.67±0.86ab	48.73±2.14ab	6.20±0.32a	3.47±0.81ab
Washed sunflower oil 600mg/kg	204.68±5.37a	26.20±3.78b	97.44±8.15c	6.95±0.58c	53.14±1.85a	4.85±0.50b	3.10±1.08ab

* The different letters in the same column indicate that there were a significant differences values (P≤ 0.05). All the rat groups feed animal fats, cholesterol in the diet and offered hydrogen peroxide in drinking water.(n=6)

Figure (1) showed that the highly significant ($p \leq 0.05$) decrease in the total lipids (TL) and triglycerides (TG) was seen in the groups treated with the dose 300 mg/ kg body weight of both trail oils, while the high significant decrease in the phospholipids (PL) was seen in the groups treated with the washed oil and crude oil 300 mg/ kg body weight, but the treatment with crude oil 600 mg/ kg body weight led to significant ($p \leq 0.05$) increase in the PL value in comparison with the other treatments which still significantly less than the value of the control group.

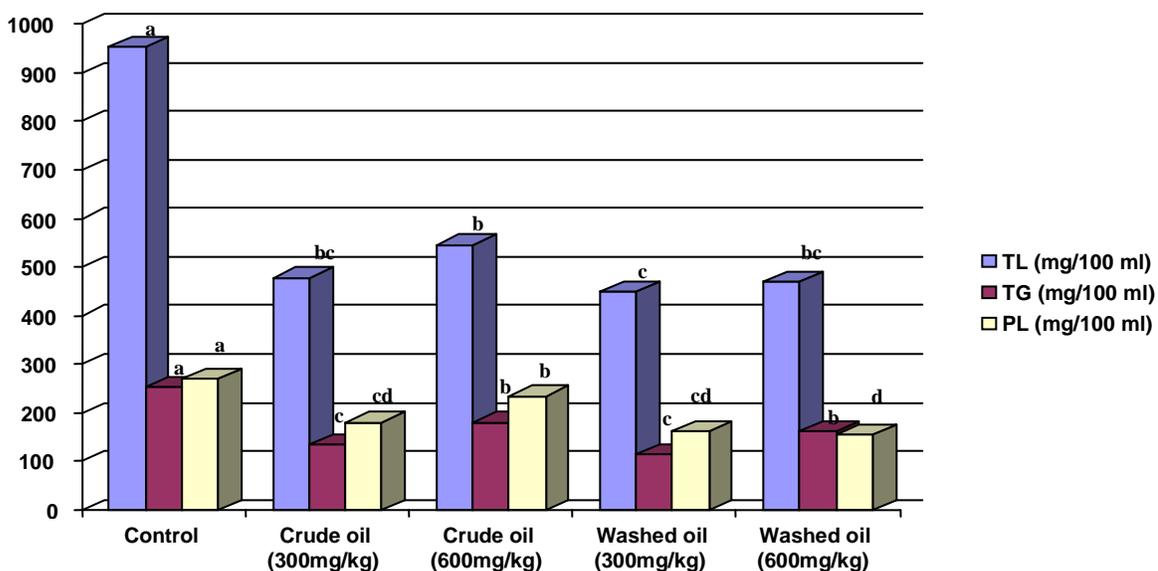


Fig (1) : Effect of crude and washed sunflower oil on total lipids, triglycerides, and phospholipids. n=6.

Figure (2) refers to the high significant ($p \leq 0.05$) difference in the serum total cholesterol (TC) was for the groups treated with the both doses of washed oil , in addition to the treatment with crude oil 300 mg/ kg body weight, the group treated with the crude oil 600 mg/ kg body weight was higher in the TC than the other treated groups but still significantly ($p \leq 0.05$) under the value of the control group.

Liver total cholesterol was decreased in the groups treated with the crude oil as well as washed oil 300 mg/ kg body weight, the group of washed oil 600 mg/ kg body weight did not differ from the dose 300 mg/ kg body weight of the two trails of sunflower oils but it considered to be declined in comparison with the control group.

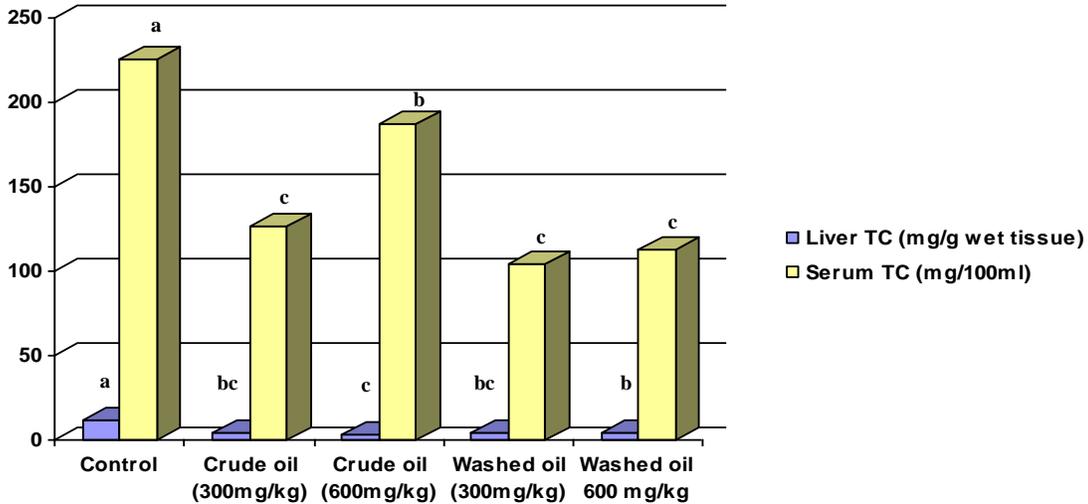


Fig (2) : Effect of crude and washed sunflower oil on serum and liver total cholesterol , n=6 .

Figure (3) show a decrease in the LDL-c in the groups treated with the two doses of the washed oil in addition to the treatment with the crude oil 300 mg/ kg body weight which did not differ from the group treated with the dose of 600 mg/ kg body weight of the same oil, but the last group value of LDL-c is significantly decreased in comparison with the control, The very low density lipoproteins (VLDL-c), was highly decreased in the groups treated with the dose 300 mg/ kg body weight for two trails of oils but the decreased value was still less significant ($p \leq 0.05$) than the treatment with the dose 600 mg/ kg body weight in comparison with the control group. High density lipoproteins (HDL-c) value was elevated in all groups treated with sunflower oils in comparison with untreated control.

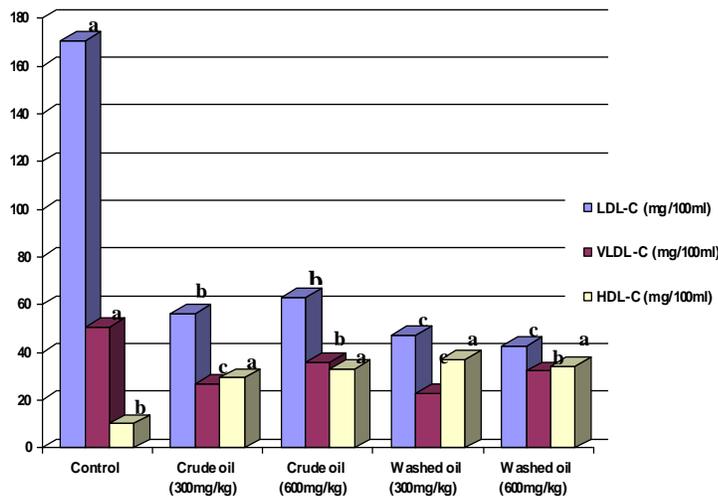


Fig (3) : Effect of crude and washed sunflower oil on lipoproteins concentration, n=6.

Table (4) showed that the more significant ($p \leq 0.05$) decline in the malondialdehyde (MDA) content in the liver seen in the group treated with washed oil 300 mg/ kg body weight which has a greater rate of decline than the group which treated with crude oil in the same dose (300 mg/ kg body weight), while the MDA content is declined more significantly in the groups of crude oil 600 mg/ kg body weight and washed oil 300 mg/ kg body weight. In the kidneys the treatment with crude and washed sunflower oil of both doses caused a significant ($p \leq 0.05$) decrease in the MDA content of the four treated groups in the same manner in comparison with control.

Table(4): Effect of crude and washed sunflower oil on the malondialdehyde of liver, heart and kidneys in the adult male rats with induced hypercholesterolemia by saturated animal fats, cholesterol and hydrogen peroxide.

Rat groups	Malondialdehyde nmol/g wet tissue		
	liver	heart	kidneys
Control	836.31±12.18a	806.01±18.21a	801.77±24.35a
Crude sunflower oil 300mg/kg	403.96±27.85c	562.94±29.44b	476.27±32.38b
Crude sunflower oil 600mg/kg	621.56±17.25b	291.31±16.71c	404.73±20.91b
Washed sunflower oil 300mg/kg	290.37±15.39d	355.66±9.19c	404.06±21.89b
Washed sunflower oil 600mg/kg	562.25±34.60b	552.93±34.80b	451.58±35.25b

The different letters in the same column indicate that there were a significant differences values ($P \leq 0.05$). All the rat groups feed animal fats, cholesterol in the diet and offered hydrogen peroxide in drinking water. (n=6), (mean±S.E).

Discussion

Results in table (2) refers to the washing process in causing a decrease in the iodine number that's means some of unsaturated fatty acids UFAs may removed by the washing solution, the international values of iodine number range between (88-135) (3). there was a decrease in the peroxide value caused by the washing process but this value remained over the international values (3). The specific gravity (Sp. Gr.) of the crude oil was higher than the reference value but washing normalized this value and increased pH value. The other characters (steroids, total cholesterol TC, triglycerides TG) did not affected with the washing process because of non polar characteristics of these compounds that did not dissolved in the polar phase (warm water). The decrease in iodine number means a decrease in the level of UFAs which was at the acidic state therefore pH value raised toward the alkaline state.

Results in table (3) showed that, the decrease in the rats body weight gain in groups treated with crude and washed oils of 600 mg/kg body weight, was resulted from the decrease in the dietary consumption which may be resulted from some interactions of oils with metabolic status especially in the high dose of sunflower oil whereas the decrease in the fat absorption which seen by the treatment with crude oil 600 mg/kg body weight may resulted from the high amount of fecal fat in this group (4) while the washed oil 600 mg/ kg body weight. showed a less amount of fecal fat with relatively high fat absorption which thought to be resulted from the increase rate of fat intestinal absorption by the treatment with washed oil which considered to be more pure and show it's effect in high doses.

Figure (1) showed that the decrease in the total lipids (TL) and triglycerides (TG) in the rat groups treated with washed and crude oils 300 mg/ kg body weight, was resulted from the high content of PUFAs (3) and (4) while the washed oil in the high dose (600 mg/ kg body weight.) caused the same effect of crude oil. This may be due to the relatively high dose administration but the greater decrease in the phospholipids (PL) in this group was resulted from the high relationship between PL and total cholesterol (TC) (4).

Figure (2) referred to the washing process success in decreasing TC even in the dose of 600 mg/ kg body weight which was the higher in the group of crude oil but it was lower than the control, it is mostly resulted from the ability of the washing process to remove some materials which it may make an impairment to the cholesterol metabolism such as lectin (4).

The action of sunflower oil on TC was resulted from it's content of PUFA and MUFA (18). In addition to the high amount of the Phytosterols (approx. 40%) (19) present in sunflower oil and the increase in the fecal excretion of steroids and bile acids which utilizes high amount of cholesterol (20) as well as to the action of sunflower oil in the modulating of intestinal fat absorption (4) and increasing insulin level (21). Washing process was not able to decrease liver TC in the same manner compared with the crude oil. The ability of Sunflower oil to decrease liver TC due to it's content of tocotrienols which is known to inhibit 3- hydroxy 3- methyl glutaryl CoA (HMG-CoA) reductase (a rate limiting enzyme in cholesterol biosynthesis) in the liver resulting in hypocholesterolemia and decrease liver cholesterol (20).

Figure (3) referred to the decrease in the low density lipoproteins (LDL-c) in the rat groups treated with washed oils was similar levels to those of TC, this decrease is related to TC which is 70% of it is carried in blood by LDL-c particles (4). The treatment with sunflower oil will lead to

increase the levels of LDL-c linoleic acid (C18:3) which aids to normalize the metabolism of LDL-c (22). As considered with very low density lipoproteins (VLDL-c) and high density lipoproteins (HDL-c), there were no significant difference between the effect of crude and washed oils. The HDL-c was elevated in the same manner without relation to dose or the type of oil, this elevation was due to the elevation in the level of apoprotein A-4 (apo A-4) which is very sensitive to the increase in dietary lipids, independent on the degree of fatty acid saturation (23) which stimulate the HDL-c biosynthesis specially HDL-3 (21).

Results in table (4) showed that the treatment with washed oil 300 mg/kg body weight which posses a peroxide value (5.50) less than that value of crude oil (7.10) caused the higher significant decrease in the malondialdehyde (MDA) concentration of the tissues especially in the liver which may lead to conclude that the washing process may cause a decrease in the ratio of the oxidized fatty acids in the oil which affect directly on the levels of lipid peroxides in the tissues and blood. The treatment with sunflower oil resulted a positive effect on the peroxidation lag time in addition to the linoleic acid (C18:2) present in the oil will enrich the LDL-c with this fatty acid which acts as antioxidant in the LDL-c particles (4).

In conclusion we can say that the washing process of crude sunflower oil did not affect serum TL, TG but positively affects the serum TC and lipoproteins in male rats and led to consume a larger amount of dietary fats without effect on serum TC in addition to the ability of the washing process to get rid of the portion of oxidized fatty acids in the oil and decreasing peroxidation products inside the cells.

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