Study of Walnut Oil Supplementation on Serum Biochemical Parameters and Histopathology of Male Rats

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Abstract

The purpose of the experiment is an investigate the association of walnut oil with lipid profiles, glucose as well as total proteins and assessment its side effect on some sensitive organs such as the liver and spleen tissues. The experiment divided into two main groups are treated group with walnut oil and the control group, where the former divided into two subgroups 0.25 and 0.5 ml of oil per each. We found a statistically non-significant difference between treated and control groups (for total protein, lipid profile, and glucose). There was no change in total protein, but cholesterol decreased by 0.25 ml but a little increased by 0.5 ml. HDL was increased for the treated group. While on 0.25 ml LDL decreased in treated animals, on another hand no change for 0.5 ml. Also, no change for VLDL between treated and control. The only triglyceride was increased but non-significant for the treated group compared with the control. Both doses decreased in treated animals for glucose. We also found an increase in whole-body weight and on sensitive organs such as the liver and spleen. Even no change in a histological study for the mentioned organs. The conclusion: By walnut oil, all parameters have changed despite the treated group was normal without any induced diseases. So, recommended the researchers induce the disorders in the liver and assessment the extracted oil on the lipid profile.

Key Words: Walnut Oil, Lipids Profile, Cholesterol, HDL, LDL, VLDL, Triglycerides, Liver and Spleen.

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Introduction

A High-lipids diet continually contributes to 'hyperlipidemia', a rise in "the total cholesterol, triglycerides, LDL-C", and a reduction in "Lipoprotein-Cholesterol (HDL-C)" in "High-Density (HDL-C)"(Hirschler et al, 2013). Lipoprotein is a complex of "triglycerides and cholesteryl esters", surrounded via a "hydrophobic phospholipid" and carried by a protein in a deferent percentage called "Apolipoprotein". The occurrence of atherosclerosis caused by hyperlipidemia, one of the triggering factors of cardiovascular disease such as hypertension; "coronary heart, and stroke" (Mark S. et al, 2008). Diet and improved lifestyles are the primary treatment for hyperlipidemia, supplemented when appropriate by drug treatment (Tangpricha V. et al, 2008). Walnut is man's oldest longevity food known for thousands of years (Tangpricha V. et al, 2008).

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Walnut came from ancient Persia, this is why the walnut is sometimes called the Persian walnut. Walnut is a large antioxidants source and have differents phytochemicals like fatty acids of omega-3, and vitamin E; minerals, iron, sodium, calcium, magnesium, manganese, copper, potassium, and phosphorus as well as a variegated food, protein, and fibres, make it riches, varied nutritious meal (Tangpricha V. et al, 2008).

The key ingredients are triglycerides, monocyclic unsaturated fatty (oleic acids mainly) acids, and polycyclic, high-level unsaturated fatty (linoleic and alpha-linolenic) acids. There has also been evidence of the existence of other bio-substances, for example, phenols and tocopherols (Earthman C.P. et al, 2012). Extracted oil of walnut is too a good exporter of the acids of essential omega-3 for human nutrition (Lagunova Z. et al, 2011). "The monounsaturated fatty acids and polycyclic unsaturated fatty acids (MUFA and PUFA)" have preventive roles against cardiovascular diseases have been identified (Turer C.B. et al, 2013). The consumption of walnut (kernel and oil) as has been mentioned lowers levels of blood cholesterol (Turer C.B. et al, 2013). Kernel of walnut is used to decrease bad lipids in the blood, while, to raise "high-density lipoprotein," and diminishing "low-density lipoprotein"(Wortsman J. et al, 2000). Also, walnut oil aims to treat type 2 diabetes and improve cardiovascular stability (Dobnig H. et al, 2008). Walnut was noted as a defence against some forms of cancer because it has a high concentration of natural antioxidants (Cosmulescu S. et al, 2009). Therefore, the main purpose of this research Is finding a way to get rid of bad lipids and replace them with good lipids as alternative drugs.

Methodology

Preparation of Walnut Oil Extraction

The extraction of alcoholic methanolic of walnut oil has been prepared depending on James Redfern (2014) and associates protocol with some modification (Deirdre K., Frank B. 2009). Briefly, 20g dry powder of walnut has been put in a flask of Soxhlet contain 400 ml of absolute ethanol. The extraction filtered two times bypassing it subsequently during a Whatman No. 1 then, a Millipore filter paper (0.22µm). The dry walnut oil extract was then gained by drying the ethanolic alcoholic extract at 60 °C using an oven then was stored at refrigerator 4 °C depending on the limited protocol mentioned above (Deirdre K., Frank B. 2009).

Experimental Animals

Nine animals of males Albino rats with an average weight of 230-270 g were used in this study and were carried out and kept in crates with lumber chips in animal facilities, "Department of Biology, Faculty of Science, University of Kufa," Najaf, Iraq. Standard diet and water were given in a breeding colony. At 25 ± 3 ° C, the room temperature was preserved. On an average day (24 hours a day), the light / dark rhythm was retained. At about 200 W from 8 am, the room was illumined by synthetic light. Towards 7 p.m. The ethics committee of the DOB approved all experimental protocols and procedures for animal handling at the faculty of science, University of Kufa. Animals were randomized divided into two main groups; control and oil-fed groups (the latter includes two subgroups where one subgroup treated with 0.25 ml of pure oil of walnut through oral administration, while the second subgroup treated with 0.5 ml of the same oil and pathway). Exposure to the oil diet was continued every 48 hours for 60 days by oral.

The Collection of Blood and Serum

At the end of each experiment, After 24 hours of the last dose and weighed the animals using an electrical balance, numb with use a mix of ketamine and xylazine, where: Mix 0.1 ml of xylazine with 0.5 ml of ketamine per weight (250 gm) through injected by muscle, and blood was sampled either from direct cardiac puncture. Blood was collected for biochemical tests. The apparatus is AFIAS-6 (AFIAS-automated fluorescent immunoassay system) and their specific strips.

Statistical Analysis

The mean ± standard deviation for all the data is expressed. The findings were analyzed using SPSS (version 23). A t-test was conducted for a 2-sample comparison between oil and their respective untreated controls after a dose of separate serum biochemical profiles of Albino Rattus rats. A comparison of various parameters studied among oil-treated, and untreated groups were carried out with the same statistical test.

The Results

Effect of Walnut Oil Supplementation on Total Proteins of Rats After Doses

Results indicate that all other studies parameter remain uninfluenced while compared to oil-treated and untreated animals after dosing for several weeks of supplementation.

It shows no significant decrease (P>0.05) in the mean serum levels of total proteins in the group of treated animals compared with the control group. Where the results in table (3-1) show: Decrease in the mean serum levels of total proteins in the animals treated with 0.25 ml (11.5) mg\dl compared with its levels in the control group (12.2) mg\dl. While, approximately, no change for a treated group with 0.5 ml (12.4) mg\dl.

Table 3.1. Levels of total proteins in the serum of treated animals and control groups

Parameter	Control groups (mg/dl) N = 3	Animals treated (mg/dl)		P-value
		0.25 ml ± Std. Deviation N = 3	0.5 ml ± Std. deviation N = 3	
Total proteins	12.2	11.5 ± 0.4243	12.4 ± 0.2828	P> 0.05

Effect of Walnut Oil Supplementation on Lipid Profile

When biochemical serum parameters have been contrasted between animals supplemented from walnut oil within 2 months and control. It was found that concentrations of cholesterol (P>0.05), TG (P>0.05) and LDL (P=0.05) concentrations in the blood were different from the oil-treated and untreated groups.

The **cholesterol** result shows no significant decrease (P>0.05) in the mean serum levels of cholesterol in the group of treated animals compared with the control group. Where the results of table (3-2) show: decrease in the mean serum levels of cholesterol in the treated group with 0.25 ml (174.5) mg/dl compared with its levels in the control group (184) mg/dl. While there is a slight increase in cholesterol with 0.5 ml (186.5) mg/dl of animals treated with oil.

Table 3.2. Levels of cholesterol in the serum of treated animals and control groups

Parameter	Control groups (mg/dl) N = 3	Animals treated (mg/dl)		P-value
		0.25 ml ± Std. Deviation N = 3	0.5 dl ± Std. Deviation N = 3	
Cholesterol	184 ± 1.41	174.5 ± 14.84	186.5 ± 7.7782	P > 0.05

The **HDL** results show no significant increase (P> 0.05) in the mean serum levels of HDL in the group of treated animals compared with the control group. Where the results in a table (3-3) show: Increase in the mean serum levels of HDL in the animals treated group (mean of both doses 0.25& 0.5 ml) was (12.5) mg\dl compared with its levels of the control (10.7) mg\dl. Whereas, no difference between the two doses of the treated animals.

Table 3-3. Levels of HDL in the serum of treated animals & control

Parameter	Control groups (mg/dl) N = 3	Animals treated (mg/dl)		P-value
		0.25 ml ± Std. Deviation N = 3	0.5 ml ± Std. Deviation N = 3	
HDL	10.7 ± 0.14	12.5 ± 1.26		P > 0.05

The results of **LDL & VLDL** show no significant decrease (P> 0.05) in the mean serum levels in the case of treated animals compared with the control group. Where the results of table (3-4) show: Decrease in the mean serum levels of LDL in the case 0.25 ml treated group (127.5) mg/dl compared with its levels in the control group (137) mg/dl. However, in this case, a 0.5 ml treated group has the same control (137) mg/dl.

The table (3-4) revealed: No decrease in the mean serum levels of VLDL in treated animals with 0.25 ml (35) mg/dl compared with its levels in the control group (36) mg/dl. As a subgroup of 0.5 ml (37) mg/dl.

Table 3.4. Levels of LDL & VLDL in the serum of treated animals & control

Parameter	Control groups (mg/dl) N = 3	Animals treated (mg/dl)		P-value
		0.25 ml ± Std. Deviation N = 3	0.5 ml ± Std. Deviation N = 3	
LDL	137 ± 1.414	127.5 ± 10.607	137 ± 5.657	P > 0.05
VLDL	36 ± 2.121	35 ± 2.828	37 ± 1.414	P > 0.05

For **triglycerides**, The result indicates no significant increase (P>0.05) in the mean serum levels of triglycerides in the group of treated animals compared with the control group. Where the results of table (3-5) show: Increase in the mean serum levels of triglycerides in the case 0.25 ml treated subgroup (197.5) mg/dl compared with

its levels in the control group (171) mg/dl. While the mean serum level of triglycerides of 0.5 ml subgroup (230) mg/dl compared with the control.

Table 3.5. Levels of triglycerides in the serum of animals treated and control groups.

Parameter	Control groups (mg/dl) N = 3	Animals treated (mg/dl)		P-value
		0.25 ml ± Std. Deviation N = 3	0.5 ml ± Std. Deviation N = 3	
Triglycerides	171 ± 1.414	197.5 ± 41.7193	230 ± 67.8823	P > 0.05

Walnut Oil Supplementation Effect Upon Blood Sugar Levels (BS)

The measures were well tolerated and it was good to stick to the prescribed regimen of diet and intake of walnut oil. Eight weeks after the experiment is beginning, it was observed a statistically decrease significantly between the BS level in the experimental category from 93 mg by 0.5 ml dosages to the intervention to 108 mg by 0.25 ml dosage after the intervention. For the control group, such observations were not found and the level of the FBS remained relatively uncharged. The level of FBS, significantly, decreased in the treated category via comparing it with the control group after the experiment. (P > 0.05).

Table 3.6. Levels of glucose in the serum of animals treated and control groups

Parameter	Control groups (mg/dl) N = 3	Animals treated (mg/dl)		P-value
		0.25 ml ± Std. Deviation N = 3	0.5 ml ± Std. Deviation N = 3	
Glucose	123	108 ± 7.071	93 ± 7.525	P > 0.05

Effect of Walnut Oil on Whole Body Weight

Table (3.7) shows the effect of oil extract on whole body weight, a clear increase in total body weight was observed for two doses of animals treated compared to the control group. When comparing two doses among them no clear increase in body weight was observed.

The mean weights were (325, 331) g. for 0.25 ml and 0.5 ml respectively compared to the control group (250) g. as follows.

Table 3.7. Effect of walnut oil on the total body weight.

Parameter	Control groups (g)	Animals treated (g)	
		0.25 ml	0.5 ml
Whole-body weight	250	325	331

Effect of Walnut Oil on some Organ's Weight

Table (3.8) shows a clear increase in the weight of some organs for two doses compared to the control group. When comparing the weight of the liver and spleen for two doses, a clear increase was observed as follows in the table.

Table 3.8. Effect of walnut oil in the liver and other sensitive organs.

Weight	Control groups (g)	Animals treated (g)	
		0.25 ml	0.5 ml
Liver	8.7177	12.18535	11.73625
Spleen	0.7835	1.3434	1.29715

Histological Changes

1. Liver

The picture (3.1) represents the liver of control and treated animals where there is no lesion of oil extract was observed on the mentioned tissue compared between two groups.

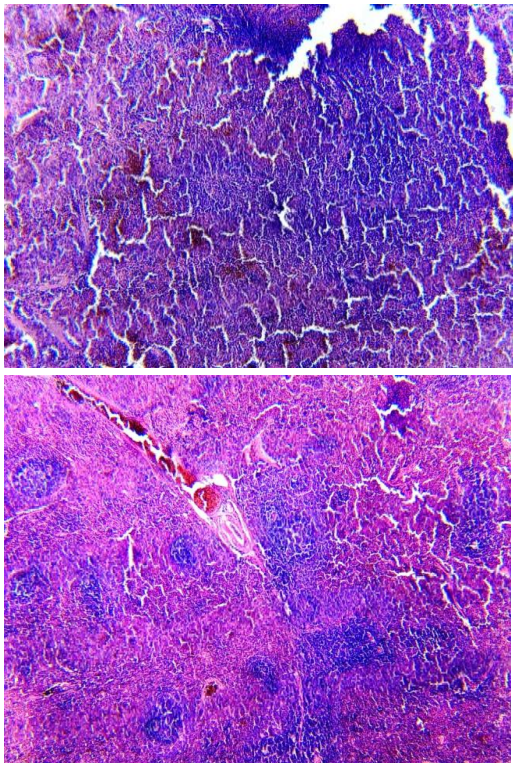

Fig. 3-1. Liver sections. The left represent the control while the right is treated groups. 0.25 ml, 4x magnification. H. & E. stain

Discussion

In this study, the walnut oil proved to be quite effective in lowering the increased blood cholesterol and the increased triglycerides in the plasma and HDL fraction. This was also the trend concerning the observed phospholipidosis in the plasma and RBC. The oil might be a pointer to the fact that it could possess some chelating properties. This agrees with the work of (Olabinri et al.,) who observed a dose-dependent increase in the chelating properties of the aqueous fraction of walnut in vitro. Those results agreed with (Esther O. Abam et al., 2013)

The results indicated that animals that were treated with doses had a high HDL level as compared with their undoing control group. Damasceno NR et al. 2011. Increased HDL level has also been reported in doses of rats after 8 weeks of oil supplementation (Table 3.3), compared with their regular dietary animals, showing that oil supplementation enhances liver function in animals. HDL levels were reported by Damasceno NR et al. 2011, this matches our results in the table (3,8), where we found an increase in liver weight.

Walnut oil is composed largely of polyunsaturated fatty acids and incorporation of polyunsaturated fatty acids in the LDL composition facilitates receptor-mediated LDL clearance by hepatocytes, which could explain cholesterol-lowering effects of walnut consumption (M J Zibaeenezhad et al., 2017). Where, no increase in VLDL in plasma.

The findings of Li et al. mentioned oil doses being one of the main mechanisms leading to hyperlipidemia. Similar findings have also been reported throughout Rajaram S. et al, 2009, which found oil to increase cholesterol in serum and LDL-C concentrations in animals fed regularly. Damasceno NR et al. 2011, mentioned the oil supplementation impact upon the profile of plasma lipid of men and women and noted a decrease in blood total cholesterol in people with high concentrations of cholesterol following oil supplementation, and that is consistent with our results.

The reason for the decrease in cholesterol level in the blood may be due to stimulating steroid hormones. While triglycerides increased maybe because the aforementioned lipids are rich in energy and the presence of the rat in a small cage with restricted movement, which led to an increase in these lipids.

The study investigated the diet role in blood sugar regulation in a patient with DM type 2nd stressed the substitution of traditional fats with PUFA-containing oils (Diana O., Labuckas D. 2008). In our finding, we examined the impact of walnut oil intake, who includes high PUFAs, in particular alpha-linolenic acid (ALA). Walnut oil intake for 8 weeks has been shown to reduce FBS levels significantly. Evident improvements in weight and BMI were observed, however. The findings of previous research are in agreement (Reiter RJ. et al.2005, Fladman EB. 2002, Qadan F. et al. 2005).

It has been shown that PUFAs oil can exert its antidiabetic action via decreasing resistance and improving insulin sensibility by the GLUT4 glucose transporter over-expression mechanism and the receptors of insulin upon the membrane of adipocyte, and too by decreasing inflammation markings in the adipose tissue (McPherson A. 2005), this agreed with our results.

In a six weeks study on cis 9 trans-11-CLA diets is riched, this CLA isomer has been shown to reduce the resistance to insulin and reduce FBS and levels of serum insulin via rising membrane of adipose plasma GLUT4. Besides, this form of CLA could decrease tumour necrotic necrosis α (TNF-α-) level of inflammation inside the adipose tissue via 50 percent. This CLA isomer has therefore been suggested to attenuate resistance of insulin through the effects of anti-inflammatory tissue(Deirdre K., Frank B. 2009). In this study, the FBS level decrease in the walnut oil group received, but substantial weight changes were observed. In the study conducted on diabetic rats, Rahimi et al. reported a decrease in the level of HbA1c in the diabetic rats receiving glibenclamide was statistical. Walnut oil was therefore suggested as antidiabetic (James Redfern et al. 2014).

Results from our investigation showed that improvements in the dietary oil composition consumed by rats and the fats include saturated fatty acids were replaced to contain ALA and PUFAs.

Some of the studies show small non-significant increases in body weight (Sharon Natoli, 2007), but results of this study indicate to there is a clear increase in body weight of treated animals. Weight gain may be caused by increasing the effectiveness of thyroid and growth hormones which are an important factor in increasing body weight by increasing the metabolic rate (Rashmi Mullur et al. 2014), and this may conflict with the result of the previous study.

The clear increase in weight of all sensitive organs after injecting the oil by oral may be interpreted

based on the increased metabolism rate Rashmi Mullur et al.2014) or by an increase of receptors of testosterone (Moisés Tolentino Bento-Silva et al., 2010).

Conclusion

Our results indicated that walnut oil supplementation after doses does not affect studied blood total proteins, however biochemical parameters of serum are non-significantly affected via doses too, in oil-treated groups, all treated animals were normal without any induced diseases, so, all values were non-significant but there was a change in different variables despite it was normal. We recommended assessing extracted oil on lipid profile with induced disorders. Studied variables had concentrations lower than control groups suggesting that oil supplementation keeps serum HDL as higher values. The increase in triglyceride might be back that triglyceride is energy riched and the animals were in a small cage and the limited move. It has been used in large quantities of dosing (0.5 ml per animal) to assess the toxicity of it and its effect on hepatocytes. Therefore it used different dosing (0.25, 0.5 per group) randomly.

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