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Original Article

The Effects of Olive Oil on Non-Alcoholic Fatty Liver Disease (NAFLD) in Male Wistar Rats

Arefeh Khaksar Jalali[1], Shahrzad Mir Ashrafi[1], Samira Shokri[2], Maryam Rezaee[3], Farzad Ebrahimzadeh[4], Ebrahim Falahi[5*]

[1] Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran
[2] Department of Nutrition, School of Health and Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran
[3] Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[4] Department of Public Health, School of Health and Nutrition,, Lorestan University of Medical Sciences, Khorramabad, Iran
[5] Nutrition Health Research Center, School of Health and Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran

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Abstract

Background and Aim: Non-alcoholic fatty liver disease (NAFLD) is a chronic condition associated with poor liver performance. No strategy for NAFLD treatment other than weight reduction and lifestyle modification, including diet, has yet been suggested. This study was performed to show the effect of olive oil on NAFLD improvement.

Materials and Methods: In this trial, 35 male Wistar rats were fed a basic diet for 14 days, and then they received a high fat – high carbohydrate diet (4۳% fat, 5۰% carbohydrates and ۱۷% protein) for 28 days to induce NAFLD. Afterwards, intervention period was started by dividing the animals into 3 groups as control (C) (induction diet), treatment 1 (T1) (10% olive oil) and treatment 2 (T2) (20% olive oil) for 28 days. Biochemical indices such as AST, ALT, TG, Total Cholesterol, HDL-C, and LDL-C were measured between each two periods. Twenty-four rats were included in the final analysis. Data were analyzed using ANCOVA and repeated measures tests.

Results: Mean AST decreased in C group from 466 UI/L to 193.3 UI/L, in T1 group from 697 UI/L to 312 UI/L, and in T2 group from 453.7 UI/L to 263.6 UI/L. Mean ALT decreased in C group from 121.3 UI/L to 80.3 UI/L, in T1 group from 178.8 UI/L to 167.3 UI/L, and in T2 group from 124.8 UI/L to 120.2 UI/L(p=0.02). Mean total Cholesterol in C, T1, and T2 groups changed from 76.3,75.8, and 78.8 mg/dl to 80.8, 77, and 73.9 mg/dl, respectively (p=0.05). Mean serum TG in C, T1, and T2 groups decreased from 87.3, 128.1, and 133.7 mg/dl to 78, 58, and 89.3 mg/dl, respectively (P=0.02).

Conclusion: All in all, the results indicated that the diet containing olive oil (10% and 20%) decreased the level of ALT enzyme and the diet with 20% olive oil reduced total serum cholesterol level in rats.

Keywords: Olive oil, Non-alcoholic fatty liver disease, Rat

***Corresponding Author:** Ebrahim Falahi. PhD, Professor, Nutrition Health Research Center, School of Health and Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran. Email: e_falahi@yahoo.com.

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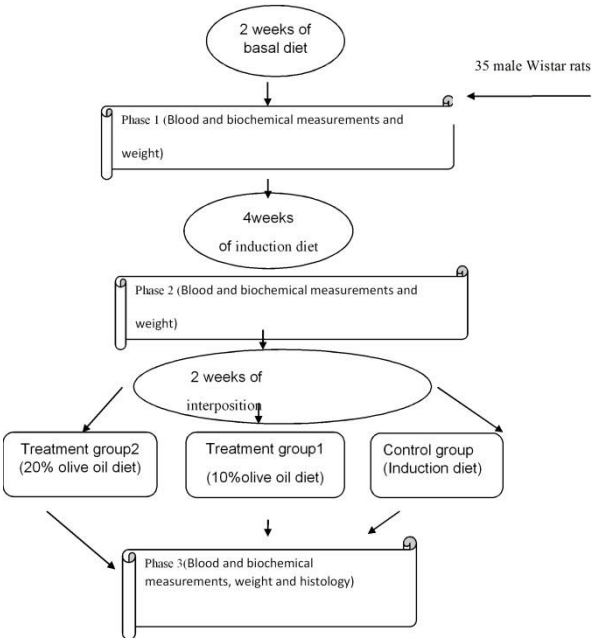
Introduction

Non-alcoholic fatty liver disease (NAFLD) is a reversible condition characterized by the pathologic accumulation of intrahepatic fat (1). It encompasses a spectrum of hepatic disorders from steatosis (simple fatty liver) to nonalcoholic steatohepatitis (NASH) (2). In epidemiologic studies, the prevalence of NAFLD has been reported to be 10-24% in US, Australia, New Zealand, and Europe and 2.4-2.9% in Iran (3-5). Iranian individuals between the ages of 30-50 are one of the most susceptible groups to this disease (6). Studies have shown that NAFLD is associated with the elevation of liver enzymes, ALT and AST. A link between increased level of AST and ALT and coronary artery disease in diabetic and nondiabetic patients has been reported in studies conducted so far (7). A study by Almed et al. found that 36-75% of the NAFLD patients also have type2 diabetes (2) and 20-80% of them suffer from hyperlipidemia (2). Risk factors for the development of NAFLD include abdominal obesity, diabetes and dyslipidemia (metabolic syndrome). The prevalence of the disease among obese and underweight individuals was reported to be 20% and 3%, respectively (8). Etiologic mechanism of this condition includes free fatty acids flow towards liver resulting from dietary triglycerides and fatty acids released from adipose tissues through starvation, diminished beta-oxidation of free fatty acids, decrease in hepatic secretion of triglycerides rich in very low density lipoproteins and high lipid peroxidation (9). Currently, lifestyle intervention, involving diet and exercise aimed at weight loss, is the primary treatment of NAFLD. Dietary pattern studies in NAFLD patients compared with controls have yielded conflicting results in terms of the importance of diet macronutrient composition. Several studies have involved dietary alteration as causal in the development of NAFLD. For instance, Bahrololumi et al. reported that the replacement of some of the daily dietary fat with olive oil along with weight loss could be effective in the prevention and treatment of NAFLD and cardiac complications. Moreover, the excess of dietary fat and lack of antioxidants lead to the elevation of lipid peroxidation which might be associated with more severe form of this disease (NASH) (10). In patients with NAFLD, the lower intake of saturated fatty acids associated with weight loss is linked to improved NAFLD pathophysiology. Recently, it has also been indicated that the dietary ratio of polyunsaturated fatty acids to monounsaturated fatty acids (PUFA/MUFA) in NAFLD patients was lower than that of healthy population. MUFAs induce decreased oxidation of low density lipoproteins (LDL), and therefore are associated with improved serum lipid profile including decreased level of LDL cholesterol, triglycerides, total cholesterol and increased level of HDL cholesterol. The replacement of dietary SFAs with MUFAs for the purpose of improving lipid profile and thereby the health status is still questionable (11). A comparison of olive oil fatty acid content with other oilseeds showed that the total content of saturated and unsaturated fatty acids in olive oil was comparable with other seeds. However, olive oil contains an average of 55-83% oleic acid which has been implicated in the prevention of metabolic syndrome, cardiovascular diseases and hypertension (12). High content of MUFAs in olive oil could be effective in improving lipid profile. However, both animal and human studies have yielded inconsistent results regarding the effect of olive oil on NAFLD. Furthermore, evidences are inconclusive (1). Studies conducted on mice have indicated that olive oil consumption leads to the triglyceride release from liver and lower accumulation of triglycerides in hepatic cells. It appears that NAFLD occurs following obesity, hyperglycemia, type2 diabetes and hyperlipidemia. Moreover, a significant association between high intake of SFAs and NAFLD occurrence has been reported in some studies. Gradual weight loss, elevated physical activity and diet (reduced consumption of dietary sources of SFAs and trans fatty acids) could be effective in NAFLD. Therefore, the present study was carried out to examine the effect of olive oil consumption on recovery from NAFLD in male rats.

Materials and Methods

Experimental Animals

In this analytical-experimental study, 35 male Wistar



rats (3 weeks old) were purchased from Pasteur Institute (Tehran, Iran). The animals were kept in an environment with controlled temperature (22±2 °C), a 40% minimum humidity and a light-dark cycle of 12 hours. This study was conducted in three phases.

Phase 1 (acclimatization proceeding)

The animals were fed with the same diet (according to the Razi Herbal Medicines Research Center guideline) for 2 weeks in order to acclimatize to the laboratory conditions. During this phase, animals were divided into 7 groups (n= 5/group) and were fed ad libitum water and a diet of the following composition: protein 20%, lipid 4-5% and carbohydrate 75% (3100 kcal per kilogram of food). These acclimatization proceedings were conducted according to the Razi Herbal Medicines Research Center guideline. The mean daily food intake was measured based on the difference between the weight of the allocated food and that remaining 24 hours later divided by the number of animals.

Phase 2 (induction phase)

In the second phase, a diet was prepared with the following composition: carbohydrate 43%, lipid 40% and protein 17% (13). Based on the calorie of a 20 gr tablet, the required calorie, and thereby the ratio of macronutrients, were calculated (20 gr was considered as the maximum amount of food consumed by the animals). At this point, the diet was prepared by a combination of white sugar, ghee and soy protein. Sugar was used because of its high content of fructose, and ghee was used for it is a rich source of saturated fatty acids. Rats were treated with 10 gr of the prepared food on a daily basis for 4 weeks. At the end of this period, the animals were weighed and blood samples were collected. Also, the mean food intake was calculated. Blood was prepared for biochemical tests as following to be described.

Phase 3 (intervention phase)

In the third phase, 26 animals, remaining after previous experiments, were randomly divided into the following groups: control group (n=9), treatment1 (n= 9) and treatment2 (n=8). Again, based on the calculated calorie, the macronutrient composition was determined and the diet was prepared using white sugar, ghee, olive oil and soy protein. A distinct dietary plan was designed for each group. Animals in the control group were treated with the induction diet. Rats in the treatment 1 group received a diet containing sugar 55%, ghee 30%, olive oil 10 and soy protein 5% (25% of fat from olive oil). Animals in treatment 2 group were fed with a diet containing sugar 55%, ghee 20%, olive oil 20% and soy protein 5% (50% of fat from olive oil).

100 gr olive oil contains following fatty acids: 73.7 gr MUFA-n9, 13.5 gr SFA, 7.9 gr PUFA n6 and 4.9 g MUFA n3.

After 4 weeks, rats were weighed and blood samples were collected again.

Biochemical Parameters Estimation

Fasted blood samples (2 cc) were drawn directly from the heart of each animal under diethyl ether anesthesia by a syringe at the end of phases 1, 2 and 3. Samples were collected from each rat and serum was separated from blood 2-3 hours after sampling by centrifugation at 2500- rpm for 10 minutes. Collected serum samples were stored at -80°C until the time of analysis.

Biochemical parameters that were determined in the samples included lipid profile total cholesterol, low-density lipoprotein (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides, and serum levels of liver enzymes aspartate aminotransferase (AST), and alanine aminotransferase (ALT), all being measured by routine spectrophotometric or enzymatic methods on an Automatic Hitachi 902 auto-analyzer.

Statistical Analysis

Data analysis was performed using SPSS, 18. The overall comparison of the 3 groups was done with

Kruskal-wallis test and Mann-Witny test in which repeated measures and 95%CI were used to compare each two groups with each other. Differences between groups were considered significant at the α= 0.05 level.

Results and Discussion

After adjusting energy intake and weight at baseline, no significant effect of time was observed on the mean total cholesterol in rats (p=0.183). Based on the results of repeated measures analysis, the interactive effect of time laps and test group on the mean total cholesterol was not significant (p=0.381). However, after adjusting mean energy intake and baseline weight, a significant distinction in terms of mean total cholesterol was found between the studied groups (p=0.050) (Table1).

After controlling energy intake and weight at baseline, the effect of time on mean serum triglyceride was not significant (p=0.868). Based on the results of repeated measures analysis, the interactive effect of time laps and test group on mean plasma triglyceride was not significant (p=0.256). Based on ANCOVA test and after adjusting energy intake and weight at baseline, no remarkable distinction with respect to mean serum triglyceride was observed among the groups (p=0.714) (Table 1).

The results of repeated measures analysis indicated that with adjustment for energy intake and weight at baseline, time had no significant effect on mean HDL cholesterol (p=0.570). According to the results obtained from repeated measure analysis, the interactive effects of time laps and test group on mean HDL cholesterol were not noticeable (p=0.620). Furthermore, ANCOVA tests demonstrated no significant difference in mean HDL cholesterol among the groups after adjustment for energy intake and weight at baseline (Table1).

After adjusting energy intake and weight at baseline, no significant effect of time was observed on the mean LDL cholesterol in rats (p=0.142). Based on the results of repeated measures analysis, the interactive effects of time laps and test group on the mean LDL cholesterol were not significant (p=0.795). Moreover, after adjusting mean energy intake and baseline weight, no significant difference in terms of mean LDL cholesterol was found among the groups (p=0.193) (Table1).

Hepatic enzymes, including ALT and AST, are indicators of hepatocellular injury. The serum concentrations of these enzymes in individuals with NAFLD could be 10 times higher than normal (<31 IU/L ALT or AST in women and <41 IU/L ALT and <37 IU/L AST in men) (13, 14). Our study revealed that consumption of olive oil could result in significant reduction in serum ALT but not AST in rats with NAFLD. Bahrololumi et al. (2013) also examined the effects of virgin olive oil on serum aminotransferases through a normal fat diet among 50 patients with NAFLD. A significant decrease in ALT and AST was observed in the intervention group as compared to the controls (10). However, the effect of olive oil alone and independent of weight loss diet was not assessed in this study. In another study, Fraser et al. (15) reported that a modified Mediterranean diet, high in MUFAs, was associated with the lowest ALT levels in 6 months. They showed that these effects were independent of changes in body mass index (BMI). As can be seen, the present study is among few studies conducted to examine the effects of olive oil on liver and hepatic transaminases, and thereby further studies on humans are required to ascertain whether the consumption of olive oil may be helpful in NAFLD patients.

NAFLD is also accompanied by dyslipidemia characterized by high serum TG and LDL-C and low HDL-C levels (16). According to our results, olive oil intake lowered serum total cholesterol significantly in the intervention group compared to the control. However, no significant changes in serum TG, LDL-C or HDL-C levels were observed. Beneficial effects of MUFAs, such as those found in olive oil on lipid profile, have been indicated in some animal and human studies. A study demonstrated that the accumulation of TGs in the liver of rats decreases by olive oil consumption. Severe fatty liver was seen in methionine choline deficient diet (MCDD), MCDD + fish oil and in MCDD + butter fat groups, but not in the MCDD + olive oil group. The hepatic TG increase in the MCDD + olive oil group was decelerated by 30% compared to the MCDD group. In comparison with the control group, the long chain PUFA n6: n3 ratio increased in the MCDD + olive oil group by 345-fold. Olive oil improved insulin resistance, increased

Table 1: The comparison of mean total cholesterol, HDL, LDL, TG, AST,ALT, Weight (mg/dl) in experimental groups in different phases of study.

			Control	Treatment1	Treatment2	Total	P**	P***
			Mean ±SE	Mean± SE	Mean ±SE	Mean ±SE		
Total Cholesterol mg/dl	Phase 1	9	57.5±7.5	70.9±12.4	60.3±10.7	62.2±11.1	-	-
	Phase 2	7	76.3±17.9	75.8±16.9	78.8±15.0	76.9±16.1	-	-
	Phase 3	8	80.8±20.1	77.0±9.7	73.9±20.4	77.3±16.9	-	-
	Total	24	71.6±9	74.9±9.4	80.0±12.7	72.5±10.1	0.381	0.05
	P*		-	-		0.183		
HDL mg/dl	Phase 1	9	39.4±4.3	51.6±7.9	44.7±7.4	44.7±8.0	-	-
	Phase 2	7	45.7±9.5	39.5±5.6	41.2±8.7	42.2±8.3	-	-
	Phase 3	8	57.1±13.7	55.2±8.5	49.3±9.8	11±54	-	-
	Total	24	47.4±4.9	48.4±4.6	45.1±5.8	47±5.1	0.620	0.349
	P*		-	-	-	0.570		
LDL mg/dl	Phase 1	9	7.8±12.8	9.8±7.8	7.1±3.8	9.8.±6.9	-	-
	Phase 2	7	13.8±5.9	14.9±16.4	14.2±17.9	14.3±13.8	-	-
	Phase 3	8	7.2±6.5	10.9±5.6	13.4±21.8	10.4±12.8	-	-
	Total	24	9.5±5.1	12.1±7.4	10.5±12.9	10.7±8.6	0.795	0.193
	P*		-	-	-	0.142		
TG mg/dl	Phase 1		94.8±103	80.1±49.5	134.4±68.8	103.2±89.7	-	-
	Phase 2		87.3±44.8	128.1±103.5	133.7±133.7	115.7±70	-	-
	Phase 3		89.3±53.3	58±29.8	78±29.1	75±40.2	-	-
	Total		90.4±53.1	90±38.5	115.4±23.5	109.5±41.9	0.256	0.714
	P*		-	-	-	0.868		
AST (IU/L)	Phase 1	9	308±224.9	187.7±71.7	152.5±38.3	221.2±155.9	-	-
	Phase 2	7	466±450.9	697±669.3	453.7±320	529.3±480.5	-	-
	Phase 3	8	193.3±72.9	312±235.4	236.6±82.5	251.4±144	-	-
	Total	24	396±76.6	450±326	290±134.3	323.5±239	0.07	0.574
	P*		-	-	-	0.05		
ALT (IU/L)	Phase 1	9	98.4±30.3	70.1±22.2	95.4±45.5	89.1±35.1	-	-
	Phase 2	7	121.3±97.6	178.8±128	124.8±46	142.3±98.1	-	--
	Phase 3	8	80.3±14.6	167.3±68	120.2±30.4	122.7±56.3	-	-
	Total	24	100±44	144.7±60.8	113.4±26.3	118.1±63.2	0.863	0.020
	P*		-	-	-	0.378		
Weight (g)	Phase 1	9	243.2±19	200.5±40	254.1±29.5	231.8±37.8	-	-
	Phase 2	7	193.2±9.7	184.7±27	206.121.4	194.2±21.7	-	-
	Phase 3	8	149.0±11.5	155.3±25.9	170.4±15.5	157.8±20.1	-	-
	Total	24	195.1±7.4	180.2±25.2	210.2±18.4	194.6±22.8	0.562	0.166
	P*	-	-	-	-	0.547	-	-

P*: the effect of time on mean total cholesterol, HDL, LDL, TG, AST,ALT, Weight based on repeated measures test

P**: the effect of time and experimental group on mean total cholesterol, HDL, LDL, TG AST,ALT, Weight based on repeated measures test

P***: the effect of experimental group on mean total cholesterol HDL, LDL, TG AST,ALT, Weight based on repeated measurse test

the release of TG from the liver and decreased the flux of FFAs from peripheral adipose tissue back to the liver (17). Another study conducted in Spain showed that treatment with a balanced diet rich in olive oil could contribute to the recovery of the liver from hepatic steatosis. This was achieved by diminishing activation of hepatic stellate cells by MUFAs, which are less susceptible to lipid peroxidation compared to PUFAs (18). Moreover, Ingestion of a virgin olive oil-based breakfast decreased postprandial glucose, triacylglycerol, and insulin concentrations, and at the same time increased

HDL cholesterol and glucagon-like peptide-1 (GLP-1) concentrations as compared to a carbohydrate rich diet (19). In human beings, dietary MUFA (oleic acid) decreased oxidized LDL, LDL-cholesterol and TG concentration without the concomitant decrease in HDL(20, 21). In contrast, Esmaeelzadeh et al. in their study found that a diet rich in olive oil caused no change in total cholesterol and LDL cholesterol (22). Similarly, Fito et al. investigated the effect of olive oil-rich diet on 50 patients with non-alcoholic fatty liver disease during 12 weeks and showed that olive oil compared to regular oil significantly attenuated mean serum TG and the ratio of HDL cholesterol to TG. No change in total cholesterol and LDL cholesterol was observed (20). A meta-analysis of studies in individuals with diabetes showed that a high fat diet with 22%-33% of the energy from MUFAs resulted in lower plasma total cholesterol, VLDL, and TG levels as compared to a low fat, high carbohydrate diet (23). Inconsistencies in these studies and also the results observed in the present study could be explained in terms of differences in dose and duration of consumption, manner and frequency of administration, environmental factors and complex interactions between dietary components, cells and metabolic pathways that are rarely mediated by a single mechanism.

Mechanistic studies attribute a direct efficient role for olive oil in improving plasma lipids (22). Unrefined, or virgin, olive oil has bioactive compounds with beneficial antioxidants action (24). Oleocanthal, a component found in extra virgin olive oil, is a natural anti-inflammatory compound that has a potency and profile considerably similar to that of ibuprofen (25). The exact mechanism through which MUFAs and olive oil could improve hepatic TG content is not clear. Oleic acid from cooking oil resulted in lower insulin resistance in the general population (26). An olive oil-enriched diet is involved in redistribution of body fat and modifies the lipolytic efficiency of fat cells (27). Furthermore, n-9 fatty acids may regulate gene expression related to peripheral insulin sensitivity, increase endothelial vaso-reactivity, up-regulation of uncoupling protein mRNA in adipose tissue and muscle, and expression of up regulates glucose transporter-2 in the liver (28). Oleic acid decreases the expression of genes involved in hepatic gluconeogenesis and lipogenesis and SREBP in Zucker fatty rats (29). Additional effects of olive oil beyond its MUFA composition can be explained by its polyphenols. Polyphenols present in olive oil, such as oleuropein, hydroxytyrosol, tyrosol and caffeic acid, possess important antioxidant and anti-inflammatory effects (30). In rat leukocytes, these molecules have been reported to inhibit leukotriene B4 generation at the 5-lipoxygenase level and to lower the generation of reactive oxygen species (31). Moreover, a diet rich in olive oil improves endothelial function compared to a high carbohydrate diet or a high linoleic acid diet (32, 33). Finally, an oleic acid-rich Mediterranean type diet may reduce the risk of atherosclerosis by decreasing the number of chylomicron remnant particles as compared to a linoleic acid-enriched diet (34). The principal mechanisms of action of olive oil include a decrease in NF-κB activation, the reduction of LDL oxidation and an improvement in insulin resistance.

Conclusion

The present study indicated some efficient effects of olive oil consumption on NAFLD. However, further studies, especially in human, are required to confirm these effects and also to elucidate the underlying mechanisms involved.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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