Journal of Home Economics
Volume 30, Number (1), 2020
http://homeEcon.menofia.edu.eg

Journal of Home Economics
ISSN 1110-2578

Effect of oil and leave olive on rats suffering from acute liver disease

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Abstract

This study aimed to investigate the effect of two levels of olive oil, olive leave and their mixture on rats suffering from acute liver disease. In addition to determine the chemical composition and phenolic compounds. Forty eight male albino rats (Sprague Dawley) used in this study. Rats divided into two main groups. First main group (6 rats) was fed on basal diet, as negative control. The second main group (42 rats) fed on basal diet and were treated with CCL4 in paraffin oil (50% v/v 4ml/kg) by a single dose subcutaneous injection to induce acute damage in the liver. After injection, AST, ALT and ALP enzymes activity were determined in the first and second main groups to insure the induction. Then the rats in the second main group were divided into seven subgroups (n = 6) according to the following: *Subgroup 1*: fed on normal basal diet, this group used as a (positive control group). *Subgroup 2&3:* fed on diet containing (3.5% and 7%) olive oil, respectively. *Subgroup 4&5:* fed on diet containing (3.5% and 7%) olive leave , respectively. *Subgroup 6*: fed on basal diet containing (3.5% olive oil and 3.5% olive leave). *Subgroup 7:* fed on basal diet containing (7% olive oil and 7% olive leave). The results revealed that, injected rats with CCl4 induced decrease in feed intake, BWG% and HDL-c, while organs weight / body weight%, serum (cholesterol, triglycerides, LDL-c, VLDL-c, AST, ALT and ALP) increased significantly, as compared to non-injected rats (control negative group). Treating acute liver disease groups with the two levels from olive oil, olive leave and their combination improved all parameters, especially the groups which were treated with (7% olive oil and 7% olive leave) and (3.5% olive oil and 3.5% olive leave). From these results, it could be concluded that, olive oil, olive leave and the combination from them improved liver enzymes and the complication resulting from CCl4.

Keywords: olive oil – olive leave – acute liver disease - rats- liver enzymes – lipid profile.

Introduction:

The liver is the main organ in the body and plays an vital role in the metabolism of foreign compounds entering the body. Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment. All these compounds produce a variety of toxic manifestations **(Athar *et al.*, 1997).**

Acute liver failure appears when your liver quickly loses its ability to function. Liver failure develops gradually over the course of years. But acute liver failure develops in a matter of days. Acute liver failure can produce many complications, including excessive bleeding and high blood pressure in the brain. Another term for acute liver failure is fulminant hepatic failure **(Larson., 2005)**.

Olive leave from *Olea europaea*, is native to the Mediterranean and has been claimed to have medicinal values including anti-diabetic and antioxidant activities **(Wojcikowski *et al.*, 2007 and Eidi et al., 2009)**.

Olive oil has traditionally been the principal oil of the Mediterranean diet. TheMono-unsaturated Fatty Acids(MUFA) diet prevents central body fat accumulation and decreases postprandial adiponectin expression induced by a carbohydrate rich diet in insulin-resistant subjects **(Paniagua *et al.*, 2007)**. Polyphenols present in olive oil, such as oleuropin, hydroxytyrosol, tyrosol and caffeic acid, have an important antioxidant and anti-inflammatory effect **(Covas *et al.*, 2006).** Previous studies carried out in fibrotic rats showed that olive oil, in contrast to polyunsaturated oils, could protect against the development of fibrosis (**Szende *et al.*, 1994)**.

Jalali *et al.*, (2017) reported that, diet containing (10 and 20% olive oil) decreased the level of ALT enzyme on the other hand the diet containing 20% olive oil reduced total serum cholesterol level in rats. Olive oil containing high amount of mono-unsaturated fatty acid, in this respect, **Fraser *et al.* (2008)** reported that a modified Mediterranean diet, high in MUFAs, was associated with the lowest ALT levels in 6 months. The purpose of this study was to clarify the effect of two levels from olive oil, olive leave and their combinations on nutritional parameters, liver enzymes and lipid profile of rats suffering from acute liver disease.

MATERIALS AND METHODS:

Materials:

(1) Casein, all vitamins, minerals, cellulose, L –Cystine, CCl4 and choline chloride were obtained from El–Gomhoriya Company, Cairo, Egypt.
(2) Starch and soy oil were obtained from local market, Cairo, Egypt.
(3) Olive (leave & oil) were obtained from Agriculture Research Center, Cairo, Egypt.
(4) Normal male albino rats (42) of Sprague Dawley Strain obtained from the Laboratory Animal Colony. Ministry of Health and Population, Helwan, Cairo, Egypt.
(5) Kits used to determine serum cholesterol, triglycerides, LDL-c, HDL-c, VLDL-c, AST, ALT and ALP.

Methods:

Chemical Composition: Concentrations of moisture, total protein, fat, fiber and ash were determined in olive leave according to (**A.O.A.C. 1990)**, while total carbohydrate estimated by deference's.

Fatty acid composition of olive oil: Fatty acid compositions of olive oil were determined according to **(Gunstone *et al.*, 1994 and Yeshajahu, 1994)**.

Determination of phenolic compounds: Phenolic compounds of olive leave s was determined according to the method described by **(Crozier *et al.*, 1997)** by using High performance liquid chromatography analysis HPLC.

Experimental Design:

Male albino rats sprague Dawley Strain (42 rats) weighing (150 ± 10 g) were housed in well aerated cages under hygienic condition and fed on basal diet for one week for adaptation, according to **Reeves et al., (1993).**

After adaptation period, the rats were divided into two main groups as follows: ***The first main group (6 rats)***: fed on basal diet, and used as a negative control group. ***The second main group (36 rat)*** fed on basal diet and were treated with CCL4 in paraffin oil (50% v/v 4ml/kg) by a single dose subcutaneous injection to induce acute damage in the liver **(Jayasekhar *et al.*, 1997)**. After injection, AST, ALT and ALP enzymes activity were determined in the first and second main groups to

insure the induction. Then the rats in the second main group were divided into six subgroups (n = 6 each) as follows:

Subgroup 1: fed on basal diet, this group used as a (positive control group). ***Subgroups 2 & 3:*** fed on basal diet containing (3.5% soybean oil + 3.5% olive oil) and (7% olive oil), respectively. ***Subgroups 4 &5:*** fed on basal diet containing (3.5% olive leave) and (7% olive leave), respectively. ***Subgroup 6:*** fed on basal diet containing (3.5% olive oil and 3.5% olive leave). ***Subgroup 7:*** fed on basal diet containing (7% olive oil and 7% olive leave).

During the experimental period (6 week), the diets consumed and body weights were recorded every week. At the end of the experiment, the rats were fasted overnight, and then the rats were anaesthetized & sacrificed, the blood samples were collected from the aorta of all rats. The blood samples were centrifuged and serum was separated to estimate biochemical parameters, i.e. serum cholesterol **(Allain *et al.*, 1974)**, triglycerides **(Fossati and Principe (1982)**, high density lipoprotein HDL-c **(Burstein 1970)**, low density lipoprotein LDL-c and VLDL-c **(Friedwald *et al.*, 1972)**, Aspartate Amine Transaminase (AST) and Alanine Amine Transaminase (ALT) **(Reitman and Frankel 1957)** , Alkaline Phosphatase (ALP) **(Belfield and Goldberg 1971).**

Liver and kidney were separated from each rat and weighted to calculate the liver and kidney weights to body weights %. Results of biological evaluation of each group were statistically analyzed (mean ± standard deviation and one way ANOVA test) using SAS package and compared with each other using the suitable test (least significant differences at $P< 0.05$ **(Steel and Torri, 1980).**

Results and Dissection:

Approximate analysis of olive leave:

Olive leave was analyzed and illustrated in table (1). The amount of moisture, ash, protein, fiber, lipid and carbohydrates of olive leave were (4.22, 4.93, 11.00, 12.50, 7.00 and 60.35 g/100g), respectively. These results agree with the findings of **(Ibrahim *et al.*, 2016)** who found that, the amounts of protein, fat, ash fiber and carbohydrates of whole olive leaves were (10.60, 7.90, 6.80, 14.50 and 60.20 g/100g), respectively. **Cavalheiro *et al*. (2015)** determined the chemical components of olive leaves from five types cultivated in Brazil. They found that protein, lipids, ash and total carbohydrates

contents in fresh leaves ranged from 10.5 to 13.1, 9.13 to 9.8, 4.37 to 6.0 and 8.74 to 32.63%, respectively.

Table (1): Chemical Composition of olive leave (g/100g)

Nutrients	Moisture	Ash	Protein	Fiber	Lipid	Other carbohydrates
Olive leave	4.22	4.93	11.00	12.50	7.00	60.35

Phenolic compounds concentration in dried olive leave s extract:

The main phenolic compounds which extracted from dried olive leaves which fractionated by using high performance liquid chromatography presented in Table (2). Rutin was found to be the major compound and amounted 203.66mg/100g, while, Quercetin and Oleuropein recorded the mediated compounds which were in 90.53 and 83.62mg/100g, respectively. On the other hand, the amounts of Catechin, Apigenin, Hydroxytyrosol and Caffeic acid were amounted (25.55, 22.00, 19.95 and 15.42 mg/100g), respectively.

Table (2): Phenolic compounds concentration in dried olive leaves extract (mg /100 g olive leaves)

Compound	mg/100g
Rutin	203.66
Quercetin	90.53
Oleuropein	83.62
Catechin	25.55
Apigenin	22.00
Hydroxytyrosol	19.95
Caffeic acid	15.42

In this respect, **Benavente-Garcia *et al.*, (2000)** reported that, some of Mediterranean diet such as fruits, vegetables, oilseeds and olive oil is known for its health benefits, especially which given to the large amount of polyphenols. Olive leaves contain higher amount of polyphenols as compared to olive oil. E.g., the amount of oleuropein, which is the most abundant phenolic compound ranges from 0.005% and 0.12% in olive oil while in olive leave s it ranges between1 and 14% **(Japon-Lujan *et al.*, 2006)**.

Fatty acids composition of olive oils (g/100g):

Gas liquid chromatography technique (GLC) was employed to identify the fatty acids composition of olive oil. The fatty acid composition of olive oil was illustrated in table (3). The results obtained from this Table indicated that, olive oil contained 17.88% saturated fatty acids (SFA). The palmitic acid (C16:0) was the major saturated fatty

acid reached 14.00% while stearic acid (C18:0) had the lowest percent of saturated fatty acid being 3.33%. Olive oil also contained about 64.48% monounsaturated fatty acids and the oleic acid (C18:1) had the predominant percent 63.24% followed by palmitoleic acid (C16:1) being 0.95%. Olive oil also contained about 17.64% polyunsaturated fatty acids, linoleic acid (C18:2) was the major polyunsaturated fatty acids being 16.89%, followed by linolenic acid (C18:3) being 0.75% respectively.

In this respect, **Scano *et al.*, (1999)** found that, fatty acids present in olive oil are palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic. Myristic, heptadecanoic and eicosanoic acids are found in trace amounts. Greek, Italian, and Spanish olive oils are low in linoleic and palmitic acids and they have a high percentage of oleic acid. Olive oil is composed of 71% oleic and 1% palmitoleic (monounsaturated fats); 10% linoleic and 1% linolenic (polyunsaturated fats); and 13% palmitic, 3% stearic, and 1% arachidic (saturated fats) **(ISEO 2016)**.

Table (3): Fatty acids composition of olive oils (g/100g):

Fatty Acids		Olive Oil g/100 g
Myristic acid (C14:0)		0.01
Palmitic acid (C16:0)		14.00
Palmitoleic acid (C16:1)		0.95
Heptadecanoic acid (C17:0)		0.08
Heptadecenoic acid (C17:1)		0.03
Stearic acid (C18:0)		3.33
Oleic acid (C18:1)		63.24
Linoleic acid (C18:2)		16.89
Arachic acid (C20:0)		0.31
Linolenic acid (C18:3)		0.75
Eicosenoic acid (C20:1)		0.26
Behenic acid (C22:0)		0.10
Lignoceric acid (C24:0)		0.05
Total	**Saturated Fatty Acids (SFA)**	17.88
	Mono-Unsaturated Fatty Acids (MUFA)	64.48
	Poly-Unsaturated Fatty Acids (PUFA)	17.64

Effect of oil and leave of olive on feed intake, body weight gain% and organs weight/body weight% of rats suffering from acute liver disease

The effect of oil and leave of olive on feed intake (g/day/each rat), body weight gain% (BWG%), and some organs weight/body

weight% including "liver and kidney" of rats suffering from acute liver disease are presented in table (3). The mean value of feed intake of rats fed on basal diet "control negative group) was 17 g/day/each rat, while the mean value of feed intake on acute liver disease rats "control positive group" was 14.770 g/day/each rat". Feed intake in the positive control group decreased by about 13.11%, than that of the control negative group.

The mean values of feed intake of all treated acute liver disease groups with (3.5% and 7%) olive oil, (3.5% and 7%) olive leave and (3.5% olive oil and 3.5% olive leave) decreased than that of the negative control group, on the other hand, these treatments increased the mean values of feed intake than that of the positive control group.

The mean value ±SD of body weight gain % (BWG%) of healthy rats fed on basal diet "control –ve group" was 33.88 ± 1.12, while the mean value of BWG% of acute liver disease group "control +ve group" was 8.047 ± 1.127. BWG% of the positive control group decreased significantly ($p \leq 0.05$), as compared to the negative control group (Table 4).

All treated groups showed significant increase in BWG%, as compared to the positive control group. While the mean values of treated groups were decreased significantly, when compared to the negative control group. The highest increase in BWG% in all treated groups recorded for the groups fed on diets containing (7% olive oil) then (3.5% olive leave) then (7% olive leave) and (3.5% olive oil and 3.5% olive leave), while the lowest Value recorded for group fed on diet containing 3.5% olive oil.

Liver weight/body weight% of acute liver disease group (control +ve group) increased significantly $P \leq 0.05$, as compared to liver weight/ body weight% of healthy rats (control negative group). Treating acute liver groups with diets containing (3.5% and 7%) olive oil, (3.5% and 7% olive leave) and (3.5% olive oil and 3.5% olive leave) induced significant decrease in liver weight / body weight%, as compared to the positive control group. The best results in liver weight/body weight% recorded for the groups which were treated with (3.5% olive leave and 3.5% olive oil) and (7% olive leave and 7% olive oil), these treatments showed non-significant changes in liver weight/body weight%, as compared to the negative control group.

Table (3): Effect of oil and leave of olive on feed intake, body weight gain% and some organs weight/body weight% of rats suffering from acute liver disease

Parameters / Groups	Feed Intake g/day/each rat	BWG%	Organs weight / body weight%	
			Liver	Kidney
Control (-ve)	17.00	33.88 [a] ± 1.12	2.89 [e] ± 0.08	0.42 [d] ± 0.01
Control (+ve)	14.77	8.05 [d] ± 1.13	5.30 [a] ± 0.19	0.63 [a] ± 0.02
3.5% olive oil	15.45	12.85 [c] ± 0.79	4.86 [b] ± 0.26	0.59 [b] ± 0.01
7% olive oil	16.86	15.84 [b] ± 0.97	4.27 [c] ± 0.18	0.48 [c] ± 0.04
3.5% olive leave	15.62	15.22 [b] ± 0.92	4.22 [c] ± 0.085	0.47 [c] ± 0.03
7% olive leave	15.41	15.16 [b] ± 0.63	3.40 [d] ± 0.18	0.42 [d] ± 0.02
3.5% olive oil and 3.5% olive leave	15.90	15.95 [b] ± 0.83	3.039 [e] ± 0.10	0.41 [d] ± 0.01
7% olive oil and 7% olive leave	16.00	16.11 [b] ± 0.54	2.97 [e] ± 0.13	0.42 [d] ± 0.05

Least significant differences at P≤0.05.

Means with the same letter are insignificantly difference.

Kidney weight/body weight% of acute liver disease group (control +ve group) increased significantly P≤0.05, as compared to kidney weight/ body weight% of healthy rats (control negative group). All treated acute liver disease groups showed significant decrease in kidney weight / body weight%, as compared to the positive control group. The best results in kidney weight/body weight% recorded for the groups which fed on diets containing (7% olive leave), (3.5% olive oil and 3.5% olive leave) and (7% olive oil and 7% olive leave), these treatments caused non-significant differences in kidney weight/body weight%, as compared to the negative control group.

From these results it could be observed that, injected rats with CCl4 induced decrease in feed intake and body weight gain %, while liver and kidney weight / body weight % increased as compared to non-injected rats (control –ve group). Also the results indicated that olive oil with the levels (3.5% and 7%), olive leave with the same levels (3.5% and 7%) and (3.5% olive oil + 3.5% olive leave) improved the mean

value of feed intake, body weight gain %, and organs weight/body weight%.

In this respect **Murthy *et al.*, (2002)** stated that the reduction of weight gain in injected rats with CCL4 might be due to gastrointestinal toxicity. **Shen *et al.*, (2014)** suggest that olive leave s extract exerts beneficial effects against obesity by regulating the expression of genes involved in adipogenesis and thermogenesis in the visceral adipose tissue of high fat diet-fed mice.

Effect of oil and leave of olive on lipid profile of rats suffering from acute liver disease.

The effect of oil and leave of olive on serum cholesterol, triglycerides, high density lipoprotein-cholesterol HDL-c, low and very low density lipoprotein-cholesterol) LDL-c and VLDL-c of rats suffering from acute liver disease are presented in table (5 and 6) .

Serum cholesterol and triglycerides (mg/dl):

The mean value of serum cholesterol and triglycerides increased significantly ($p<0.05$) in acute liver disease group which fed on basal diet, as compared to healthy group fed on the same diet table (4). The mean values ± SD of serum cholesterol and triglycerides in these groups were (145.666 ± 7.094 and 78.666 ± 3.214 mg/dl) (87.333 ± 8.386 & 38.333 ± 4.618 mg/dl), respectively. The mean value of serum cholesterol and triglycerides increased in the positive control group by about 66.79% and 105.21%, than that of the negative control group.

Feeding acute liver disease groups on diet containing (olive oil, olive leave and olive oil & olive leave) led to significant decrease in serum cholesterol and triglycerides, as compared to the positive control group fed on basal diet. Treating acute liver disease group with diet containing 7% olive oil decreased the mean values of serum cholesterol and triglyceride, as compared to the group which treated with diet containing 3.5% olive oil. The same trend was observed when compared the mean values of cholesterol and triglyceride in the group which treated with (7% olive leave) and (3.5% olive leave).

Feeding acute liver disease groups on diet containing (3.5% olive oil and 3.5% olive leave), (7% olive oil) and (7% olive leave) decreased the mean values of serum cholesterol and triglycerides, as compared to the positive control group and other treated groups, especially the group which treated with (7% olive oil and 7% olive

leave) , this treatment decreased the mean values of serum cholesterol and triglyceride by about 40.960% and 42.796%, than that of the positive control group.

Serum lipoprotein-cholesterol (mg/dl):

The effect of oil and leave of olive on serum lipoprotein-cholesterol including (high density lipoprotein-cholesterol HDL-c, low and very low density lipoprotein-cholesterol) LDL-c and VLDL-c of acute liver disease rats are presented in table (6). Injected rats with CCl4 to induced acute liver disease " decreased the mean values of HDL-c, while LDL-c and VLDL-c increased significantly ($p \leq 0.05$), as compared to the negative control group (32.000 ± 2.645, 97.933 ± 6.132 & 15.733 ± 0.642) vs. (52.333 ± 2.516, 27.100 ± 6.060 & 7.666 ± 0.923 mg/dl), respectively. The mean value of serum LDL-c and VLD-c increased in the positive control group by about 261.376% and 105.230%, while HDL-c decreased by about 38.849%, than that of the control negative group.

Table (5): Effect of oil and leave of olive on serum cholesterol and triglyceride of rats suffering from acute liver disease

Parameters / Groups	Cholesterol	Triglycerides
	mg/dl	
Control (-ve)	87.33 ^{e} ± 8.39	38.33 ^{g} ± 4.62
Control (+ve)	145.66 ^{a} ± 7.09	78.66 ^{a} ± 3.21
3.5% olive oil	130.66 ^{b} ± 6.11	65.33 $^{b\ c}$ ± 2.52
7% olive oil	116.33 $^{c\ d}$ ± 5.50	51.33 ^{e} ± 1.53
3.5% olive leave	123.33 $^{b\ c}$ ± 6.11	66.33 ^{b} ± 4.51
7% olive leave	110.00 ^{d} ± 7.00	58.00 ^{d} ± 3.00
3.5% olive oil and 3.5% olive leave	92.00 ^{e} ± 3.00	59.33 $^{c\ d}$ ± 3.055
7% olive oil and 7% olive leave	86.00 ^{e} ± 8.19	45.00 ^{f} ± 4.58

Significant at $p<0.05$ using one-way ANOVA test.

Values which have different letters differ significantly, while those with have similar or partially are non-significant.

All treated acute liver disease groups with olive oil, olive leave and (olive oil & olive leave) improved the mean values of serum HDL-c, LDL-c and VLDL-c, as compared to the positive control group.

The data presented in this Table revealed that, the mean values of LDL-c and VLDL-c decreased gradually with increasing the levels of olive oil or olive leave and or (olive oil + olive leave) in the diet.

Using olive leave with the level (3.5%) in the diet improved lipoprotein-cholesterol, as compared to olive oil (3.5%), the same trend

was observed when compare (7% olive leave with olive oil). The best results in HDL-c, LDL-c and VLDL-c recorded for the acute liver disease group treated with (7% olive oil and 7% olive leave), this treatment improved these parameters, as compared to the positive control group and the other treated groups. This treatment increased the mean value of serum HDL-c by about 52.081% and decreased the mean values of serum LDL-c and VLDL-c by about 71.341% and 42.796%, than that of the positive control group.

Table (5): Effect of oil and leave of olive on serum lipoprotein-cholesterol of rats suffering from acute liver disease

Parameters / Groups	HDL-c	LDL-c	VLDL-c
	mg/dl		
Control (-ve)	52.33 ^{a}± 2.52	27.10 ^{e}± 6.060	7.67 ^{f}± 0.92
Control (+ve)	32.00 ^{e}± 2.65	97.93 ^{a}± 6.13	15.73 ^{a}± 0.64
3.5% olive oil	38.67 ^{d}± 3.21	78.80 ^{b}± 2.71	13.07 ^{bc}± 0.503
7% olive oil	43.00 ^{bcd}± 6.08	62.93 ^{c}± 3.64	10.07 ^{e}± 0.12
3.5% olive leave	40.33 $^{c d}$± 1.53	69.53 ^{c}± 5.44	13.27 ^{b}± 0.90
7% olive leave	45.00 $^{b c}$± 2.89	52.93 ^{d}± 6.93	11.60 ^{d}± 0.60
3.5% olive oil and 3.5% olive leave	46.67 ^{ab}± 0.58	33.27 ^{e}± 2.19	11.87 $^{c d}$± 0.61
7% olive oil and 7% olive leave	48.67 ^{ab}± 3.055	28.066 ^{e}± 4.56	9.00 ^{e}± 0.916

Significant at p<0.05 using one-way ANOVA test.

Values which have different letters differ significantly, while those with have similar or partially are non-significant.

From these results, it could be concluded that, injected rats with CCl4 induced elevation of serum cholesterol, triglycerides, LDL-c and VLDL-c, while HDL-c decreased than that of non-injected rats. Treating acute liver disease rats with olive oil, olive leave and their combination decreased the mean value of serum cholesterol, triglycerides, LDL-c and VLDL-c, also these treatments increased HDL-c, as compared to the positive control group.

Olive oil and olive leave improved lipid profile in rats which suffer from acute liver disease. In this respect, **Yubero-Serrano & Garcia-Rios (2011)** also **Venturini Simao (2015)** reported that, mono-unsaturated fatty acids MUFA concentration of extra virgin olive oil EVOO, decreased total cholesterol TC, low density lipoprotein LDL cholesterol and the ratio TC/HDL cholesterol when replace it with saturated fats. Treating the CCl_4 induced hepatotoxicity in male rats with olive oil very high significantly ($P < 0.001$) decreased the mean values of TC, TG, LDL-C and VLDL-C compared with that of the positive

control. In addition, the mean values of HDL were very high significantly ($P < 0.001$) higher than that of the positive control **(Al-Seeni *et al.*, 2016)**.

Phenolic compounds in olive oil were associated with increased levels of HDL-cholesterol and in improvements in endothelial function **(Servili *et al.*, 2013)**. Oxidative stress due to CCl_4 injection caused an increase in free fatty acid distribution to the liver and elevated hepatic TG accumulation and diet rich with olive oil reduced the accumulation of TG in the liver **(Ilhan and Seçkin., 2005).**

Olive leaves extract has an important role in preventing formation of atherosclerosis and coronary heart disease in patients suffering from diabetes **(Zoair, 2014)**. Olive leave s extract contains bio functional components, such as oleuropein, which may play as a regulatory lipid agent and have anti-atherosclerotic effect. On the same context, the anti-atherosclerotic effect of olive leaves extract was also demonstrated in rabbits on a high-lipid diet **(Wang *et al.*, 2008).**

Effect of oil and leave of olive on liver enzymes of rats suffering from acute liver disease.

The effect of two levels from (olive oil, olive leave and their combination) on liver enzymes (Aspartate Amino Transferase AST, Alanine Amino Transferase ALT and Alkaline Phosphatase ALP) u/l of acute liver disease rats presented in Table (7).

Aspartate Amino Transferase "AST" (U/l):

The mean value of serum AST enzyme of acute liver disease rats fed on basal diet (+ve control group) increased significantly $p<0.05$, as compared to healthy rats fed on the same diet (115.334 ± 11.015 u/l vs. 57.667 ± 4.932 u/l), respectively. AST enzyme increased by about 100% in the positive control group, than that of the negative control group (Table 7).

Treating acute liver disease groups with diets containing (3.5 and 7% olive oil), (3.5 and 7% olive leave) and the combination between these oil and leave with the same level, led to significant decrease in AST enzyme, as compared to the positive control group. The highest decrease in AST enzyme recorded for acute liver disease group fed on diet containing (7% olive oil and 7% olive leave), this treatment decreased the mean value of serum AST by about 42.19%, than that of the positive control group.

The mean value of serum ALT enzyme of rats suffering from acute liver disease increased significantly $p<0.05$, as compared to

healthy rats fed on normal diet (38.66 ± 4.618u/l vs. 13.66 ± 2.081 u/l), respectively. Injected rats with CCL4 increased the mean value of serum ALT enzyme by about 182.943%, than that of the negative control group (Table 6).

Table (6): Effect of oil and leave of olive on liver enzymes of rats suffering from acute liver disease

Parameters / Groups	AST	ALT	ALP
	U/l		
Control (-ve)	57.68^{c}± 4.938	13.67^{c}± 2.08	91.00^{e}± 6.56
Control (+ve)	115.338^{a}±11.02	38.67^{a}± 4.62	266.33^{a}± 10.97
3.5% olive oil	84.00^{b}± 7.00	17.66^{c}± 0.58	145.00^{b}± 11.00
7% olive oil	80.33^{b}± 6.11	15.00^{c}± 1.00	113.67^{cd}± 6.03
3.5% olive leave	104.00^{a}± 4.58	24.67^{b}± 3.21	115.67^{c}± 6.51
7% olive leave	82.67^{b}± 9.29	17.33^{c}± 1.53	114.00^{cd}± 8.19
3.5% olive oil and 3.5% olive leave	81.00^{b}± 8.66	15.00^{c}± 2.08	100.67^{de}± 6.03
7% olive oil and 7% olive leave	66.67^{c}± 3.51	15.67^{c}± 2.08	94.67^{e}± 4.51

- *AST: Aspartate Amino Transferase - ALT: Alanine Amino Transferase*
- *ALP: Alkaline Phosphatase ALP - Significant at p<0.05 using one-way ANOVA test.*

Values which have different letters differ significantly, while those with have similar or partially are non-significant.

All treated acute liver disease groups with olive oil, olive leave and their combination showed significant decrease p≤0.05, as compared to the positive control group. On the other hand, the mean values of serum ALT enzyme of acute liver disease groups which were treated with (3.5% olive oil, 7% olive oil, 7% olive leave , (3.5% olive oil and 3.5% olive leave) and (7% olive oil and 7% olive leave) showed non-significant changes in this parameter, as compared to the negative control group.

Injected rats with CCl4 in induced acute liver disease increased the mean value of serum ALP significantly p<0.05, as compared to healthy rats fed on normal diet (266.334 ± 10.965u/l vs. 91.00 ± 6.557u/l), respectively. The mean value of serum ALP increased in the positive control group by about 192.674%, than that of the negative control group (Table 7).

The mean values of ALP in all treated groups decreased significantly p≤0.05, as compared to the positive control group. The highest decrease in ALP recorded for the group which were treated with diet containing (7% olive oil and 7% olive leave), followed by the group treated with diet containing (3.5% olive oil and 3.5% olive leave), these treatments decreased the mean value of serum ALP by about 64.455% & 62.203%, than that of the positive control group, respectively. The data

in this Table revealed that, the group which treated with (7% olive oil and 7% olive leave) and (3.5% olive oil and 3.5% olive leave) showed non-significant differences in ALP, as compared to the negative control group.

From these results in (Table 7) we could be concluded that, CCl4 increased the mean values of serum AST, ALT and ALP. Feeding rats which were suffer from acute liver disease with diets containing 3.5% and 7% (olive oil), 3.5% and 7% (olive leave) and (3.5% olive oil + 3.5% olive leave) decreased the mean values of (AST, ALT and ALP) significantly, as compared to the positive control group.

In this respect, injected rats with CCl_4 induced hepatotoxicity and acute liver disease this treatment increased the aminotransferase and ALP activities and similar observation was found in group administrated with CCl_4 and caused significant increase in ALT and AST enzymes in Wistar rats **(Jaswal and Shukla 2015 and Wunjuntuk & Kettawan 2016)**.

Carbon tetrachloride CCl_4 treatment is frequently used in rats to produce an experimental model to study liver fibrosis **(Safer *et al.*, 2012).** The elevation serum enzymes AST, ALT and ALP levels has been attributed to the hepatocellular degeneration and impairs different enzymatic systems **(Kim *et al.*, 1990).**

Jalali *et al.*, (2017) reported that, diet containing (10 and 20% olive oil) decreased the level of ALT enzyme on the other hand the diet containing 20% olive oil reduced total serum cholesterol level in rats. **Khalil, (2004)** studied the hepatoprotective activity of an aqueous extract of olive leave s against overdose paracetamol in male albino rats. The researcher concluded that an aqueous extract of olive leave s has antioxidant property which can protect liver damage occurred by overdose paracetamol in male albino rats.

The hepatoprotective effect of aqueous extract of olive leave s may be due to its antioxidant property **(Peirce, 1999)**. The phenolic structure of olive leave extract **(Briante *et al,* 2002)** helps to reduce the free radicals which, resulted from hepatotoxin paracetamol. previous research works on olive leave extracts showed that CCl4 caused significant elevation of ALT, AST, ALP activities, while pretreatment with olive leave extracts significantly suppresses the increase in their levels **(Soliman and Soliman, 2019).**

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تأثير زيت وأوراق الزيتون على فئران التجارب المصابة بأمراض الكبد الحادة

المستخلص

هذه الدراسة تهدف إلى بحث تأثير مستويين من زيت الزيتون ، أوراق الزيتون وخليط من زيت الزيتون وأوراقه علي الفئران المصابة بأمراض الكبد الحادة. هذا بالإضافة إلى تقدير التركيب الكيمائي والمركبات الفينولية للزيت والأوراق. استخدمت في هذه الدراسة (48 فأرا) من نوع الالبينو. تم تقسيم الفئران إلى مجموعتين رئيسيتين. المجموعة الرئيسية الاولي (6 فئران) تم تغذيتها علي غذاء أساسي واستخدمت كمجموعة ضابطة سالبة (غير مصابة). المجموعة الرئيسية الثانية (42 فأرا) تم تغذيتها علي غذاء أساسي وتم حقنها بجرعة واحده أسفل الجلد بمادة رابع كلوريد الكربون المذاب في زيت البرافين (50% حجم / حجم) بجرعة 4 مللي / كيلو جرام وزن فأر وذلك لإحداث الإصابة بأمراض الكبد الحادة. تم تقدير كل من AST, ALT and ALP بعد الحقن للمجموعتين للتأكد من حدوث الإصابة. تم تقسيم فئران المجموعة الثانية المصابة إلى سبع مجموعات فرعية (كل مجموعة 6 فئران) كالتالي: **المجموعة الفرعية الأولي**: تم تغذيتها علي غذاء أساسي واستخدمت كمجموعة ضابطة إيجابية (مصابة). **المجموعات الفرعية (2 و 3):** تم تغذيتها علي غذاء يحتوى علي (3.5% و 7% زيت زيتون)، علي التوالي. **المجموعات الفرعية (4 و 5):** تم تغذيتها علي غذاء يحتوى علي (3.5% و 7% أوراق الزيتون)، علي التوالي. **المجموعة الفرعية (6):** تم تغذيتها علي غذاء يحتوى علي (3.5% زيت زيتون و 3.5% أوراق الزيتون). **المجموعة الفرعية (7):** تم تغذيتها علي غذاء يحتوى علي (7% زيت زيتون و 7% أوراق الزيتون). أشارت النتائج إلى حقن الفئران بمادة رابع كلوريد الكربون أدت إلى حدوث انخفاض في المتناول من الطعام والنسبة المئوية للزيادة في الوزن وكوليسترول الليبوبروتينات عالية الكثافة، في حين أحدثت ارتفاع في مستويات النسبة المئوية لأوزان الاعضاء و مستويات كل من (الكولسترول، الجلسريدات الثلاثية، وكوليسترول الليبوبروتينات منخفضة الكثافة و كوليسترول الليبوبروتينات منخفضة الكثافة جدًا وانزيمات الكبد مقارنة بالمجموعة الضابطة غير المحقونة (المجموعة الضابطة السالبة). معاملة المجموعات المصابة بأمراض الكبد الحادة بمستويين من زيت الزيتون ، وأوراق الزيتون والخليط منهم أدت إلى حدوث تحسن في كل التقديرات، وخاصة المجموعات التي تم معاملتها بخليط من زيت الزيتون وأوراق الزيتون (7% زيت زيتون و 7% أوراق زيتون) و (3.5% زيت زيتون و 3.5% أوراق زيتون). من النتائج السابقة يمكن استنتاج أن زيت الزيتون وأوراق الزيتون وخليطهما أدى إلى تحسين إنزيمات الكبد والمضاعفات الناتجة عن مادة رابع كلوريد الكربون.

الكلمات المفتاحية: زيت زيتون – أوراق الزيتون – أمراض الكبد الحادة – فئران – انزيمات الكبد – صورة دهون الدم