Effect of feeding olive oil combined with thyme leaves on the health status of male rats

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Abstract: The present study aimed to investigate the effect of olive oil combined with thyme leaves on the health status of male rats. Seventy five adult rats were divided into 15 groups (n=5) which fed basal diet contained extra virgin olive oil (EVOO), virgin olive oil (VOO), thyme leaves or their combination EVOO or VOO characterized with peroxide value of zero and < 0.01acidity. Body weight gain and organs relatively to body weight were determined and the data revealed an increment in weight gain due to feeding either EVOO or VOO combined with thyme. A slight increment in the organs relative ratio to body weight was noticed. Calcium and phosphorus were also determined in either blood or bone and the data revealed an improvement due to feeding either EVOO or VOO combined with thyme leaves. Bone mineral density (BMD) of femurs, body mineral concentration (BMC) and bone length (L) were determined and improved in parallel with Ca and p content. Serum lipid profile decreased significantly except that of HDL-C which increased significantly at the end of the experiment (3 months). Kidney and liver functions should a slight effect concerning kidney function which showed an increase in urea and creatinine. Regarding liver function, there are an improvement in ALT, AST and ALP. It could be concluded that olive oil either EVOO or VOO combined with thyme leaves (1 or 2.5 % w/v) improved the health status.
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1. Introduction

Olive oil is more widely used throughout the world than it ever has been, and it is increasingly being used as a substitute for other fats. Chetty *et al*. (1999) found that rats fed olive oil supplemented diet modifies iron concentration in serum and liver tissues. Gorinstein *et al*. (2002) reported that the intake of phenol rich virgin olive oil decrease total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) levels and substantially increases high density lipoprotein cholesterol (HDL-C) concentration as reported by Mangas-Cruz (2001). The hypocholesterolemic effect of olive oil is genuine and is most likely mediated through increases in bile flow and biliary cholesterol and bile acid concentration and subsequent increases in fecal excretion (Krzeminski *et al*., 2003). The biological activities of olive oil phenolic compounds have promoted several studies on their potential activities in the prevention of cardiovascular diseases and cancer. However, the increased HDL-C and decreased oxidative damage to lipids were related to the phenolic content of the extra virgin olive oil (EVOO) in a doses- dependent manner (Coras *et al*., 2006). Moreover, they found that the phenol rich virgin olive oil beneficially modulated the balance between reduced glutathione (GSH) and oxidized one (GSSG), while Weinbrenner and Colleagues (2004) found an increase in glutathione peroxidase (GSH-Px) after phenol-rich virgin olive oil administration in human subjects. Moreover, a decrease in lipoprotein oxidase (LPO) after olive oil phenolic administration has been noted. More recently olive oil phenolic compounds from olive oil mill waste water were found to increase GSH concentration in human blood (Visloli *et al*., 2009). Human, animals *in vitro* and *in vivo* studies have demonstrated that olive oil phenolic compounds have positive effect on certain physiological parameters, such as plasma lipoprotein oxidative damage, inflammatory markers and cell function, antimicrobial activity and bone (Cicerale *et al*., 2010).

Hogiwara *et al*. (2011) found that the olive oil polyphenols oleuropein and hydroxytyrosol may have critical effect on the formation and maintenance of bone, and can be used as effect remedies in the treatment of osteoporosis symptoms.

Aromatic plants have used since ancient times, in food flavouring, pharmaceutical, cosmetic and perfumery due to the presence of essential oils. Several biological activities, including antimicrobial and antioxidant properties are usually assigned to those oil or some of their constituents (Prakash, 1990). To assess whether dietary supplementation of thyme oil could adress the unfavourable antioxidant-pro-oxidant balance that occurs with age, Youdim and Deans (1999) found that there were significant declines in the superoxide dismutase activities in the

liver and heart of old rats, although kidney showed no-decline. Liver glutathione peroxidases (GSHPx) activity was found to have increased significantly in old rats, while a significant decrease was observed in kidney. Heart GSHPx activity not significantly differ between young and old rats. They also found significant declines in the total antioxidant status in each tissue examined. Ali *et al*. (2007) found that adding thyme to hen's rations significantly decreased plasma HDL, total cholesterol triglycerides and total lipids. Meanwhile, dietry thyme increased body weight and body weight gain and improved food conversion ratio of broiler chickens (El-Ghousein and Al-Beitowi, 2009). They also found that the serum level of glucose, total protein and globulin were significantly increased by addition of thyme to the diet. Serum triglycerides and cholesterol were significantly decreased.

The present study aimed to investigate the effect of olive oil combined with thyme leaves on the health status of male rats.

2. Materials and Methods

Materials:

The virgin olive oil (VOO) was obtained from privet sector (peroxide value= 0.00) and acidity < 0.01) at Sadat city, Egypt. The extra virgin olive oil (EVOO) was obtained from Food Technology Research Institute, A.R.C. Egypt. (peroxide value= 0.0 and acidity < 0.01). Thyme herbs was obtained from Horticulture Research Institute, A.R.C. Egypt. Casein, vitamins, minerals, cellulose, choline were obtained from El-Gomhoria Company, Cairo, Egypt. Starch and corn oil were purchased from local market, Giza, Egypt. Kits used to determine serum cholesterol, triglycerides, high density lipoprotein cholesterol, urea, creatinine, AST, ALT, ALP, Ca and P were purchased from Gamma Trade Company, Cairo, Egypt.

Biological experiment:

Seventy- five adult Sprague - Dawely male rats (133.6 - 138.0g) were purchased from the Lab. Of Animal Dept. Ophthalmology Res. Inst. Giza, Egypt. The animals were housed in well aerated cages under hygienic conditions (22 ±2°C and 40-60% relative humidity) and fed basal diet (AOAC 2000) for one week as adaptation period. After the adaptation period, the rats were randomly divided into 15 group (n=5) according to the following diet scheme (Table 1) for 3 months. At the end of the experiment, (3 months), rats were fasted overnight before sacrificing. Blood samples collected from eye plexuses of each rat, and centrifuged at 3000 rpm for 15 min at room temperature (22 ±2ºC) to separate the serum. Serum was carefully separated and transferred into dry clean Eppendorf tubes and kept frozen at -18°C till analysis. Another portion of blood was heparinized and digested for mineral determination. Liver, kidney heart and spleen and also femur were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline and dried using filter paper, the weighed and kept in formalin solution (10% v/v) according to Drury and Wallington (1980).

Methods:

Serum total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) were determined according to the methods described by Allian *et al*. (1974), Fossati and Principe (1982) and Burstein (1970), respectively. Meanwhile, low and very low density lipoprotein cholesterol (LDL-C and VLDL-C) were calculated according to the following equations reported by Friedwald *et al*. (1972) as follows:

$$\text{LDL-C (mg/dl)} = \text{TC} - \left(\frac{\text{TG}}{5} + \text{HDL-C}\right)$$

$$\text{VLDL-C (mg/dl)} = \frac{\text{TG}}{5}$$

Serum urea and creatinine as a kidney function were determined using the methods described by Patton and Crouch (1977) and Bohmer, (1971), respectively. Serum ALT and AST were assayed according to the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) and bilirubin were determined according to the methods of Belfied and Goldberg (1971) and Schreiber (2003), respectively. Blood Ca and P were determined according to the methods described by Dreux (1977), Gindler and King (1972) and El-Merzabani *et al* (1977), respectively. While bone Ca and P were determined after digestion according to the methods outlined in AOAC (2001). Liver, kidney, heart and spleen and femur were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline and dried using tissue paper, weighed and kept in formalin solution (10%,v/v) according to Drury and Wallington (1980). Bone length of each femur was measured using a Varnier caliper according to the method of Arjmandi *et al* (1996). Bone mineral density (BMD) was determined using Dual energy X-ray absorptiometer (Norland XR-46) as described by Blum *et al* (2003).

The statistical analysis was carried out using SPSS, PC statistical software (version 10.0 SPSS Inc, Chicago, USA). Data were analyzed by one way analysis of various (ANOVA). The differences (LSD) test at ($P \geq 0.05$) according Steel and Torri (1980).

3. Results and Discussion

Weight gain and relative ratio of organs to body weight as affected by feeding olive oil combined

with thyme leaves were recorded in Table (2). The data revealed that the initial weight of the experimental animals ranged from 133.6 to 138.0 g. At the end of the experimental, the rat's weight ranged from 305 to 400 g with weight gain ranged from 167.7 to 263.7 g and relative percentage of 121.8 to 193%. The highest relative percentage were found due to the presence of 100% EVOO + 2.5% thyme leaves followed by 100% VOO + 2.5% thyme, 50% EVOO + 1% thyme, 50% EVOO + 2.5% thyme, and 50% VOO + 2.5% thyme. Concerning the relative ratio of organs to body weight, it could be observed that a slight variation between treatments was found. In this respect, Zarchi and Babci (2006) reported that the consumption of thyme plant resulted in partial or complete anti-flatuous, anti-phlegmasia effect, in addition to regulating digestion system. Calcium and phosphorus in serum and bone were determined after 3 months of treatments (Table 3) to show the effect of olive oil combined with thyme leaves on its concentration compared with control. At zero time, calcium and phosphorus amounted in 11.11, 4.11 and 24.06 and 10.48 mg/dl for serum and bone, respectively. The data revealed that the control (basal diet containing corn oil) resulted in serum calcium and phosphorus amounted in 11.53 and 4.23 mg/dl. Feeding VOO instead of corn oil resulted in higher amounts of calcium (15.65 and 16.25 mg/dl), meanwhile EVOO showed a slight decrease than control. Feeding thyme only resulted in slight decrease than control. Feeding either VOO or EVOO combined with thyme resulted in a remarkable increase in calcium compared with control. Concerning serum phosphorus, the differences was found in the 1St or second decimal with significant differences. Similar trend was observed regarding bone minerals. Hagiwara *et al*. (2011) found that the olive polyphenols oleuropein and hydroxytyrosol may have effects on the formation and maintenance of bone, and can be used as effective remedies in the treatment of osteoporosis symptoms.

The effect of feeding olive oil combined with thyme leaves on bone mineral density (BMD), bone mineral concentration (BMC) and bone length (L) were studied for 3 months and results were recorded in Table (4). The BMD, BMC and L at zero time were 0.1012, 0.2114 and 3.25, respectively. After 3 months, the BMD of control was 0.1109 g/cm^2 which decreased to 0.1035, 0.1085, 0.1051 and 0.1092 due to feeding VOO (50%), VOO (100%), thyme (1%) and 100% VOO + 1% thyme, respectively. Meanwhile, other treatments increased BMD. The highest increase was found due to feeding 100% EVOO, 50% VOO + 1% thyme, 50% EVOO + 2.5% thyme. BMC resulted in 0.2564 mg. Treatments of 50% VOO, 1% thyme, 100% VOO + 1% thyme, and 100% EVOO + 1% thyme decreased BMC while a remarkable increase was found due to other treatments. Bone length (L) showed a slight increase as a result of all treatments except that of 50% VOO which showed a slight decrease than control. The study of Puel *et al*. (2008) revealed that both tyrosol and hydroxytyrosol increased bone formation in rats significantly.

Also, Kontogianni *et al*. (2009) concluded that adherence to a Mediterranean dietary pattern was not associated with indices of bone loss mass of adult women. Whereas, adherence to a dietary pattern close in the Mediterranean diet, i.e., high consumption of fish and olive oils and low red meat intake, was positively related to bone mass, suggesting potential bone preserving properties of this pattern through adult life.

The effect of feeding olive oil combined with thyme leaves on the serum lipid profile of male rats was studied and the results shown in Table (5). At zero time TG, TC, HDL, LDL, and VLDL amounted in 200.2, 153, 40.2, 129.6 and 30.6 mg/dl, respectively. After one month of feeding triglycerides amounted in 139.33 mg/dl. Feeding treatments resulted in triglycerides ranged from 101.67 to 137.33 mg/dl. The highest decrease was found due to 100% VOO + 2.5% thyme < 100% VOO + 1% thyme < 50% VOO + 2.5% thyme < 100% VOO. Total cholesterol showed 186.00 mg/dl for control. The most effective treatment which decrease total cholesterol was 100% VOO < 100% VOO + 2.5% thyme < 100% EVOO + 2.5% thyme. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) resulted in 114.47 and 27.87 mg/dl for control, respectively. The decreasing order showed similar trend as that of TC. An opposite trend was observed regarding high density lipoprotein cholesterol (HDL-C) which increased according the olive oil treatment. All treatments increased HDL-C by about 6.9 to 36.6%. The highest increase was found due to treatment with 100% VOO + 1% thyme. Meanwhile, a decrease in HDL-C was found due to EVOO (50 and 100%), 100% VOO + 2.5% thyme, 100% EVOO + 1% thyme, 50% VOO + 2.5% thyme and 1% thyme, respectively. After three months of feeding, a gradual decreasing in lipid profiles except that of HDL-C which showed a slight decrease than control except that of 1% thyme, 100% VOO + 1% thyme, 50% EVOO + 2.5% thyme and 100% EVOO + 1% thyme. From the aforementioned data, it could be concluded the VOO or EVOO either alone or combined with thyme (1 or 2.5%) improved the health status. Several mechanisms have been proposed to explain the preventive effects of olive oil on atherosclerosis development. Among them, the reduction of LDL susceptibility to oxidation has been

one of the main mechanism addressed. Consequences of this reduction are protection on cellular oxidation stress, thrombogenicity and atheroma plaque formation (Perona *et al*, 2006 and Visioli *et al*, 2006). These data confirmed the feeding of Mangas-cruz (2001) and Gorinstein *et al* (2002) who reported that the intake of phenol rich virgin olive oil decrease TC, LDL-C and TG levels, and substantially increase HDL-C.

Kidney and liver function were determined after one and three months of the experiment and the results were represents in Table (6). At zero time, serum, urea, creatinine and bilirubin showed a values of 24.35, 0.83 and 0.53 mg/dl, respectively. Meanwhile, AST, ALT and ALP resulted in 18.25, 20.90 and 124.0 U. After one month of feeding olive oil combined with thyme leave at different levels, the serum of control showed urea, creatinine and bilirubin amount in 24.67, 0.79 and 0.59 mg/dl, while AST, ALT and ALP showed an amount of 18.33, 21.33 and 122.67 U, respectively. Feeding olive oil combined with thyme leaves resulted in a range of 26.66 to 37.68 mg/dl except that of 50% VOO + 2.5% thyme which showed a value of 23.67 mg/dl which slightly decreased than the control. The highest amount of urea was found due to 100% EVOO followed by 2.5% thyme and 50% EVOO + 2.5% thyme. Creatinine ranged between 0.79 and 1.08 mg/dl of which 2.5% thyme resulted in the highest amounted followed by 50% EVOO + 2.5% thyme, 100% VOO + 2.5% thyme, 1% thyme, 100% EVOO, 100% VOO and 50% VOO + 1% thyme (1.08 to 1.01 mg/dl). Meanwhile, 50% VOO + 2.5% thyme showed an amount equal to the control (0.79 mg/dl). Concerning liver function, bilirubin amounted in 0.59 mg/dl. Feeding 50% EVOO + 2.5% thyme showed bilirubin value as that of control meanwhile, 1% thyme resulted in highest value (0.61 mg/dl). On the other hand, all treatments resulted in decreasing bilirubin and the highest decrease resulted due to feeding 100% EVOO followed by 2.5% thyme, 50% VOO + 2.5% thyme, 100% EVOO + 1% thyme and 100% EVOO + 2.5% thyme. AST, ALT and ALP were determined (Table 6) and the control showed a values of 18.33, 21.33 and 122.67 U, respectively. The maximum values of AST were found according the following descending order, 100% VOO + 1% thyme > 50% EVOO + 2.5% thyme > 50% VOO + 1% thyme > 50% EVOO + 1% thyme > 2.5% thyme >100% EVOO + 2.5% thyme which were highly significant than control. The minor values were found in the range of 19.67 to 16.33 U which showed non-significant differences compared with control. On the other hand, feeding 100% EVOO + 1% thyme, 50% VOO + 2.5% thyme, 1% thyme and 100% VOO resulted in significant decrease than the control (12.00 to 14.33 U). ALT showed a maximum value ranged from 20.33 to 26.00 U of which 100% VOO + 1% thyme and 50% EVOO + 2.5% thyme were the superior (26.00 U) meanwhile, 100% EVOO + 2.5% thyme were the low. On the other hand, the lowest values ranged from 10.67 U to 17.67 U. ALP increased to it maximum value due to feeding100% VOO + 1% thyme (166.0 U) followed by 1% thyme (164.67), 100% EVOO (159 U), 50% VOO + 2.5% thyme (149.66 U) and 100% EVOO + 1% thyme (140.33 U).The lowest values resulted due to feeding (100% and 50%) VOO and 50%EVOO+ 2.5% thyme, respectively. After 3 months of feeding, continuous improving were found in similar trend as that of one month feeding.

Table (1): Diet scheme of the experimental animals.

Groups	Basal diet without oil (g)	Corn oil (g)	Virgin olive oil (g)	Extra virgin olive oil (g)	Thyme leaves (g)
1	90.0	10.0	--	--	--
2	90.0	5.0	5.0	--	--
3	90.0	--	10.0	--	--
4	90.0	5.0	--	5.0	--
5	90.0	--	--	10.0	--
6	89.0	10.0	--	--	1.0
7	87.5	10.0	--	--	2.5
8	89.0	5.0	5.0	--	1.0
9	87.5	5.0	5.0	--	2.5
10	89.0	--	10.0	--	1.0
11	87.5	--	10.0	--	2.5
12	89.0	5.0	--	5.0	1.0
13	87.5	5.0	--	5.0	2.5
14	89.0	--	--	10.0	1.0
15	87.5	--	--	10.0	2.5

Youdin and Deans (1999) assess whether dietary supplementation of thyme oil could address the unfavourable antioxidant-pro-oxidant balance that occurs with age. They found that there were significant decline in the superoxide dismutase activity in the liver and heart of old rats, although kidney showed no-decline. Liver glutathione peroxidases (GSHPX) activity was found to have increased significantly in old rats, while a significant decrease was observed in kidney. There was also significant declines in the total antioxidants status in each tissue examined. A general feature of these various antioxidants parameters measured that their activities remained higher in rats whose diets were supplemented with thyme oil, suggesting that they retained a more favorable antioxidant capacity during their life span.

Nakabi *et al.* (2012) concluded that EVOO may be a potential functional food source of antioxidants can decrease the frequency of cardiovascular disease and liver damage.

Table (2): Body weight gain percentage and relative ratio of organs to body of experimental rats as affected by feeding olive oil combined with thyme leaves.

Treatment		Body weight (g)				Organs relative ratio %			
		Initial	End	Gain	Gain %	Heart	Liver	Kidney	Spleen
1	Control	137.3^{a}	321.3^{b}	184.0^{f}	134.0	0.26	2.49	0.45	0.23
2	50% Virgin Olive Oil (VOO)	138.0^{a}	324.0^{b}	186.0^{f}	134.7	0.34	3.46	0.58	0.32
3	100% Virgin Olive Oil (VOO)	138.0^{a}	359.3^{cd}	221.3^{dc}	160.4	0.34	3.48	0.51	0.32
4	50% Extra Virgin Olive Oil (EVOO)	137.6^{a}	305.3^{a}	167.7^{g}	121.8	0.28	2.46	0.67	0.35
5	100% Extra Virgin Olive Oil (EVOO)	135.3^{a}	354.7^{cd}	219.4^{dc}	162.2	0.23	3.06	0.51	0.36
6	1%Thyme	137.0^{a}	305.0^{a}	168.0^{g}	122.6	0.26	3.09	0.60	0.32
7	2.5%Thyme	137.0^{a}	349.3^{c}	212.3^{c}	154.9	0.31	3.11	0.53	0.35
8	50% VOO+1%Thyme	136.0^{a}	354.3^{c}	218.3^{de}	160.5	0.36	3.54	0.61	0.27
9	50% VOO+2.5%Thyme	136.0^{a}	361.6^{cd}	225.0^{cd}	165.4	0.23	2.67	0.51	0.29
10	100% VOO+1%Thyme	133.6^{a}	320.7^{b}	187.3^{f}	140.2	0.32	3.05	0.51	0.29
11	100% VOO+2.5%Thyme	136.6^{a}	380.6^{d}	244.0^{b}	178.6	0.28	2.54	0.57	0.30
12	50% EVOO + 1%Thyme	137.3^{a}	372.3^{d}	235.0^{bc}	171.2	0.31	2.14	0.56	0.20
13	50%EVOO + 2.5%Thyme	136.7^{a}	362.3^{cd}	225.6^{cd}	165.0	0.32	2.78	0.54	0.31
14	100% EVOO + 1%Thyme	136.3^{a}	322.0^{b}	185.7^{f}	136.2	0.26	3.04	0.57	0.20
15	100% EVOO + 2.5%Thyme	136.3^{a}	400.0^{e}	263.7^{a}	193.5	0.26	2.70	0.49	0.26

Each value in a column followed by the same letter are not significantly different at p≤0.05.

Table (3):Some blood and bone minerals of the experimental rats affected by feeding olive oil combined with thyme leaves.

Groups		Blood		Bone	
		Ca	P	Ca	P
1	Control	11.53^{h}	4.23^{ef}	24.23^{g}	10.51^{j}
2	50% Virgin Olive Oil	15.65^{d}	4.91^{a}	26.47^{b}	13.87^{a}
3	100% Virgin Olive Oil	16.25^{c}	4.07^{g}	24.78^{f}	12.52^{f}
4	50% Extra Virgin Olive Oil	11.13^{i}	4.10^{ab}	27.97^{a}	14.25^{a}
5	100% Extra Virgin Olive Oil	12.96^{fg}	4.24^{def}	25.12^{e}	13.73^{bc}
6	1%Thyme	10.73^{j}	4.21^{efg}	22.48^{i}	12.15^{g}
7	2.5%Thyme	11.40^{hi}	4.37^{cd}	25.47^{d}	13.64^{bc}
8	50% VOO+1%Thyme	13.32^{f}	4.18^{efg}	25.04^{e}	13.52^{c}
9	50% VOO+2.5%Thyme	15.60^{d}	4.17^{efg}	23.47^{h}	12.77^{e}
10	100% VOO+1%Thyme	17.91^{b}	4.21^{efg}	25.70^{c}	12.38^{fg}
11	100% VOO+2.5%Thyme	19.41^{a}	4.52^{b}	22.48^{i}	11.33^{i}
12	50% EVOO + 1%Thyme	12.66^{g}	4.46^{bc}	21.40^{i}	10.40^{i}
13	50% EVOO + 2.5%Thyme	15.63^{d}	4.25^{de}	25.51^{c}	13.62^{bc}
14	100% EVOO + 1%Thyme	11.57^{h}	4.18^{efg}	24.71^{f}	11.67^{h}
15	100% EVOO + 2.5%Thyme	13.73^{e}	4.55^{b}	25.06^{e}	14.11^{a}

Each value in a column followed by the same letter are not significantly different at $p \leq 0.05$.
Control at zero time for Ca and P were (11.11 and 4.11mg/dl) in blood and (24.06 and 10.48 mg/dl) in bone, respectively.

Table (4): Effect of fed on olive oils combine with thyme on bone measurements (X-ray and length) for male rats.

Groups	BMD[1] (g/cm^2)	BMC[2]	L (mm)
1 Control	0.1109	0.2564	3.34
2 50% Virgin Olive Oil	0.1035	0.2125	3.12
3 100% Virgin Olive Oil	0.1085	0.2467	3.40
4 50% Extra Virgin Olive Oil	0.1122	0.2629	3.43
5 100% Extra Virgin Olive Oil	0.1252	0.3088	3.56
6 1%Thyme	0.1051	0.2179	3.41
7 2.5%Thyme	0.1138	0.2796	3.40
8 50% VOO+1%Thyme	0.1216	0.2849	3.71
9 50% VOO+2.5%Thyme	0.1167	0.2797	3.50
10 100% VOO+1%Thyme	0.1092	0.2185	3.46
11 100% VOO+2.5%Thyme	0.1148	0.2697	3.76
12 50% EVOO + 1%Thyme	0.1144	0.2760	3.50
13 50% EVOO + 2.5%Thyme	0.1252	0.3330	3.71
14 100% EVOO + 1%Thyme	0.1113	0.1981	3.57
15 100% EVOO + 2.5%Thyme	0.1186	0.2894	3.59

Each value in a column followed by the same letter are not significantly different at $p \leq 0.05$.
"BMD": bone mineral density of the femurs (g/cm^2 bone vol.). "BMC": bone mineral concentration. "L": bone length.

Control at zero time for BMD,BMC and L were 6.1012, 0.2114 and 3.25, respectively.

Table (5): Effect of fed on olive oils combined with thyme for nutritional period on lipid profile for albino male rats

Groups	A month later					Three months later				
	T. C. mg/dl	T.G. mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	T. C. mg/dl	T.G. mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
1 Control	186.00^{f}	139.33^{j}	43.67^{f}	114.47^{g}	27.87^{j}	155.67^{j}	125.67^{h}	47.33^{cd}	84.20^{h}	25.20^{j}
2 50% Virgin Olive Oil (VOO)	167.00^{cd}	116.33^{def}	49.00^{de}	94.73^{cd}	23.27^{def}	143.67^{g}	107.67^{d}	45.00^{ef}	76.20^{g}	21.47^{e}
3 100% Virgin Olive Oil (VOO)	151.00^{a}	110.33^{bc}	53.00^{bc}	75.93^{a}	22.07^{bc}	143.83^{g}	112.53^{ef}	44.67^{efg}	75.37^{fg}	22.80^{g}
4 50% Extra Virgin Olive Oil (EVOO)	171.33^{d}	128.67^{i}	37.00^{i}	108.60^{f}	25.73^{i}	128.00^{b}	97.33^{b}	42.67^{gh}	65.47^{bc}	19.53^{b}
5 100% Extra Virgin Olive Oil (EVOO)	160.33^{b}	121.00^{gh}	37.33^{hi}	98.80^{de}	24.20^{gh}	140.33^{f}	115.00^{f}	41.33^{h}	71.60^{def}	23.40^{h}
6 1%Thyme	167.32^{d}	137.33^{j}	41.33^{fg}	98.53^{de}	27.47^{j}	143.33^{g}	103.33^{c}	52.33^{a}	69.13^{cd}	20.87^{d}
7 2.5%Thyme	158.67^{b}	115.67^{def}	46.67^{e}	88.87^{b}	23.13^{def}	130.00^{c}	97.67^{b}	47.67^{cd}	63.27^{b}	19.73^{b}
8 50% VOO+1%Thyme	168.33^{d}	113.00^{cd}	54.33^{b}	91.40^{bc}	22.60^{cd}	160.00^{k}	120.00^{g}	39.00^{i}	97.27^{i}	24.07^{i}
9 50% VOO+2.5%Thyme	160.33^{b}	107.33^{b}	41.00^{fg}	97.87^{de}	21.47^{b}	119.83^{a}	94.67^{a}	43.33^{fgh}	57.33^{a}	19.00^{a}
10 100% VOO+1%Thyme	162.33^{bc}	106.00^{b}	59.67^{a}	81.47^{a}	21.20^{b}	132.50^{d}	125.00^{h}	53.67^{a}	53.60^{a}	25.07^{j}
11 100% VOO+2.5%Thyme	152.33^{a}	101.67^{a}	40.00^{gh}	92.00^{bc}	20.33^{a}	147.67^{h}	101.67^{c}	49.00^{bc}	78.33^{g}	20.33^{c}
12 50% (EVOO) + 1%Thyme	177.00^{e}	124.33^{h}	52.67^{bc}	99.47^{de}	24.87^{h}	135.00^{e}	107.67^{d}	38.00^{i}	74.93^{efg}	21.73^{e}
13 50% (EVOO) + 2.5%Thyme	159.00^{b}	119.67^{fg}	57.67^{a}	77.40^{a}	23.93^{fg}	152.50^{i}	111.67^{e}	53.00^{a}	76.93^{g}	22.40^{f}
14 100% (EVOO) + 1%Thyme	167.00^{cd}	118.33^{efg}	40.33^{g}	103.00^{e}	23.67^{efg}	150.67^{i}	114.67^{f}	50.33^{b}	77.47^{g}	22.87^{g}
15 100% (EVOO) + 2.5%Thyme	153.67^{a}	114.33^{cde}	50.33^{cd}	80.47^{a}	22.87^{cde}	140.33^{f}	114.67^{f}	46.67^{de}	70.87^{de}	23.13^{gh}

Each value in a column followed by the same letter are not significantly different at $p \leq 0.05$.
Control at zero time for TG, TC, HDL, LDL and VLDL were 200.2, 153, 40.2, 129.6 and 30.6 mg/dl, respectively.

Table (6): Effect of fed on olive oils combined with thyme for nutritional period on liver and kidney for albino male rats

Groups	A month later						Three months later					
	Kidney function		Liver function				Kidney function		Liver function			
	Urea mg/dl	Creat. mg/dl	Bil. mg/dl	AST U.	ALT U.	ALP U.	Urea mg/dl	Creat. mg/dl	Bil. mg/dl	AST U.	ALT U.	ALP U.
1 control	24.67^{ab}	0.79^{a}	0.59^{f}	18.33^{ef}	21.33^{c}	122.67^{g}	17.27^{a}	0.52^{a}	0.71^{b}	22.50^{b}	22.23^{a} b	140.00^{a}
2 50% Virgin Olive Oil (VOO)	27.66^{cd}	0.89^{ab} c	0.43^{d}	17.33^{ef}	16.00^{e}	96.67^{j}	26.00^{h}	0.93^{e}	0.84^{c}	19.83^{cd}	21.50^{b} c	106.00^{g}
3 100% Virgin Olive Oil (VOO)	29.00^{cd} e	1.02^{bc} d	0.51^{e}	12.00^{h}	10.67^{g}	99.33^{j}	17.50^{a}	0.63^{b}	0.70^{b}	18.60^{de} f	16.93^{e}	98.00^{i}
4 50% Extra Virgin Olive Oil (EVOO)	26.66^{bc}	0.90^{ab} c	0.50^{e}	16.33^{fg}	18.00^{d}	115.33^{h}	19.67^{bc}	0.71^{c}	0.81^{c}	20.53^{c}	18.03^{e}	105.50^{g}
5 100% Extra Virgin Olive Oil (EVOO)	37.68^{h}	1.03^{cd}	0.31^{a}	18.67^{ef}	17.67^{d} e	159.00^{b}	18.50^{ab}	0.63^{b}	0.72^{b}	19.00^{de}	19.93^{d}	101.67^{h}
6 1%Thyme	29.00^{cd} e	1.03^{cd}	0.61^{f}	14.33^{g} h	13.33^{f}	164.67^{a}	18.50^{ab}	0.73^{c}	0.86^{c} d	17.33^{fg} h	14.67^{f}	126.00^{c}
7 2.5%Thyme	34.67^{g}	1.08^{e}	0.33^{ab}	22.67^{c}	24.67^{a} b	123.67^{f} g	24.00^{g}	0.83^{d}	0.91^{e}	17.67^{ef} g	15.33^{f}	131.67^{b}
8 50% VOO+1%Thyme	29.33^{de}	1.01^{bc} d	0.54^{ef}	25.66^{b}	25.00^{a} b	130.33^{e}	17.67^{a}	0.64^{b}	0.72^{b}	25.00^{a}	23.00^{a}	118.00^{e}
9 50% VOO+2.5%Thyme	23.67^{a}	0.79^{a}	0.33^{ab} c	14.00^{g} h	12.67^{f}	149.66^{c}	21.67^{ef}	0.84^{d}	0.92^{e}	20.00^{cd}	18.00^{e}	95.00^{j}
10 100% VOO+1%Thyme	30.68^{ef}	0.99^{bc} d	0.54^{ef}	31.00^{a}	26.00^{a}	166.00^{a}	24.00^{g}	0.83^{d}	0.90^{d} e	16.67^{gh}	17.50^{e}	130.00^{b}
11 100% VOO+2.5%Thyme	29.00^{cd} e	1.03^{cd}	0.57^{ef}	19.67^{d} e	20.67^{c}	109.33^{i}	21.00^{cd} e	0.75^{c}	0.84^{c}	22.00^{b}	20.00^{d}	112.00^{f}
12 50% (EVOO)+ 1%Thyme	31.67^{f}	0.95^{bc} d	0.51^{e}	23.00^{c}	23.33^{b}	105.67^{i}	26.00^{h}	0.92^{e}	0.71^{b}	16.00^{h}	14.33^{f}	119.00^{d} e
13 50% (EVOO)+ 2.5%Thyme	32.33^{f}	1.05^{d}	0.59^{f}	26.67^{b}	26.00^{a}	89.33^{k}	21.33^{def}	0.73^{c}	0.64^{a}	19.00^{de}	20.67^{c} d	131.00^{b}
14 100% (EVOO)+1%Thyme	29.00^{cd} e	0.89^{ab}	0.39^{bc} d	14.33^{g} h	13.33^{f}	140.33^{d}	22.67^{fg}	0.84^{d}	0.62^{a}	23.00^{b}	22.67^{a} b	120.00^{d}
15 100% (EVOO) +2.5%Thyme	28.33^{cd} e	0.96^{bc} d	0.40^{cd}	21.00^{c} d	20.33^{c}	127.00^{e} f	20.00^{cd}	0.73^{c}	0.64^{a}	19.00^{de}	20.67^{c} d	131.00^{b}

Each value in a column followed by the same letter are not significantly different at $p \leq 0.05$.
Control at zero time for Urea, Creat., Bil., AST, ALT, and ALP were 24.35, 0.83, 0.53, 18.25, 20.90, and 124.00, respectively.

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