

Effect of effective dose (ED50) of olive oil on heart and liver parameters in normal New-Zealand rabbits.

الجرعة المؤثرة لزيت الزيتون في بعض معايير القياسية للقلب والكبد في الأرانب النيوزلندية السليمة

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Summary

This study was carried out to investigate the effect of effective dose (ED50) of olive oil on some parameters related to cardiac and hepatic functions in normal rabbits .The effective dose (ED50) of olive oil was determined in this study by studying the dose response curve . Twenty five adult male rabbits were divided randomly into five equal groups (5/group) and were intubated orally for four weeks with four successive increasing daily doses of olive oil (0.2 , 0.5 , 0.75 and 1 ml/kg B.W) . Fasting blood samples were collected of 7,14,21 and 28 days of experiment to study the following parameters : Serum total cholesterol (TC) , Triacylglycerol (TAG) , high density lipoprotein (HDL-C) and reduced glutathione (GSH) concentrations and Aspartate Transaminase (AST) activity .

The results revealed that oral intubation of male rabbits with different doses of olive oil for four weeks caused significant ($p<0.01$) decrease in (TC) and TAG) concentrations and (AST) activity with significant ($p<0.01$) increase in GSH and HDL-C concentrations .The (ED50) of olive oil was found to equal to 0.51 ml/kg B.W.

الخلاصة

شملت الدراسة الحالية لمعرفة تأثير قياس الجرعة المؤثرة (ED50) لزيت الزيتون على بعض المعايير المتعلقة بوظيفة القلب والكبد من خلال دراسة منحنى الجرعة المؤثرة حيث تم استخدام (25) من ذكور الأرانب البالغة والتي قسمت عشوائياً إلى خمس مجاميع متساوية (خمس حيوانات /مجموعة) و جرعت فمويماً أربعة جرعات متزايدة من زيت الزيتون يومياً (0.2, 0.5, 0.75, 1 مل/كغم.و.ج) ولمدة أربعة أسابيع. جمعت عينات الدم في الأيام (7,14,21,28) من التجربة لدراسة المعايير التالية : تركيز الكولسترول الكلي (TC) , الشحوم البروتينية ذات الكثافة العالية (HDL-C) , ثلاثي اسيل الكليسيرول (TAG) , والكلوتاثيون المختزل (GSH) وكذلك قياس فعالية الأنزيم الناقل للامين (AST) في مصل الدم. اشارت الدراسة الحالية الى ان اعطاء جرعة مختلفة من زيت الزيتون لمدة اربع اسابيع تسبب في حدوث انخفاض معنوي ($p<0.01$) في تركيز الكولسترول الكلي (TC) و ثلاثي اسيل الكليسيرول (TAG) وفعالية انزيم (AST) مع زيادة معنوية ($p<0.01$) في تركيز كولستيرول الشحوم البروتينية ذات الكثافة العالية (HDL-C) وتركيز الكلوتاثيون المختزل (GSH) , وكانت الجرعة المؤثرة لزيت الزيتون تساوي (0.51 مل /كغم.و.ج) .

Introduction

Olive oil is a complex compound made of fatty acid , vitamin , volatile components and water soluble components (1,2) , Primary fatty acids of olive oil are oleic and linoleic acid with small amount of linolenic acid (3,4) . Tocopherols are normally present in olive oil in the forms α , β and γ where α is the most abundant 90-95%. Tocopherols carry out an anti oxidant action in oils exposed to light especially ultra violet radiation (5). Each table spoon of olive oil contain 1.6 mg or 2.3 IU of vitamin E(6) In addition to vitamin E ,olive oil contains many antioxidants such as vitamins , A, K and phenols(7,8) .Research has proved that only breast milk can be more easily digested than olive oil by the human body (9).

Phenolic compounds of olive oil are responsible for the particular or stability of the oil (10,11). The most abundant phenolic compound in the drupe is oleuropein, a bitter glycoside that constitutes up to 14% of the fruit's dry weight.(12).Olive oil has beneficial effect on gastritis, ulcers and gall stones also helps the body to absorb calcium (13).

The research cited by thesis of the first researcher

Studies on olive oil and atherosclerosis reveal that particles of LDL-cholesterol (the potentially harmful cholesterol) that contain the monounsaturated fats of oil are less likely to become oxidized (14). A recent in vitro study, also showed that polyphenolic compound present in olive oil including oleuropein inhibit the adhesion of monocytes cells to the blood vessel lining a process that is involved in the development of atherosclerosis (15). where the antioxidants properties of olive oil may be one of the key reason for the lowering incidence of cancer in the Mediterranean region (16,17).

Olive is good source of edible oil and is also used for table particularly for pickles. In addition to it is nutritional and medicinal value, its fat content is also free from cholesterol (18).

This study aimed to determination of the effective dose of olive oil on some parameters related to cardiac and hepatic function was achieved in this experiment.

Material and methods

Different doses (more or less than the recommended one) of olive oil were intubated to male rabbits by using gavage needle. Five groups of rabbits (each of five) were randomly divided and were handled as follows for four weeks :- Group G (rabbits of this group were intubated ordinary tap water and served as control), Group GI (animals in this group were intubated 0.2ml /kg B.W of olive oil). Group GII (rabbits of this group were intubated 0.5 ml/kg B.W of olive oil). Group GIII (animals in this group were intubated 0.75 ml/kg B.W of olive oil). Group GIV (animals in this group were intubated 1ml/kg B.W of olive oil). Fasting blood samples were collected weekly via cardiac puncture technique along the experimental period (4weeks) for measuring the following parameters:- Total serum cholesterol (TC) concentration, serum Triacylglycerol (TAG) concentration, serum High Density Lipoprotein Cholesterol (HDL-C) concentration using enzymatic assay kits (Biocon, Germany) and serum Glutathione (GSH) concentration using standard curve according to (19) and serum Aspartate Transaminase (AST) activity by using enzymatic assay kit (Rendox company, Switzerland).

Statistical analysis of data of present study was performed on the basis of two way analysis of variance (ANOVA) using significant level of ($P < 0.01$). Differences were determined using least significant differences (LSD) (20)

Then from the dose response curve, the ED₅₀ of olive oil were determined and their arithmetic means were computed.

Results

The effective dose which produce half of the maximum response (ED₅₀) was measured by studying the dose response curve. Four successive increasing doses of olive oil were given to rabbits to study its effects on serum total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), Aspartate transaminase (AST) and Glutathione (GSH) concentrations. These parameters have been used as a guide for the detection of the (ED₅₀) of olive oil as follows :- The effect of different doses of olive oil on mean values of serum TC (mg/dl) concentration of male rabbits were shown in table (1). With exception of group GIV, Statistical difference ($p > 0.05$) were absent between treated groups in the first two weeks comparing to control, however, oral intubation of 0.5, 0.75, and 1ml of olive oil /kg to male rabbits caused a significant depression in total serum TC concentration in groups GII, GIII, and GIV comparing to other treated groups and control. Such reduction were started at the third week of treatment till the end of experiment which lasted for four weeks.

Highest reduction in this parameter were observed in GIV group at the end of the third week of the experiment (97.8 ± 8.1) comparing to control (130 ± 5.2).

Different concentration of olive oil caused significant depression in serum TAG concentration of male rabbits at different intervals (table 2). Such decrement was started at the end of the 2nd week in groups GII and GIV which received 0.75 and 1 ml of olive oil respectively. Highest significant reduction ($P < 0.05$) were observed in group of rabbits received 1ml of olive oil at the end of the third week of the experiment comparing to other olive oil treated group and control

Serum HDL –C concentration in the control and olive oil treated rabbits were present in table (3) .The results showed that different doses of olive oil caused a significant increase ($p<0.05$) in the concentration of serum HDL-C in all treated groups as compared to the control group . Such increment began from first weeks of the treatment in groups GII,GIII, and GIV which received 0.5, 0.75 , and 1ml of olive oil respectively , and continued till the end of the experiment , while animal received 0.2ml of olive oil (group GI) showed significant increase ($P<0.05$) in serum HDL-C concentration at the beginning of the third week till the end of the four weeks of the experiment , when compared to control .

Within the time , significant increase ($p<0.05$) in this parameter were observed in all treated groups (at different intervals) comparing to the pretreated period .

The data pertaining to the serum GSH concentration ($\mu\text{mol/l}$) of control and olive oil treated groups were depicted in the table (4) . Comparing to control different doses of olive oil caused significant increase ($p<0.05$) in serum GSH concentration $\mu\text{mol/l}$ at different intervals , highest increment in serum GSH concentration were observed at the third week of the experimental in animal received 1ml of olive oil (group GIV) , the mean value of serum GSH concentrations of group GIV was (49.6 ± 1.6) comparing to mean value of the control (22.0 ± 0.6) .

Serum AST activity in the control and olive oil treated rabbits were present in table (5) .The result showed that different doses of olive oil caused a significant decrease ($P<0.05$) in the activity of serum AST in all treated groups at different intervals as compared to the control group . Such decrement was significant from the beginning of the experiment in groups received 0.5, 0.75, and 1ml of olive oil till the end of the experiment .

Depending on the results shown in tables (1,2,3,4 and 5),a maximal significant changes(suppression or elevation) in the previous parameters were recorded after three weeks of olive oil intubation . Accordingly , results obtained at week three were used for the estimation of(ED_{50}) of olive oil , from the equation of straight line, the(ED_{50}) of olive oil that cause marked depression in TC concentration was found to be equal to (0.37ml) figure (1) , while from figure 2,3,4,and 5 the estimated (ED_{50}) of TAG , HDL-C , GSH concentration and ASR activity were equal to 0.56, 0.53,0.58 and 0.51ml/kg B.W. respectively .

To determine the ED_{50} of olive oil which will be used in the succeeding experiment , the arithmetic mean of ED_{50} of TC, TAG, HDL-C, GSH , and AST values were computed . It was found to be equal to (0.51 ml) . N.B the log dose mentioned in figures (1,2,3,4and 5) where obtained by multiplying different doses of olive oil by 1000 to avoid negative results . e. g 1ml of olive oil \times 1000 = 1000 . $\log 1000 = 3$.

Table(1) Serum Total Cholesterol (TC)concentration (mg/dl) in adult male rabbits treated with different doses of olive oil for four weeks.

Dose	G		GI		GII		GIII		GIV	
	Control	0.2	ml/kg	0.5ml/kg	0.75ml/kg	m/kg	B.W	B.W	B.W	B.W
Week			B.W	B.W	B.W	B.W	B.W	B.W	B.W	B.W
1	140		139	134	133	110				
	±8.8		±7.0	±2.8	±1.8	±21.2				
	A	a	A	a	A	a	B	a		
2	131		130.8	125.8	124.8	108.6				
	±6.9		±11.4	±1.4	±2.4	±10.7				
	A	A	A	ab	A	a	B	a		
3	130		125.8	120.4	106	97.8				
	±5.2		±3.3	±2.6	±6.4	±8.1				
	A	a	AB	b	B	b	C	b	C	b
4	129		126.8	123.8	108	103.6				
	±4.8		±9.1	±3.8	±8.1	±11.0				
	A	a	A	b	A	b	B	b	B	ab

Values are expressed as mean ± SE .n= 5/ group. Small letters denote within group difference p<0.05.Capital letters denote between groups difference p<0.05.

Table (2) Serum Triacylglycerol (TAG)concentration(mg/dl) in adult male rabbits treated with different doses of olive oil for four weeks.

Dose	G		GI		GII		GIII		GIV	
	Control	0.2	ml/kg	0.5ml/kg	0.75ml/kg	1m/kg	B.W	B.W	B.W	B.W
Week			B.W	B.W	B.W	B.W	B.W	B.W	B.W	B.W
1	132.8		130.2	130.0	129.0	127.0				
	±5.1		±2.9	±3.9	±4.3	±3.3				
	A	a	A	a	A	a	A	a	A	a
2	131.6		129.8	128.8	127.2	125.8				
	±4.3		±3.2	±6.0	±3.7	±5.0				
	A	a	AB	ac	AB	a	B	a	B	a
3	130.0		124.8	120.4	118.4	114.8				
	±6.1		±3.8	±3.1	±5.3	±9.3				
	A	a	B	b	BC	b	CD	b	D	b
4	129.2		125.8	121.4	120.0	118.8				
	±4.3		±4.6	±6.1	±6.1	±10.1				
	A	a	B	bc	C	b	C	b	C	b

Values are expressed as mean ± SE .n= 5/ group. Small letters denote within group difference p<0.05.Capital letters denote between groups difference p<0.05.

Table (3) Serum High Density Lipoprotein -Cholesterol(HDL-C)(mg/l) in male rabbits treated with different doses of olive oil for four weeks.

Dose Week	G	GI	GII	GIII	GIV
	Control	0.2 ml/kg B.W	0.5ml/kg B.W	0.75ml/kg B.W	1m/kg B.W
1	37.4 ±1.2 A a	42.00 ±2.0 AB a	45.40 ±1.3 BC a	48.00 ±1.0 BC a	51.80 ±2.2 C a
2	38.6 ±1.6 A a	43.60 ±1.8 A a	51.40 ±1.5 B ab	54.80 ±2.0 B ac	55.60 ±2.3 B a
3	38.4 ±1.1 A a	51.80 ±2.2 B b	58.28 ±2.1 BC b	62.20 ±2.7 CD b	67.80 ±3.1 D b
4	36.82 ±1.0 A a	49.01 ±1.9 B ab	55.62 ±1.8 BC b	60.00 ±3.0 CD bc	62.43 ±3.0 D b

Values are expressed as mean ± SE .n= 5/ group. Small letters denote within group difference p<0.05.Capital letters denote between groups difference p<0.05

Table (4) Serum Reduced glutathione (GSH) concentration (µmol/L) in male rabbits treated with different doses of olive oil for four weeks.

Dose Week	G	GI	GII	GIII	GIV
	Control	0.2ml/kg B.W	0.5ml/kg B.W	0.75ml/kg B.W	1ml/kg B.W
1	20.0 ±0.5 A a	21.0 ±0.3 A a	22.8 ±1.0 AB a	25.8 ±0.4 BC a	27.0 ±1.0 C a
2	21.4 ±0.3 A a	22.4 ±0.5 A ac	24.4 ±0.8 AB a	24.4 ±0.3 AB a	27.6 ±0.8 B a
3	22.0 ±0.6 A a	30.2 ±0.4 B b	41.2 ±0.9 C b	47.2 ±1.2 D b	49.6 ±1.6 D b
4	22.4 ±0.4 A b	25.6 ±0.6 A c	38.2 ±0.6 BC b	38.0 ±1.4 BC c	40.4 ±1.4 C c

Values are expressed as mean ± SE .n= 5/ group. Small letters denote within group difference p<0.05.Capital letters denote between groups difference p<0.05.

Table (5) Serum Aspartate transaminase (AST)concentration (IU/L) in male rabbits treated with different doses of olive oil for four weeks

Dose Week	G Control	GI 0.2ml/kg B.W	GII 0.5ml/kg B.W	GIII 0.75ml/kg B.W	GIV 1m/kg B.W
1	54.0 ±1.3 A a	50.0 ±0.8 A a	47.8 ±1.0 A a	47.0 ±0.9 A a	37.4 ±1.4 B a
2	52.20 ±1.6 A a	48.6 ±0.7 AB ab	46.6 ±1.1 B a	45.6 ±1.7 B ac	36.3 ±0.6 C a
3	50.60 ±0.9 A a	44.4 ±0.6 B b	42.6 ±0.8 B a	39.4 ±0.8 BC b	35.8 ±1.3 C a
4	50.00 ±1.0 A a	47.0 ±0.6 A ab	46.8 ±1.2 A a	40.4 ±0.9 BC bc	37.4 ±1.2 C a

Values are expressed as mean ± SE .n= 5/ group. Small letters denote within group difference p<0.05.Capital letters denote between groups difference p<0.05.

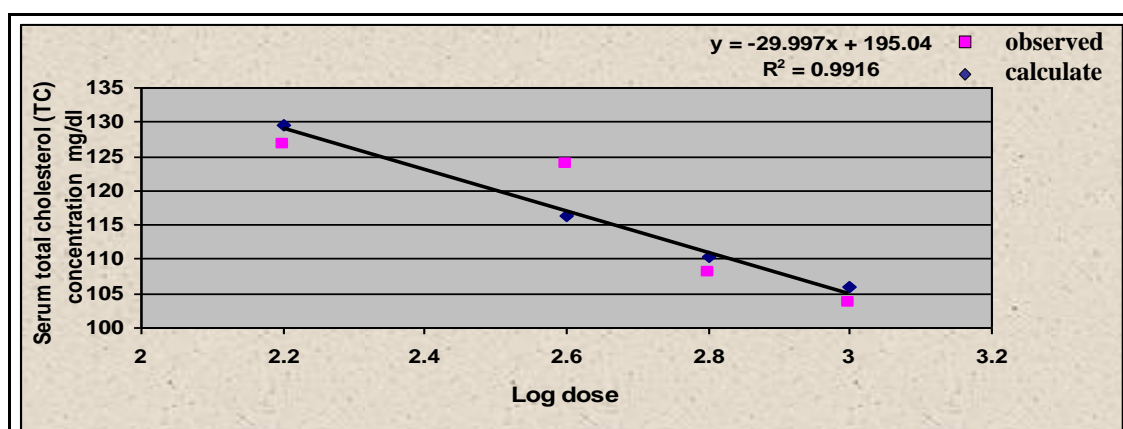


Figure (1) Effect of different doses of olive oil on serum total cholesterol(TC) concentration (mg/dl) after 4weeks of treatment in adult male rabbits . n=5, ED₅₀ = 0.37 ml/kg.

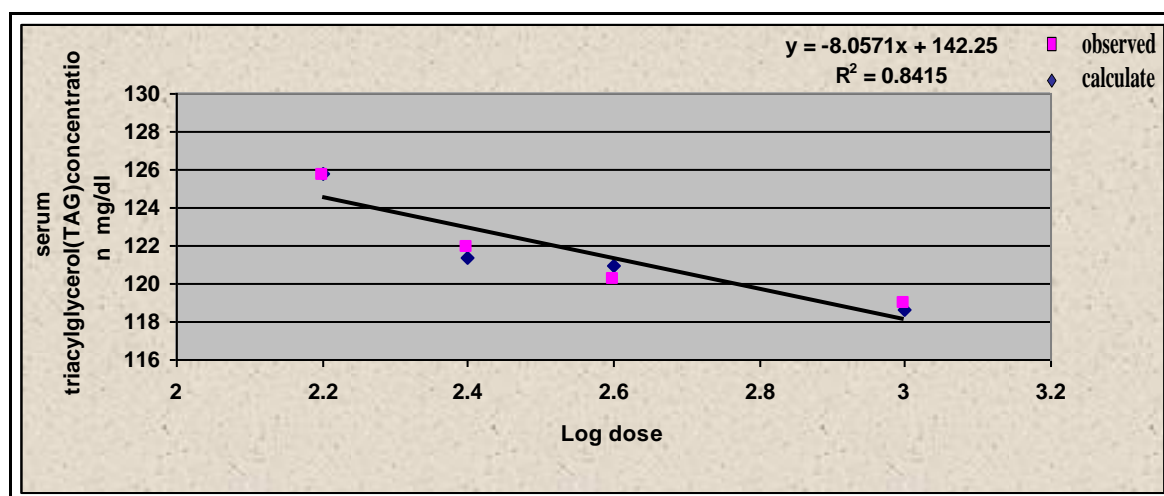


Figure (2) Effect of different doses of olive oil on serum triacylglycerol (TAG)concentration (n mg/dl) after 4weeks of treatment in adult male rabbits. N=5, ED₅₀=0.56 ml/kg

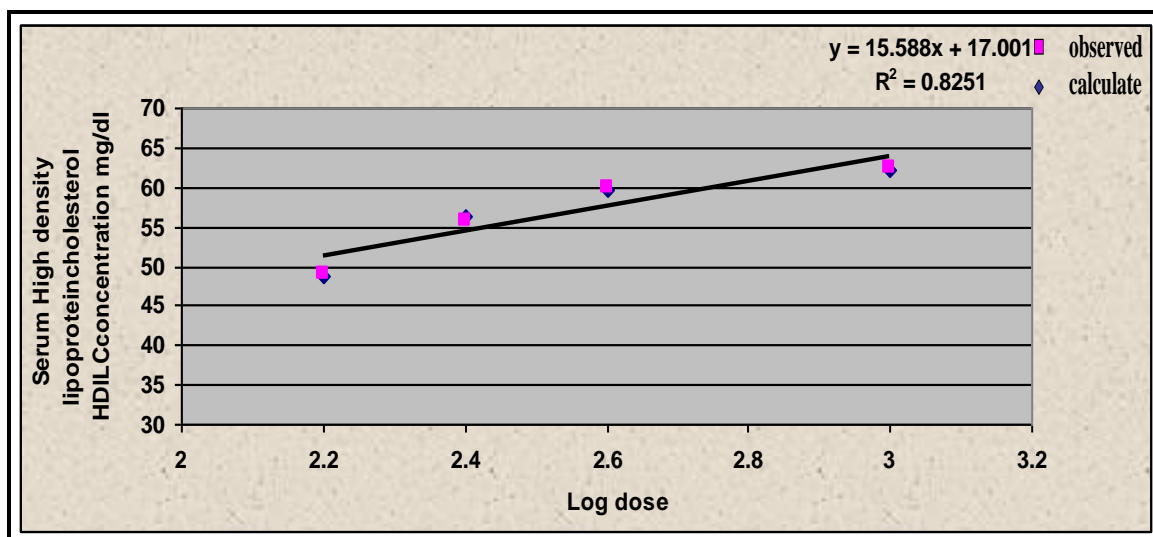


Figure (3) Effect of different doses of olive oil on serum high density lipoprotein(HDL-C)concentration (mg/dl) after 4weeks of treatment in adult male rabbits. N=5, ED₅₀=0.53ml/kg .

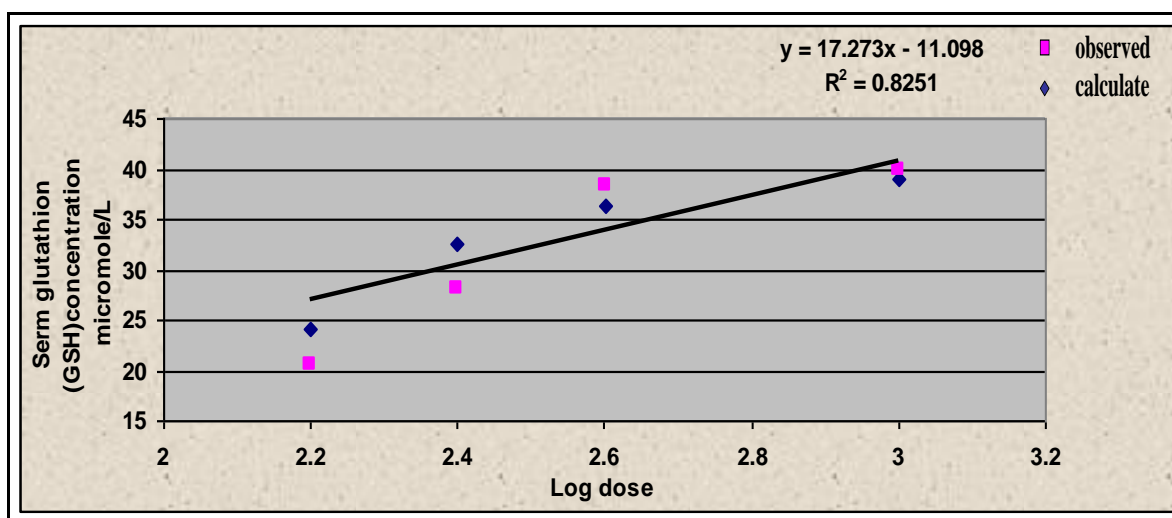


Figure (4) Effect of different doses of olive oil on serum glutathione (GSH)concentration (μmol/L) after 4 weeks of treatment in adult male rabbits. n=5, ED₅₀=0.58ml/kg.

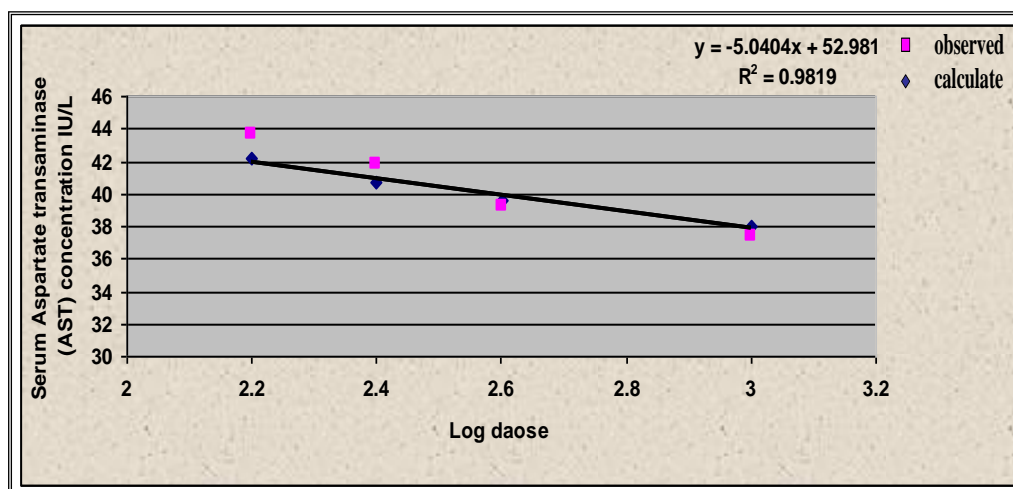


Figure (5) Effect of different doses of olive oil on serum AST activity IU/L after 4 weeks of treatment in rabbit. N=5 ED₅₀= 0.51 ml/kg.

Discussion

Our results showed that oral intubation of 0.51 ml of olive oil for one month, exerts hypolipidemic effects (decrease in serum TC, LDL-C and VLDL-C and TAG concentrations). This result is in agreement with other recent studies on olive oil (21) and those obtained by (22,23,24) on olive leaves.

Monounsaturated fatty acids found in olive oil is responsible for its hypolipidemic effect. Oleic acid of olive oil may increase the break down of fats in adipocytes (25) and cause reduction on plasma LDL-C concentration (16).

Furthermore, polyunsaturated fatty acids (PUFA) present in olive oil may be responsible for its hypolipidemic effect. Olive oil contains two essential fatty acids with suggested ratio of 10:1 for omega-6 (Linoleic acid) to omega-3 (Linolenic acid) (12). The essential fatty acid have a noticeable effect on lowering cholesterol concentration more than oleic acid and palmitic acid when plasma cholesterol levels are higher than ($>200\text{mg/dl}$) (26). The hypolipidemic role of PUFA may be either through inhibition of hepatic synthesis and secretion of TAG and reduction of chylomicron secretion from intestinal cell, while their role in limiting VLDL-C secretion can be mediated by a lowering rate of VLDL-C lipoprotein B conversion to LDL-C (27). Besides, Omega 3 and 6 fatty acids of olive oil may be also responsible for the capability of olive oil in elevation of HDL-C (good cholesterol) concentration through elevation hepatic HDL-C receptor activity and facilitating transition of fatty acids from VLDL-C to HDL-C (28).

There are usually high concentrations of squalene in olive oil as compared with other common fats and oils consumed by humans. Squalene is also has an antioxidant effect. It inhibits farnesylation by inhibiting β -hydroxylation β -methylglutaryl-CoA reductase activity leading to significant decrease in serum cholesterol concentration (11).

The present study showed that oral administration of olive oil caused a significant depletion of AST activity. Olive oil consist of high MUFAs which is resistant to oxidation (10,29). The presence of high MUFA, phenols, tocopherol and other natural antioxidants in olive oil prevent tissue oxidation, within the body, eliminating the formation of free radicals, inhibit cell destruction and prevent enzymes leakage (30). Oleuropin content of olive oil might decrease protein carbonyl, reducing oxidative stress, protein modification and maintained cellular membrane integrity. Furthermore, the aromatic and the flavour compound of olive oil, as well as the chlorophylls and pheophytin pigments increase

stomach secretion and facilitated the absorption of natural antioxidants, protect the tissues from oxidation and restoring enzymes activities (31)

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