The Effect of Different Level of Flaxseed Oil on Biochemical Changes on Hypercholesterolemic Rats

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Abstract

Flaxseed oil has beneficial effects on many chronic diseases in human and animals. Besides, flaxseed oil has also received increasing attention for its potential role in preventing lipid disorders. However, some studies have shown that whole flaxseed oil lower serum cholesterol in animal and human. so we worked This study to determine the effect of different level of flaxseed oil on biochemical changes on hypercholesterolemic rats. The study used (24) white Albino rats and were divided into two main groups first set of mice infected with hypercholesterolemic, Second Group of negative control, a non-mice group infected and was then the first and second main group is divided into four sub-groups, including two groups fed with different concentrations of (20% and 25%) flaxseed oil and one group control positive infected with the disease do not feed on the experimental diet and another control negative non-infected this disease means that all mice are divided into four groups of six mice in each group. The result showed that non significant differences in HDL levels between 20% ,25% flaxseed oil and control negative when compared with control positive Also, there were significant decreases in ALP between the groups replaced with different percentage of flaxseed oil compared with control (+) group . The results suggests to use different levels of flaxseed oil for hypercholesterolmic patients , also suggests future studies may be to evaluate the efficacy and advantage of using flaxseed oil as extracts.

Keywords: Flaxseed oil - *Biochemical changes* - Hypercholesterolmic

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INTRODUCTION

Flaxseed oil has beneficial effects on many chronic diseases in human and animals. Besides, flaxseed oil has also received increasing attention for its potential role in preventing lipid disorders. However, some studies have shown that whole flaxseed oil lower serum cholesterol in animal and human. (**Anonymous , 2017).** Flaxseed has recently gained attention in the area of cardiovascular disease primarily because it is the richest source of both ALA and phytoestrogen, lignans, as well as being a good source of soluble fiber. Human studies have shown that flaxseed can modestly reduce scrum total and low-density lipoprotein cholesterol concentrations, reduce postprandial glucose absorption, decrease some markers of inflammation and raise serum levels of the omega-3.(**Hall, 2013)** Flaxseed oil differs from whole and ground flaxseed by being devoid both fiber and lignans. Besides, the oil in flaxseed is unique in that it is composed of 73% polyunsaturated fatty acids (PUFA), 18% monounsaturated fatty acids (MUFA) and 9% saturated fatty acids, making it a low-saturated fat food **(Morris, 2014).** Flaxseed oil has been reported to have beneficial effects on many chronic diseases in human and animals **(Bhathena et al.,2013).** Besides, flaxseed oil has also received increasing attention for its potential role in preventing lipid disorders. However, some studies have shown that, whole flaxseed oil lower serum cholesterol in animal and human (**Ratnayake et al., 1992 and Bierenbaum, 1993)**. (**Lagrange ,1995)** observed that the amount of crude oil,

protein, dietary fibers and ash content of whole flaxseed were 40 - 45%o, 17 - 22%o, 4% and 6%, respectively and carbohydrates were 25%o.(**Oomah and Mazza ,1993)** found that total lipid of flaxseed was 46%. Also, **(Nag , 2015)** reported that flaxseed had the following percentage composition: moisture, 8.3; protein, 26.9; total dietary fiber, 42.8 and the oil content in the seed was about 45% by weight. **(Malcolmson et al., 2010)** mentioned that the moisture and oil content of whole flaxseed were 6.5 and 43.4% in mixed variety. **(Carter ,1993)** reported that flaxseed oil is the richest known source of the omega 3 (n-3) fatty acid, alpha linolenic acid (ALA) which comprises approximately 55% of total fatty acids and alpha- linolenic acid (ALA) in flaxseed is 5.5 times higher than that in the next-highest sources, walnuts and canola oil.

AIM OF STUDY:-

This work aims to show the effect of different level of flaxseed oil on biochemical changes on hypercholesterolemic rats.

MATERIALS AND METHODS:-

1- Materials:

A- Flaxseeds oil (Linum usitcitissiwmvn) were obtained from Agriculture Research Center, Oil Crops Department, Giza, Egypt .

B-Experiraental animals: twenty four male albino rats, Sprague Dawley strain, weighing 150±10g were used in the study.

C-Used chemicals: Cholesterol powder (obtained from Local markets in Jeddah. KSA).

2- Methods:

A- Biological experiment

Basal diet composition of tested rats:

The basal diet in the experiment consisted of casein(10 %) , com oil (10 %), vitamin mixture (1 %), salt mixture (4 %), choline chloride (0.2 %), methionine (0.3 %), cellulose (5%) and the remained is corn starch (69.5%) according to **(Campbell ,1963)** as seen in table (1)

Table (1): **Composition of basal diet:**

Ingredients	**Amounts**
Protein (casein)	10%*
Corn oil	10%
Mineral mixture	4%
Vitamin mixture	1 %
Cellulose	5%
Choline chloride	0.2 %
Methionine	0.3 %
Corn starch	Up to 100%

* 12.3g casein gives l0g protein.

Source: **(Campbell ,1963).**

Table (2): **Composition of salt mixture%:**

Compound	**Amount**
Caco3	600 mg
K2HPo4	645 mg
CaHPo4.2H2O	150 mg
MgSo4.2H2O	204 mg
Na CL	334 mg
Fe(C6H5O7)26H2O	55 mg
KI	1.6 mg
Mnso4.4H2O	10 mg
ZnCL2	0.5 mg
CuSo4.2H2O	0.06 mg

Source**: (Hegsfed et al, 1941).**

Table (3): **Composition of vitamin mixture:**

Vitamin	**Amount**
Vitamin E	10 Iu
Vitamin K	0.50 Iu
Vitamin A	200 Iu
Thiamin	0.50 mg
Riboflavin	1.0 mg
Pyridoxine	0.40 mg
Niacin	4.00 mg
Vitamin C	20.0 mg
Panathothenic acid	4.0 mg
Vitamin D	100 Iu
Choline chloride	200 mg
Folic acid	0.02 mg
Inositol	25 mg
Para- amino- benzoic acid	0.02 mg
Vitamin B12	2.00 mg
Biotin	0.02 mg
Corn starch	Up to 100 g

- Preparation of hypercholesterolmic rats:

Normal rats fed a special diet for inducing hypercholesterolemia, the diet was prepared from fine ingredients per 100 g according to **(Rashwan ,1994).** Diet had the following composition:

Fat 10% (corn oil 10%); sucrose 10%; salt mixture 4%; vitamin mixture 1%; choline chloride 0.2%; cholesterol powder 1.5% (obtained from Morgan Co. Cairo, Egypt) and neutral casein (obtained from Morgan Co. Cairo, Egypt)16.28g (protein content 12%), corn starch up to 100.

Table (4): Composition of hypercholesterolemia diet:

Compound	Amounts
Protein	12 %
Fat(corn oil)	10 %
Sucrose	10 %
Methionine	0.3 %
Salt mixture	4 %
Vitamin mixture	1 %
Choline chloride	0.2 %
Cholesterol powder	1.5 %
Corn starch	Up to 100%

Experimental Design and Animal Groups:

twenty four Sprague Dawley white male albino rats, weighing about 150 ± l0g were used in the study. Rats were housed in wire cages under the normal laboratory condition and fed on basal diet for a week as adaptation period. Diet was given in non scattering feeding cups to avoid loss or contamination of food, water was provided to the rats by means of glass tubes projecting through the wire cage from an inverted bottle supported to one side of the cage.

The rats were divided into 4 groups each of 6 rats. The groups of rats were as follows:

- Group (1): Control negative group, in which the normal rats fed on basal diet (control "-").
- Group (2): Hypercholesterolemic, control positive group, in which injected rats fed on basal diet (control "+").
- Group (3): Hypercholesterolemic group fed on basal diet + 20% flaxseed oil.
- Group (4): Hypercholesterolemic group fed on basal diet + 25% flaxseed oil **Biological evaluation:**

During the experimental period (28 days), the consumed feed was recorded every day, and body weight recorded weekly .The body weight gain (B.W. G. %), food efficiency ratio (F.E.R) and also organs weight were determined according to **(Chapman *et al,* 1959)**

Blood sampling:

Blood samples were collected after 12 hour fasting at the end of the experiment. Using the retro - orbital method by means of a micro capillary glass tubes, blood was collected into a dry clean centrifuge tube and left to clot in a water bath (37ºC) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum a part of was subjected to glucose determination and the reminder was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20ºC) until analysis. The organs (liver, kidney, heart, and spleen) were removed and washed in saline solution, weighted and kept in formalin solution (10%) according to methods described by **(Drury and Wallington ,1980).**

Biological evaluation:

Food intake (consumption), body weight gain% (BWG %), feed efficiency ratio (FER) according to (**Chapman *et al,*1959).** Using the following equation .

$$BWG\% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

$$FER = \frac{Gain\ in\ body\ weight\ (g/day)}{Food\ Intake (g/day)}$$

$$\text{Relative weight of organs} = \frac{\text{Organs weight}}{\text{Animal body weight}} \times 100$$

Biochemical analysis:

1) Determination of serum glucose: serum glucose was determined using chemical kits according ***(Trinder, 1969).***

2) Determination of serum lipids:

2.1) Triglycerides:

Enzymatic calorimetric determination of Triglycerides was carried out according to(***Fassati and Prencipe ,1982).***

2.2) Total cholesterol

The principle use of total cholesterol determination according to (***Allain, 1974).***

2.3) HDL-cholesterol:

Phosphotungstic acid and magnesium ions selectivity precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant can be determined by the same method used for total cholesterol, according to ***(Lopez,1977).***

2.4) V-LDL and LDL- cholesterol:

The determination of VLDL (very low density lipoprotins) and LDL were carried out according to the method of (***Lee et al ,2008)***

3) Determination of liver functions:

3.1) Determination of Alanine transferase (ALT):

Determination of (ALT) was carried out according to the method of ***(Tietz ,1976).***

ALT catalyzes the transfer of the amino group from L-alanine to a-Ketoglutarate resulting in the formation of pyruvate and L-Glutamate .

Lactate dehydrogenase catalyzes the reduction of pyruvate and the stimultaneous oxidation of NADH to NAD. The resulting rate of decrease in absorbance is directly proportional to ALT activity.

3.2) Determination of Asartate Transferase (AST):

Determination of (AST) was carried out according to the method of ***Henry (1974) and Yound (1975).***.

4- Determination of Some parameters for:

4.1) Determination of Creatinine

Creatinine was determined according to kinetic method of (***Henry ,1974)***, by following reaction:-

4.2) Determination of urea:

Urea was determined according to the enzymatic method of ***Patton and Crouch (1977)***.

5) Organs weight :

After taking retro orbital blood samples, each rat was rapidly opened, the organs (liver, kidney, heart, spleen, lungs, brain) were removed and washed in saline soluation, weighed and kept in formalin solution (10% V/V) according to methods described by(**Drury and Wallington ,1980)**, then compared to control group .

6-Statistical Analysis:

Statistical analysis were calculated using one way classification. Analysis of variance (ANOVA),and least significant difference (LSD) according to **(Snedcor and Cochran ,1967).**

RESULTS AND DISCUSSION

This work aims to show the effect of different level of flaxseed oil on biochemical changes on hypercholesterolemic rats

biological results:

- **Effect of flaxseed oil on Hypercholestrolemic treated groups with Feeding flaxseed oil (20% and 25 %).**

Effect of flaxseed oil on Body weight gain (BWG%)of change on Hypercholestrolemic treated rats- Data in table (5) indicate Body weight gain in both normal and Hypercholestrolemic treated rats after 4 weeks of feeding . Body weight gain in normal rats group was (52.2±11.43)gm/100gm. While Hypercholestrolemic treated rats groups fed a diet at different levels (positive control , 20%, and 25% flaxseed oil) showed an increase . Body weight gain (21±8, 55.6± 10.64 and 52.2±7.07) gm/100gm, respectively.

Food intake values in normal rats group was (478.8±11.43). While in Hypercholestrolemic treated rats groups fed a diet at different levels of flaxseed oil were (544.2±32.19 , 516.6±17.11 , and 562.8±38.9). the result showed that non significant between 25% flaxseed oil and control positive when compared with control negative .

Feed efficiency ratio (FER) value in normal rats group was (0.194±0.03) gm/100gm .While in Hypercholestrolemic treated rats group fed diet different levels of flaxseed oil (positive control, 20% , and 25%)showed that (0.09±0.04 , 0.198±0.05, and 0.21±0.04) gm/100gm ,respectively the results showed non significant between groups (20% and 25%) flaxseed oil ($P<0.05$) when compared with control negative .

Table(5): Body weight gain(BWG%)/ Food intake (FI)(g/28day)/and Feed efficiency ratio (FER) for Negative Control/positive Control/and Hypercholestrolemic treated groups with feeding flaxseed oil (20%and 25 %).

Groups / Parameters	Control (-)	Control (+)	20% Flaxseed oil	25% Flaxseed oil	SIG	LSD
	Mean±SD	Mean±SD	Mean ± SD	Mean± SD		
Body weight gain	52.2 [a]± 11.43	21 [b]± 8	55.6 [a]± 10.64	52.2 [a]± 7.05	*	12.674
Food intake	478.8 [b]± 31.86	544.2 [a]± 32.19	516.6 [ab]± 17.11	562.8 [a]± 38.9	*	41.635
Feed efficiency ratio	0.194 [a]± 0.03	0.09 [b]± 0.04	0.198 [a]± 0.05	0.21 [a]± 0.04	*	0.055

- Data in table (6) indicates the effect of level flaxseed oil on organ weight and organ weight /body weight in both normal and Hypercholestrolemic treated rats after 4 weeks of feeding .The liver in normal rats group was (2.75±0.37)gm/100gm. While Hypercholestrolemic treated rats groups fed a diet at different level of flaxseed oil

(positive control , 20%, and 25% flaxseed oil) showed an decrease relative weight of liver (2.48±0.2, 3.008± 0.23 and 3.12±0.46) gm/100gm, respectively. the results showed that non significant in all groups

Relative kidney weight values in normal rats group was (065±0.05). While in Hypercholestrolemic treated rats groups fed a diet at different levels of flaxseed oil were (0.59±0.05 , 0.56±0.02 , and 0.52±0.07). the result showed that group of 20% and 25% flaxseed oil were significantly increase (P<0.05) when compared with control negative . While non significant between control positive and control negative

- Relative heart weight value in normal rats group was (0.32±0.01) gm/100gm .While Hypercholestrolemic treated rats group fed diet different levels of flaxseed oil (positive control, 20 % and 25 %)showed that (0.29±0.03 , 0.29±0.01 ,and 0.286±0.03) gm/100gm ,respectively the results showed that non significant between 20% and 25% olive oil .
- Relative lung weight values in normal rats group was (0.55±0.08). While in Hypercholestrolemic treated rats groups fed a diet at different levels of flaxseed oil were (0.54±0.05 , 0.58±0.11 , and 0.56±0.11). the result showed non significant between all groups.
- Relative spleen t weight value in normal rats group was (0.45±0.01) gm/100gm .While Hypercholestrolemic treated rats group fed diet different levels of flaxseed oil (positive control, 20% and 25%)showed that (0.48±0.16 , 0.55±0.04 ,and 0.47±0.12) gm/100gm ,respectively the results showed that non significant between all groups .

Table(6): Relative Weight of internal organs for Negative Control/positive Control/and Hypercholestrolemic treated groups with feeding flaxseed oil (20% and 25%).

Groups / Parameters	Control (-)	Control (+)	20% Flaxseed oil	25% Flaxseed oil	Sig	LSD
	Mean±SD	Mean±SD	Mean ± SD	Mean± SD		
LUNG	0.55 [a] ± 0.08	0.54 [a] ± 0.05	0.58 [a] ± 0.11	0. 56 [a] ± 0.11	NS	0.092
LIVER	2.75 [b] ± 0.37	2.48 [b] ± 0.2	3.008 [a] ± 0.23	3.12 [a] ± 0.46	*	0.288
HEART	0.32 [b] ± 0.01	0.29 [b] ± 0.03	0.29 [b] ± 0.01	0.286 [b] ± 0.03	*	0.031
KIDNEY	0.65 [a] ± 0.05	0.59 [a] ± 0.05	0.56 [a] ± 0. 02	0.52 [a] ± 0.07	NS	0.065
SPLEEN	0.45 [a] ± 0.01	0.48 [a] ± 0.16	0.55 [a] ± 0.04	0.47 [a] ± 0.12	NS	0.134

Table (7) represent the effect of feeding different levels of flaxseed oil on T.Lipids, PH.Lipids, and T-Cholesterol , in both normal and Hypercholestrolemic treated rats after 4 weeks of feeding . The total Lipids in normal rats group was (237.6±10.5)mg/dl. While Hypercholestrolemic treated rats groups fed supplement diet at different levels (positive control, 20%, and 25% flaxseed oil) showed total Lipids values (318.8±3.19 , 260.2± 1.79 , and 243.8±2.88) mg/dl respectively .

- PH. Lipids values in normal rate group was (102.2±2.17) mmol/L .While in Hypercholestrolemic treated rats groups fed a diet with different levels of flaxseed oil were (111±2.83 ,102.2±2.49 and 84.8±3.03)mg/dl at levels (positive control, 20% , and 25%)mg/dl, respectively.
- Total Lipids and Ph.Lipids in table (7) concerning serum total Lipids the results showed that all groups were significantly more (P<0.05) when compared with control negative . Also Ph.Lipids showed that rats fed on 25% flaxseed oil was non significant (P<0.05) when compared with control negative .

- Cholesterol values in normal rate group was (81.6±5.13)mmol/L .While in Hypercholestrolemic treated rats groups fed a diet with different levels of flaxseed oil were (119.6±2.19 , 90.4±6.99 and 84.8±30.3)mg/dl at levels (positive control, 20% , and 125%) mg/dl, respectively.

Table(7): Serum Lipids profile and Atherogenic index for Negetive Control , positive Control and Hypercholestrolemic treated groups with feeding flaxseed oil (20% and 25 %).

Groups / Parameters	Control (-)	Control (+)	20% Flaxseed oil	25% Flaxseed oil	sig	LSD
	Mean±SD	Mean±SD	Mean ± SD	Mean± SD		
T.LIPIDS	237.6 c+ 10.5	318.8 a+ 3.19	260.2 b+ 1.79	243.8 c+ 2.88	*	7.669
PH.LIPIDS	102.2 b+ 2.17	111 a+ 2.83	102.2 b+ 2.49	101 b+ 1.41	*	3.065
CHOLESTEROL	81.6 c+ 5.13	119.6 a+ 2.19	90.4 b+ 6.99	84.8 bc+ 3.03	*	6.327

Table (8) represent the effect of feeding different levels of flaxseed oil T.G, HDL, LDL , and VLDL in both normal and liver disorder rats after 4 weeks of feeding . The T.G in normal rats group was (53.2±5.85) mg/dl. While Hypercholestrolemic treated rats groups fed supplement diet at different levels (positive control, 20%, and25% flaxseed oil) showed T.G values (91.4±5.13 , 60.4± 3.58 , and 56.2±2.68)mg/dl respectively .

- HDL values in normal rate group was (46±1.41) mmol/L .While in Hypercholestrolemic treated rats groups fed a diet with different levels of flaxseed oil were (41.6±0.89 , 44.6±0.89 and 44.4±.55)mg/dl at levels (positive control, 20% , and 25%)mg/dl, respectively. the result showed that non significant differences between 20% ,25% flaxseed oil and control negative when compared with control positive .

- LDL values in normal rate group was (25.56±3.24)mg/dl .While in Hypercholestrolemic treated rats groups fed a diet with different levels of flaxseed oil were (61.4±3.88 , 36.28±3.8 and 25.56±2.27)mg/dl at levels (positive control, 20% , and 25%)mg/dl, respectively. groups of 20% and 25% flaxseed oil high significantly ($P<0.05$) when compared with control negative .

- VLDL values in normal rate group was (10.84±1.17)mmol/L .While in Hypercholestrolemic treated rats groups fed a diet with different levels of flaxseed oil were (18.28±1.03 ,12.08±0.72 and 11.24±0.54) mg/dl at levels (positive control, 20% , and 25%)mg/dl, respectively. The result showed non significant differences between 20%, 25% and control negative when compared with control positive .

The current results in a good agreement with those of **Vijaimohan *et al.,* (2016).** Concerning the LDL-C / HDL-C ratio, the value of LDL-C / HDL-C ratio of hyperlipidemic group (2.58 mg / dl) was significantly higher than all groups. In addition to that, the control (-) group was no significantly ($p > 0.05$) compared with rats fed diet content 75% flaxseed oil. There were significantly ($p < 0.05$) differences between all groups replaced with flaxseed oil (25, 50 and 75%) groups. Our results in a good agreement with those obtained by **(Brizzi *et al.*, 2017)** reported that, LDL/HDL ratio can be used as predictive parameters of in vivo LDL oxidation. A significant increase in TC / DHL-C and LDL-C / HDL-C ratios were observed in high fat fed rats that has an effect on cardiovascular diseases. Flaxseed oil may have inhibited the apo- lipoprotein, (3-synthesis or increased its catabolism which explains the reduction of these ratios in rats. ALA rich diet resulted in the significant decrease of a number of proatherogenic factors, such as TC, LDL-C and LDL-C / HDL-C **.**

Table(8): T.G, HDL, LDL and VLDL for Negative Control positive , Control negative and Hypercholestrolemic treated groups with feeding flaxseed oil (20%and 25 %).

Groups / Parameters	Control (-)	Control (+)	20% Flaxseed oil	25% Flaxseed oil	sig	LSD
	Mean±SD	Mean±SD	Mean ± SD	Mean± SD		
T.G	53.2 ^{b}± 5.85	91.4 ^{a}± 5.13	60.4 ^{b}± 3.58	56.2 ^{b}± 2.68	*	6.015
HDL	46 ^{a}± 1.41	41.6 ^{b}± 0.89	44.6 ^{a}± 0.89	44.4 ^{a}± 0.55	*	1.323
LDL	25.56 ^{c}± 3.24	61.4 ^{a}± 3.88	36.28 ^{b}± 3.8	25.56 ^{c}± 2.27	*	4.501
VLDL	10.84 ^{b}± 1.17	18.28 ^{a}± 1.03	12.08 ^{b}± 0.72	11.24 ^{b}± 0.54	*	1.203

- Table (9) reflects the effect of different level of flaxseed oil on creatinine , urea and uric acid values in both normal and Hypercholestrolemic treated rats fed diet with different level of flaxseed oil. urea values recorded (27.6±4.77)mg/100ml in normal rats group .While Hypercholestrolemic treated rats fed flaxseed oil at values (positive control , 20 % 25%) showed serum urea levels (28.8±0.45 , 23±1.41 , and 22.4±0.55)mg/100ml , respectively. As for urea the results revealed that non significant between (20% and 25% flaxseed oil when compared with control negative. - Accorded to the same table normal rats group recorded serum creatinin level (0.598±0.07)mg/100ml. While flaxseed oil supplemented with different levels to the diet were (positive control, 20% ,and 25% ,) presented the values (0.69±0.03 , 0.672±0.02 , and 0.644±0.05)mg/100ml , respectively . the results showed that significantly higher (P<0.05) 25% flaxseed oil when compared with control negative.

- Also in the same table normal rats group recorded U.acid level (1.68±0.39)mg/100ml. While flaxseed oil supplemented with different levels to the diet were (positive control, 20% ,and 25% ,) presented the values (22.68±0.39 , 1.56±0.09 , and 1.44±0.09)mg/100ml , respectively the result showed that non significant between all groups.

Table(9):Some renal Functions for Negative Control positive , Control negative and Hypercholestrolemic treated groups with feeding flaxseed oil (20%and 25 %).

Groups / Parameters	Control (-)	Control (+)	20% Flaxseed oil	25% Flaxseed oil	sig	LSD
	Mean±SD	Mean±SD	Mean ± SD	Mean± SD		
UREA	27.6 ^{a}± 4.77	28.8 ^{a}± 0.45	23 ^{b}± 1.41	22.4 ^{b}± 0.55	*	3.372
CREATININ	0.598 ^{b}± 0.07	0.69 ^{a}± 0.03	0.672 ^{a}± 0.02	0.64 ^{ab}± 0.05	*	0.060
U.ACID	1.68 ^{a}± 0.39	2.26 ^{a}±.14	1.56 ^{a}± 0.09	1.44 ^{a}± 0.09	NS	0.316

- Table (10) revealed the effect of flaxseed oil on enzymes activity (.GOT, GPT ,and ALP) on both normal and Hypercholestrolemic treated rats groups . GOT level in normal rats group was (94.8±6.61)u/l . while liver disorder rats groups were fed diet containing (positive control, 20% and 25% flaxseed oil) recorded (102.8±1.79 , 95.4±3.55 and 92.6±3.97)u/l , respectively. The result showed non significant between 20% and 25% olive oil when compared with control negative

- Normal rats group represented GPT level (42.8±3.89) u/l .While Hypercholestrolemic treated rats groups were fed a diet supplemented with flaxseed oil containing (positive control, 20% , and 25%) showed a values (48.2±1.3 , 44.4±1.52 , and 40.4±0.89)u/l .

- With regard to table 10 for enzymes activity normal rats group respresented ALP level (104.6±7.96) u/l .While Hypercholestrolemic treated rats groups were fed a diet supplemented with flaxseed oil containing (positive control, 20% , and 25%)showed a values (111.4±7.4, 113.8±3.03 and 106.2±6.26)u/l . The results showed that non significant differences between all geoups . This results on the same line with those obtained by **(Bhatia et al., 2018).** Concerning ALT there were significant increases between control (+) group by mean (31.75 u/L) compared with control (-) group (19.00 u/L). Also, there were significant decreases between the groups replaced with different percentage of flaxseed oil compared with control (+) group.

Table(10): Some Liver Functions for Negative Control positive , Control negative and Hypercholestrolemic treated groups with feeding flaxseed oil (20%and 25 %).

Groups / Parameters	Control (-)	Control (+)	20% Flaxseed oil	25% Flaxseed oil	sig	LSD
	Mean±SD	Mean±SD	Mean ± SD	Mean± SD		
S.GOT	94.8 ^{b}± 6.61	102.8 ^{a}± 1.79	95.4 ^{b}± 3.55	92.6 ^{b}± 3.97	*	5.825
S.GPT	42.8 ^{bc}± 3.89	48.2 ^{a}± 1.3	44.4 ^{b}± 1.52	40.4 ^{c}± 0.89	*	2.998
ALP	104.6 ^{a}± 7.96	111.4 ^{a}± 7.4	113.8 ^{a}± 3.0	106.2 ^{a}± 6.26	NS	8.650

RECOMMENDATIONS

1. It is suggested to use different levels of flaxseed oil for hypercholesterolmic patients.
2. different levels of flaxseed oil, may be suggested for lowering LDL and atherogenig index levels.
3. Future studies may be suggested to evaluate the efficacy and advantage of using flaxseed oil as extracts.

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