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STUDY THE NUTRITIONAL AND THERAPEUTIC EFFECT OF FLAXSEED, SUNFLOWER AND PUMPKIN SEEDS ON THE LEVEL OF BLOOD LIPIDS AND WEIGHT GAIN ON RATS

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ABSTRACT: ***This study was conducted to investigate the use of flaxseed, sunflower and pumpkin seeds to improve the level of serum lipid profile, high liver enzymes, renal function and antioxidant enzymes. Fifty four mature male albino rats weighing 150±10g were divided into two main groups; control negative group (6 rats) was fed on basal diet and the second main group (48 rats) which was fed on hyperlipidemic diet for four weeks to induce hyperlipidemia. Rats on the second main group supplements were divided into eight subgroups (each group consisted of 6 rats and fed on basal diet. Group were as a following: The first group was left as a control positive, the second group fed diet containing 5 % flaxseeds / kg in diet. The third group fed diet containing 5 % sunflower seeds / kg in diet. The fourth group fed diet containing 5 % pumpkin seeds kg in diet. The fifth group fed diet containing 5 % mix of pumpkin and sunflower seeds. The sixth group fed diet containing 5 % mix of pumpkin and flaxseeds. The seventh group fed diet containing 5 % mix of sunflower and flaxseeds. The eighth group fed diet containing 5 % mix of sunflower, flaxseeds and pumpkin. Feeding experiment lasted for 28 days. Serum total cholesterol, triglycerides, lipoprotein fractions (HDLc, LDLc and VLDLc), atherogenic index (AI), liver enzymes(ALT, AST, ALP),total protein, albumin, globulin, albumin/globulin (A/G) ratio, uric acid, urea, creatinine, antioxidant levels superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and malondialdehyde (MDA) were determined. Histopathological changes of heart was examined. The obtained results concluded that feeding with flaxseed (5%), sunflower (5%) and pumpkin seeds (5%) improved liver, kidney functions, lipid profile, antioxidant levels (SOD), (CAT), (GPX), (MDA), heart tissues changes. According to the results, flaxseed, sunflower and pumpkin seeds could be used to improve the health situation of obese rats along with weight loss.***

Key words: ***Obesity, flaxseed, sunflower, pumpkin, lipid profile, liver enzymes, antioxidant levels superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA), histopathological changes.***

INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated to an extent that it may have a negative effect on health (WHO,2016).People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, is over 30 kg/m2; the range 25–30 kg/m2 is defined as overweight (Haslam and James, 2005).

Obesity increases the likelihood of various diseases and conditions, particularly cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer, osteoarthritis, and depression (Luppino *et al*., 2010). Obesity is most commonly caused by a

combination of excessive food intake, lack of physical activity, and genetic susceptibility (Yazdi *et al*., 2015).

A few cases are caused primarily by genes, endocrine disorders, medications, or mental disorder (Bleich *et al.,* 2008). On average, obese people have a greater energy expenditure than their normal counterparts due to the energy required to maintain an increased body mass (Kushner, 2009). Obesity is mostly preventable through a combination of social changes and personal choices. Changes to diet and exercising are the main treatments (Yanovski and Yanovski, 2014).

Diet quality can be improved by reducing the consumption of energy-dense foods, such as those high in fat or sugars, and by increasing of fruit vegtables foods. Medications can be used, along with a suitable diet, to reduce appetite or decrease fat absorption (Colquitt *et al*., 2014). If diet, exercise, and medication are not effective, a gastric balloon or surgery may be performed to reduce stomach volume or length of the intestines, leading to feeling full earlier or a reduced ability to absorb nutrients from food (Imaz *et al*., 2008).

Flaxseeds help to reduce belly fat by increasing thermogenesis (a process by which fat is burnt). It perfectly balances the hormones which contribute to weight loss. The fiber in flaxseed promotes healthy bowel function. Hence, maintaining the colon health with prevention of constipation and further complications (Garg *et al*., 2017).

Sunflower seeds appear to be a perfect melting pot of nutrients that are conducive for weight loss, including those such as fiber and the B vitamins. Fiber adds bulk in the stomach, slowing down glucose absorption and keeping you fill for longer, while the B vitamins help to ensure macronutrients are broken down in more efficient fashion. Both of these help in the weight loss process, and can help keep energy levels high while calories are restricted (Marcene, 2018).

Pumpkin seeds are full of fiber, which discourage more eating because of giving the feeling of full stomach. One cup of its seeds has about 600 calories, thus it fulfills your calorie intake to a greater extent of your body. Not only this, it also boosts your fiber intake. Therefore, the regular use of such seeds helps to control as well as management of weight (Megan, 2018).

Thereupon, this experiment was conducted to study the effect flaxseed, sunflower and pumpkin seeds on hyperlipidemic rats.

MATERIALS AND METHODS

1- Food material:

Flaxseed, sunflower and pumpkin seeds were obtained from Minstry of Agricultur. Basal diets were supplemented with flaxseed, sunflower and pumpkin seeds at concentration 5 % for 28 days.

2- Hyperlipidemic diet

Hyperlipidemic diet was prepared from fine ingredients per 100g according to Rashwan (1994). The diet had the following composition fat 20%(sunflower oil10% + sheep tallow10%), sugar 10%, salt mixture 4% vitamin mixture1%, casein (protein content 14%), mithionine 0.3%, and corn starch up to 100.

3- Chemicals:

Casein, vitamins mixture, salt mixture and all other basal ingredients diet were purchased from El-Gomhoria Company, Cairo, Egypt.

4- Experiments animals:

Fifty four male albino rats, weighing 150±10 g, of Sprague Dawley Strain were used. They were obtained from the Animal House Laboratory of Ophthalmic Research Institute, Giza.

Methods:

Biological experiments:

Diet: The basal diet (casein – basal diet) was composed of 12.3g casein (80% protein), 10g corn oil (10% fat), 4g cellulose (4% fiber), mineral mixture (4%). vitamin in mixture (1%) and corn starch up to 100g according to NRC (1995) . The salt mixture used in the experiment was composed according to Hegsted (1941). vitamin mixture used in the experiment was that of Campbell (1963) .

Experimental design and animal groups:

After one week of adiption , the rats were divided into two main groups as follows:-

- The first main group (6 rats) was fed on basal diet and kept as a control negative .
- The second main group (48 rats) was fed on hyperlipidemic diet for four weeks to induce hyperlipidemia and increased negative weight according to Rashwan (1994).

Rats in the second main group (48 rats) were divided into eight groups (each group consisted of 6 rats). Grouping of all rats was as followings:

- The first group was left as a control positive and fed on hyperlipidemic diet. The second group was fed on basal diet and supplemented standard diets with 5% Flaxseeds. The third group was fed on basal diet and supplemented standard diets with 5% sunflower seeds. The fourth group was fed on basal diet and supplemented standard diets with 5% pumpkin seeds. The fifth group was fed on basal diet and supplemented standard diets with 5% mix of pumpkin and sunflower seeds. The sixth group was fed on basal diet and supplemented standard diets with 5% mix of pumpkin seeds and flaxseeds. The seventh group was fed on basal diet and supplemented standard diets with 5% mix of sunflower and flaxseeds. The eighth group was fed on basal diet and supplemented standard diets with 5% mix of flaxseeds, sunflower and pumpkin seeds.

During the experiment period (28 days), the quantities of diet consumed and / or wasted were recorded every day. In addition, rats weight was recorded weekly.

At the end of the experiment period, the rats were fasted overnight before sacrificing and the blood samples were collected from each rat and centrifuged to obtain the serum. Serum was carefully separated and transferred into dry clean Ebendorf tubes and kept frozen at-20ºC till analysis as described by Schermer (1967).

Liver, heart and kidneys were removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution, wiped by filter paper, weighed and kept in formalin solution (10%), according to the method described by (Drury and Walling, 1980).

Biochemical analysis of serum:-

1- Total cholesterol was determined according to Allain *et al.,* (1974).
2- Triglycerides was determined according to Trinder and Ann (1969).
3- HDL-C was determined according to Lopes - Virella *et al.,* (1977).
4- Serum VLDL-C was determined according to Friedwald *et al.,* (1972) using the following equation:

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

5- Serum LDL-C was determined according to Friedwald *et al.,* (1972) using the following equation:

LDL-C = Total Cholesterol – [(VLDL-C) + (HDL-C)]

6- Serum LDL /HDL cholesterol, CRR cholesterol, AC cholesterol, AIP cholesterol and Atherogenic index was determined according to Bhardwaj *et al.*, (2013) using the following equation:

Atherogenic Index (AI)= (VLDL+ LDL)/HDL cholesterol

LDL /HDL cholesterol= LDL-C / HDL-C

CRR cholesterol= TC/HDL-C

AC cholesterol=(TC– HDLC)/HDL-C

AIP cholesterol= log (TG/HDL-C)

Determination of liver functions:

1- AST (GOT) was determined according to Reitman and Frankel (1957).
2- ALT (GPT) was determined according to Reitman and Frankel (1957).

Determination of kidney functions:

1- Total protein was determined according to Sonnenwirth and Jaret (1980).
2- Albumin was determined by Drupt (1974).
3- Globulin was calculated from formula: Globulin= Total protein – Albumin.
4- A/G ratio was calculated from formula according to Catherine *et al.,* (2003): A/G ratio =Albumin/ Globulin.
5- Urea nitrogen was determined by Patton and Crouch (1977).
6- Createnine was determined by Faulkner and King (1976).
7- Uric acid was determined by Barham and Trinder (1972) and Fossati *et al*., (1980).

Determination of serum antioxidant status:-

1- superoxide dismutase (SOD) was determined by Fossati *et al*., (1980).
2- catalase (CAT) was determined by Aebi (1984).
3- Glutathione peroxidase (GPX) was determined by Beutler *et al.,* (1963).
4- Malondialdehyde (MDA) was determined by Ohkawa *et al.,* (1979).

Statistical analysis: Data were expressed as (Mean ± SD). Differences between control and treated groups were tested for significance using a one way analysis of variance (ANOVA test) according to Armitage and Berry (1987) followed by Duncan's range multiple test. Differences were considered of significance at a level of $P \leq 0.05$ using SPSS (version 20.0) computerized program.

Histopathological examination of hearts :

The hearts, of sacrificed rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin according to Carleton (1979).

RESULTS AND DISCUSSION

<u>Nutrition evaluations:</u>

A-Biological changes:

a- Boody weight gain, feed intake and feed efficiency ratio:

Feed intake (FI) values showed significant increases ($P \leq 0.05$) for positive control group (C+) as compared to normal rats group (23.72±0.23 & 23.37±0.29 g/day, respectively). The higher values were recorded in pumpkin seeds group (23.86±0.20 g/day) when compared to normal rats group, as shown in Table (1).

In relation to body weight gain (BWG %), it could be observed that the mean value of the negative control group was

non- significantly lower than the positive control group (1.44±0.24 & 1.75±0.45 %, respectively).Sunflower & Flaxseeds revealed a higher value for increasing BWG% level of obesitic rats when compared to negative control group (2.02±0.49%) as shown in Table (1).

Calculation of feed efficiency ratio (FER) results of feed efficiency ratio (FER) which illustrated non-significant decreases between negative control group and positive control group (0.09± 0.01& 0.11±0.02 g, respectively).The best result found in pumpkin & flax seeds group which closed to normal rats values, as shown in table (1).

The obtained results are in agreement with Bean and Leeson, (2003) found that feed intake was less (P ≤0.05) for hens fed flaxseed as compared to those consuming the control diet. In accordance to the present study, Salari *et al.,* (2009) showed that weight gain, feed intake and feed conversion ratio (FCR) were improved (p≤0.05) when broilers were fed various levels of full fat sunflower seeds (FFSS) in the starter and finisher diets. Also,present results (table 1) agree with those of Cassani *et al.,* (2015) who indicated that flaxseed diet group experienced weight loss and systolic blood pressure reduction. A decrease in inflammatory markers (CRP and TNF-α) was observed after flaxseed intake. Moreover, results agree with those of Abd El-Ghany *et al.,* (2018) who showed that a significant decrease in weight gain; feed efficiency ratio in the pumpkin extract rat group .

b- Relative organs weight:

Relative liver weight value showed non-significant increases in normal rats group as compared to control (C+) group, it was 3.50 ±0.39 and 3.25 ±0.44 g, respectively. Mixed seeds revealed the lower value for decreasing liver of obesitic rats as compared to negative control group (2.64 ±0.16 g), as shown in Table (2) .

Table (1): Effect of different plant seeds diets on feed intake, body weight gain (BWG %), and feed efficiency ratio (FER), of obese and treated rat groups (n= 6 rats)

Groups	FI g/day	BWG%	FER g
Negative control	23.37 ± 0.29^{c}	1.44 ± 0.24^{bc}	0.09 ± 0.01^{bc}
Positive control	23.72 ± 0.23^{a}	1.75 ± 0.45^{ab}	0.11 ± 0.02^{ab}
Flaxseed	23.68 ± 0.20^{ab}	1.24 ± 0.26^{c}	0.08 ± 0.01^{c}
Sunflower seeds	23.78 ± 0.15^{a}	1.62 ± 0.29^{abc}	0.10 ± 0.01^{bc}
Pumpkin seeds	23.86 ± 0.20^{a}	1.55 ± 0.31^{abc}	0.10 ± 0.01^{bc}
Pumpkin & Sunflower seeds	23.73 ± 0.15^{a}	1.55 ± 0.46^{abc}	0.10 ± 0.02^{b}
Pumpkin & Flax seeds	23.46 ± 0.17^{bc}	1.63 ± 0.28^{abc}	0.11 ± 0.01^{a}
Sunflower & Flax seeds	23.47 ± 0.17^{bc}	2.02 ± 0.49^{a}	0.13 ± 0.02^{b}
Mixed seeds	23.47 ± 0.18^{bc}	1.49 ± 0.40^{bc}	0.10 ± 0.02^{b}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤ 0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

Table (2): Effect of different plant seeds on relative organs weight% of obese and treated rat groups (n= 6 rats)

Groups	Liver	Heart	Kidneys
Negative control	3.50 ±0.39^{a}	0.53 ±0.09^{b}	1.26 ±0.23^{a}
Positive control	3.25 ±0.44^{ab}	0.54 ±0.25^{b}	1.14 ±0.19^{a}
Flaxseed	2.98 ±0.26^{bc}	0.28 ±0.12^{c}	0.56 ±0.09^{d}
Sunflower seeds	2.78 ±0.14^{cd}	0.51 ±0.14^{b}	0.80 ±0.16^{c}
Pumpkin seeds	3.05 ±0.11^{bc}	0.61 ±0.16^{b}	0.51 ±0.05^{d}
Pumpkin & Sunflower seeds	2.84 ±0.14^{cd}	0.49 ±0.08^{b}	0.49 ±0.03^{d}
Pumpkin & Flax seeds	2.89 ±0.17^{cd}	0.89 ±0.05^{a}	0.48 ±0.02^{d}
Sunflower & Flax seeds	2.83 ±0.21^{cd}	0.48 ±0.05^{b}	0.93 ±0.04^{bc}
Mixed seeds	2.64 ±0.16^{d}	0.55±0.05^{b}	0.96 ±0.09^{b}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p \leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non-significantly different.

Relative heart weight value showed non-significant increases in control (C+) group as compared to normal rats group, it was 0.54 ±0.25 & 0.53 ± 0.09 g, respectively. The best result found in Sunflower seeds and Mixed seeds groups, they were near to normal rats values. Pumpkin & Flax seeds group revealed the higher value for increasing heart of obesitic rats as compared to negative control group (0.89 ±0.05 g), as shown in Table (2).

Relative Kidneys weight value showed non-significant decreases in control (C+) group as compared to normal rats group, it was 1.14 ±0.19 &1.26 ±0.23 respectively). The best result found in Mixed seeds which closed to negative control group values, as shown in Table (2) .

B- Biochemical analysis:

a- Serum lipid profile:

Data of Table (3) revealed that the mean values of cholesterol showed a significant increases in positive control group as compared to negative control group (258.5±11.43 and 123.83±13.07c mg/dl, respectively). The best result found in mixed seeds groups which was near to healthy rats values. The higher values were recorded in group of Sunflower seeds, it was 157.17 ± 27.32 mg/dl when compared to normal rats group as shown in Table (3).

In the same table, the mean values of triglycerides showed a significant increases in positive control group as compared to negative control group, it was 170.17±9.91 and 95.17±3.32 mg/dl, respectively. The higher values were recorded in groups of Sunflower seeds and Pumpkin seeds, it was 123.17±37.86 and 116.17 ±19.32 mg/dl, respectively when compared to normal rats group as shown in Table (3). Lowest triglycerides recorded for flaxseeds, pumpkin &flaxseeds and sunflower &flaxseeds groups.

The obtained results are in agreement with Khalesia *et al.,* (2011) who found that 30 days consumption of flaxseed may significantly reduce total cholesterol.

Data of Table (3) are also in accordance to the present study of Abuelgassim and Showayman, (2012) who indicated that atherogenic rats supplemented with pumpkin seeds showed a significant decrease (p≤0.001) in their serum concentrations of total cholesterol. Also, result are in agreement with those of Hussein *et al.*, (2014) they showed that, treatment with flaxseed oil to high cholesterol diet induced hypercholesterolemia rats, lowered serum total cholesterol, triacylglycerols. Moreover, data agree with those of Atefeh *et al*., (2018) who revealed that significant reductions in total cholesterol, low-density lipoprotein cholesterol [5.6% in flaxseed oil (FO) and 10.8% in sunflower seed oil (SO)], and triglyceride levels were seen within each group after treatment with (FO) and (SO) (*P* < .05).

Data of Table (4) revealed that the levels of HDL which were significantly higher in (C-) rats declined in case of (C+) group (44.5 ± 4.6 & 34 ± 1.67 mg/dl, respectively). Most of treated groups indicated non-significant increases as compared to positive control group except Sunflower seeds, Pumpkin seeds and Pumpkin & Flax seeds which showed significant increases (P≤0.05). The best result found in Pumpkin seeds groups which closed to normal rats values, as shown in Table (4).

The mean value of LDL in (negative control) group was extremely significantly lower than the (C+) group (60.3±15.20 & 190.47±10.37 mg/dl, respectively). Mixed seeds revealed the best result for decreasing LDL of obesitic rats when compared to positive control group, as shown in Table (4).

The mean value of VLDL in (negative control) group was extremely significant lower than the (C+) group (19.03±0.66 &34.03±1.98 mg/dl, respectively). All treated groups showed a significant decrease (P≤0.05) as compared to positive control group rats. The best result found in Flaxseed seeds, Pumpkin & Flax seeds and Sunflower & Flax seeds groups which indicat nearly to normal rats values, as shown in Table (4)

Table (3): Effect of different plant seeds on lipid profile of obese and treated rat groups (n= 6 rats)

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
Negative control	123.83±13.07^{c}	95.17 ± 3.32^{d}
Positive control	258.5 ± 11.43^{a}	170.17 ± 9.91^{a}
Flaxseed	141.83 ± 41.04^{bc}	92.17± 18.41^{d}
Sunflower seeds	157.17 ± 27.32^{b}	123.17± 37.86^{b}
Pumpkin seeds	152.83 ± 14.25^{bc}	116.17 ± 19.32^{bc}
Pumpkin & Sunflower seeds	141.33 ± 28.35^{bc}	109.17 ± 7.31^{bcd}
Pumpkin & Flax seeds	146.17 ±10.87^{bc}	93.17± 3.97^{d}
Sunflower & Flax seeds	147.83 ± 11.96^{bc}	93.5± 6.09^{d}
Mixed seeds	136.83 ± 30.42^{bc}	100.5 ± 7.82^{cd}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p\leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non−significantly different.

Table (4): Effect of different plant seeds on lipoproteins profile obese and treated rat groups (n= 6 rats)

Groups	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Negative control	44.5 ±4.6[a]	60.3 ±15.20[c]	19.03 ±0.66[c]
Positive control	34 ± 1.67[c]	190.47 ± 10.37[a]	34.03 ±1.98[a]
Flaxseed	36.17 ± 3.55[c]	87.23 ± 38.41[bc]	18.43 ± 3.68[b]
Sunflower seeds	42.5 ± 4.76[ab]	90.03 ± 24.15[bc]	24.63 ±7.57[bc]
Pumpkin seeds	44.17 ± 5.49[a]	85.43 ± 13.83[bc]	23.23 ± 3.86[bc]
Pumpkin & Sunflower seeds	34.83 ± 3.76[c]	84.67 ± 28.72[bc]	21.83 ±1.46[b]
Pumpkin & Flax seeds	42.5 ± 4.23[ab]	85.03 ± 10.03[bc]	18.63 ± 0.79[bc]
Sunflower & Flax seeds	34.17 ± 3.31[c]	94.97 ± 12.27[b]	18.70 ±1.22[b]
Mixed seeds	38.83 ±3.97[bc]	77.9 ± 31.67[bc]	20.10± 1.56[bc]

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p \leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non−significantly different.

The obtained results are in agreement with Hussein *et al*., (2014) who showed that treatment with flaxseed oil accompanied high cholesterol diet induced hypercholesterolemia rats, lowered serum LDL-C, VLDL-C, phospholipid, endothelin-1 and homocysteine concentration in addition to increasing HDL-C. These results suggest that, flaxseed oil may be effective in controlling cholesterolemic status & improving dyslipidemia and has the potential in reducing cardiovascular complications due to hypercholesterolemia. Also, data agree with those of Jalbani *et al.,* (2016), they reported that in two months therapy by Flaxseeds decreased LDL-cholesterol 6.2% and increased HDL-cholesterol 7.7%.). Moreover, agree with those of Mohamed *et al*., (2011) who revealed that the rats in the treated groups apricot kernel oil (AO) and pumpkin kernel oil (PO) showed significantly lower levels of total cholesterol (TC), total triglycerides (TG), low density lipoprotein-cholesterol (LDL-C). It could be concluded that AO and PO under study are useful for the treatment of hypercholesterolemia.

Data in Table (5) revealed that the level of LDL/ HDL which was significant lower in (C-) rats increased in case of (C+) group (1.38±0.44 &5.62±0.50 mg/dl, respectively). The best result found in Pumpkin seeds group which closed to negative control group values. Sunflower & Flax seeds revealed the higher value for increasing LDL/ HDL of obesitic rats when compared to negative control group (2.81 ± 0.47 mg/dl), as shown in Table (5).

The mean value of Cardiac Risk Ratio (CRR) in (C-) group was extremely significant lower than the (C+) group (2.82±0.48 & 7.62±0.59 mg/dl, respectively). All supplemented diets showed a significant decreases ($P \leq 0.05$) as compared to positive control group rats. The higher values were recorded in group of Sunflower & Flax seeds (4.36 ± 0.52 mg/dl) when compared to normal rats group. Pumpkin & Flax seeds revealed the best result for decreasing CRR of obesitic rats when compared to negative control group, as shown in Table (5).

Table (5): Effect of different plant seeds on LDL-C/HDL-C ratio, Cardiac Risk Ratio (CRR), Atherogenic Coefficient (AC) and Atherogenic Index of Plasma (AIP) of obese and treated rat groups (n= 6 rats).

Groups	LDL /HDL (mg/dl)	CRR (mg/dl)	AC (mg/dl)	AIP (mg/dl)
Negative control	1.38 ± 0.44^{c}	2.82 ± 0.48^{d}	1.82 ± 0.48^{c}	0.33 ±0.049^{d}
Positive control	5.62 ±0.50^{a}	7.62 ± 0.59^{a}	6.62 ±0.59^{a}	0.69 ±0.043^{a}
Flaxseed	2.41 ± 1.01^{bc}	3.92 ±1.03^{b}	2.92 ±1.03^{b}	0.40 ±0.07^{cd}
Sunflower seeds	2.16 ± 0.66^{bc}	3.73 ±0.74^{bc}	2.73 ±0.74^{bc}	0.45±0.11^{bc}
Pumpkin seeds	1.96 ± 0.43^{b}	3.51 ±0.56^{bc}	2.51 ±0.56^{bc}	0.42±0.12^{bcd}
Pumpkin & Sunflower seeds	2.48 ± 0.92^{bc}	4.11 ±0.97^{b}	3.11 ±0.97^{b}	0.49 ±0.04^{b}
Pumpkin & Flax seeds	2.02 ± 0.30^{b}	3.46 ±0.33^{bc}	2.46 ±0.33^{bc}	0.34 ±0.04^{d}
Sunflower & Flax seeds	2.81 ± 0.47^{b}	4.36 ± 0.52^{b}	3.36 ± 0.52^{b}	0.44 ±0.05^{bc}
Mixed seeds	2.04 ±0.88^{bc}	3.56 ±0.90^{bc}	2.56 ±0.90^{bc}	0.41±0.04^{bcd}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p \leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non−significantly different.

The mean value of Atherogenic Coefficient (AC) in (C-) group was extremely significant lower than the (C+) group (1.82±0.48&6.62±0.5 mg/dl, respectively). All treated groups showed significant decrease (P≤0.05) as compared to positive control group rats. The best result found in Pumpkin & Flax seeds group which closed to normal rats values, as shown in Table (5).

In the same table, the obtained results showed that there was significant increase atherogenic index of plasma (AIP) in positive control group as compared to normal rats. In rats feed on all treatment diets, there was significant decrease (P≤0.05) in Atherogenic Index of Plasma (AIP) as compared to (C+). The best result found in Pumpkin & Flax seeds groups which was closed to normal rats values as shown in Table (5).

b- Liver function:

1- Liver enzymes(GOT& GPT)

The mean value of GPT (ALT) showed a significant increase in positive control group as compared to negative control group, it was 86.83±17.84 and 23.5±2.34 U/L, respectively. The best result found in Sunflower & Flax seeds group which closed to negative control group values. Sunflower seeds revealed the higher value for increasing GPT of obesitic rats when compared to negative control group (50±10.43 U/L), as shown in Table (6).

In relation to GOT(AST), it could be observed that the mean value of positive control group was significantly higher than negative control group (69.67±18.12 & 23.67±8.48 U/L, respectively). The best result found in Pumpkin & Sunflower seeds and Sunflower & Flax seeds groups which closed to normal rats values. Sunflower seeds revealed the higher value for increasing GOT of obesitic rats when compared to negative control group (40 ±8.41 U/L), as shown in Table (6).

Table (6): Effect of different plant seeds on liver enzymes of obese and treated rat groups (n= 6 rats)

Groups	GPT (U/L)	GOT (U/L)
Negative control	26.83 ± 5.74^{de}	23.67 ± 8.48^{c}
Positive control	86.83 ± 17.84^{a}	69.67 ± 18.12^{a}
Flaxseed	31.33 ± 3.14^{cde}	20 ± 3.16^{c}
Sunflower seeds	50 ± 10.43^{b}	40 ± 8.41^{b}
Pumpkin seeds	39.50 ± 6.06^{c}	30.50 ± 6. 95 ^{bc}
Pumpkin & Sunflower seeds	30.83 ± 4.79^{cde}	22 ± 3.03^{c}
Pumpkin & Flax seeds	35.33 ± 2.94^{cde}	35.67 ±7.39 ^{b}
Sunflower & Flax seeds	25.50 ± 3.33^{e}	24.33 ± 4.84 ^{c}
Mixed seeds	36.16 ± 4.31^{cd}	35.5 ± 2.74^{b}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p \leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non−significantly different.

The obtained results are in agreement with Cheenam and Leena (2016) found that the patients that were being fed on the sunflower seeds showed a positive and a faster decrease in their liver function test (LFT) levels (both SGOT and SGPT). The SGOT and SGPT levels were found to be decreased from 89.7 mg/dl to 38.4 mg/dl in the Case group and 37.6 mg/dl to 40.5 mg/dl in Control group. Moreover, Al-Okbi *et al.,* (2014) reported that pumpkin seed oil reversed all histopathological changes that occur in liver tissue which became comparable to normal in some rats.

2-Protein fractions: (Total Protein, Albumin, Globulin and Albumin/Globulin (A/G) ratio):

The mean value of total protein showed a significant decrease in positive control group as compared to negative control group, it was 6.02±0.81 and 6.97±0.46 mg/dl, respectively. All treated groups indicated non- significant increases (P≤0.05) as compared to positive control group. The best result found in Pumpkin seeds and Mixed seeds groups which closed to negative control group values. Sunflower & Flax seeds revealed the lower value for decreasing total protein of obesitic rats when compared to negative control group (5.88±0.23 mg/dl), as shown in Table (7).

In the same table, results presented the mean values of albumin and showed a significant decrease in positive control group as compared to negative control group, it was 3.83±0.32 and 4.38±0.37 mg/dl, respectively. All supplemented diets caused a significant increases (P≤0.05) as compared to positive control group. The best result found in Pumpkin &Sunflower seeds group which closed to negative control group values. Sunflower & Flax seeds revealed the lowest value of decreasing albumin level of obesitic rats when compared to negative control group (3.73±0.19 mg/dl), as shown in Table (7).

Table (7): Effect of different plant seeds on serum proteins of obese and treated rat groups (n= 6 rats)

Groups	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A/G ratio (mg/dl)
Negative control	6.97 ± 0.46^{a}	4.38 ± 0.37^{a}	2.58 ± 0.54^{a}	1.77 ± 0.44^{a}
Positive control	6.02 ± 0.81^{b}	3.83 ± 0.32^{b}	2.18 ± 0.64^{ab}	1.86 ± 0.43^{a}
Flaxseed	6.03 ± 0.26^{b}	4.05 ± 0.41^{ab}	1.98 ± 0.25^{b}	2.09 ± 0.49^{a}
Sunflower seeds	6.17 ± 0.59^{b}	3.97 ± 0.39^{ab}	2.2 ± 0.35^{ab}	1.83 ± 0.28^{a}
Pumpkin seeds	6.45 ± 0.73^{ab}	3.93 ± 0.46^{ab}	2.52 ± 0.49^{ab}	1.62 ± 0.41^{a}
Pumpkin & Sunflower seeds	6.28 ± 0.22^{ab}	4.2 ± 0.36^{ab}	2.08 ± 0.46^{ab}	2.18 ± 0.91^{a}
Pumpkin & Flax seeds	6.23 ± 0.53^{b}	4.18 ± 0.45^{ab}	2.05 ± 0.43^{ab}	2.13 ± 0.57^{a}
Sunflower & Flax seeds	5.88 ± 0.23^{b}	3.73 ± 0.19^{b}	2.15 ± 0.19^{ab}	1.75 ± 0.19^{a}
Mixed seeds	6.35 ± 0.78^{ab}	3.9 ± 0.47^{ab}	2.43 ± 0.33^{ab}	1.61 ± 0.10^{a}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p \leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non−significantly different.

In relation to globulin, it could be observed that the mean value of positive control group was non-significantly lower than negative control group (2.18±0.64 & 2.58±0.54 mg/dl, respectively). All treated groups indicated non-significant differences as compared to positive control group. The best result found in Pumpkin seeds group which closed to normal rats values. Flaxseed seeds revealed the lower value for decreasing globulin level of obesitic rats when compared to negative control group (1.98±0.25 mg/dl), as shown in Table (7).

Also in the same table, the obtained results showed that there was non-significant increase in albumin/globulin (A/G) ratio in (C+) as compared to normal rats (1.86±0.43 & 1.77±0.44 mg/dl, respectively). In rats fed on all treatment diets, there was non-significant differences in A/G as compared to positive control group. The best result found in Sunflower & Flax seeds group which closed to negative control group values, as shown in Table (7).

C- Kidney functions :

1- Uric acid, urea and creatinine

The mean value of urea values showed non- significant increase in control (C+) group as compared to normal rats group (37.5±5.54 & 32±5.22 mg/dL, respectively). All treated groups indicated non-significant differences as compared to positive control group except Pumpkin & Sunflower seeds and Pumpkin & Flax seeds which showed significant differences. The best result found in Sunflower & Flax seeds and Pumpkin seeds groups which nearly to normal rats values as shown in Table (8).

In relation to creatinine, it could be observed that the mean value of positive control group was significantly higher than negative control group (1.29±0.44 & 0.65±0.104 mg/dL, respectively). All treated groups indicated significant decreases (P≤0.05) as compared to positive control group. The best result found in Pumpkin & Sunflower seeds and Sunflower seeds group which nearly to normal rats values. Mixed seeds group revealed the higher values in increasing creatinine of obesitic rats when compared to negative control group (0.92 ±0.16 & 0.65±0.104 mg/dL, respectively) as shown in Table (8).

Calculation of uric acid results illustrated non-significant increase in control (C+) group as compared to normal rats group (1.83±0.39 and 1.73±0.22 mg/dL, respectively). All treated groups recorded non-significant increase ($P \leq 0.05$) values except Pumpkin & Sunflower seeds and Mixed seeds groups which showed significant increase as compared to positive control group. The best result found in Flaxseed seeds and Sunflower seeds groups which closed to normal rats values as shown in Table (8).

d- Antioxidant enzymes and lipid peroxidation

The mean value of GPx showed a significant decrease in positive control group as compared to negative control group, it was 2.37±1.59 and 3.94±0.21 µu/mL, respectively. The best result found in Pumpkin & Flax seeds and Sunflower & Flax seeds group which closed to negative control group values. Sunflower seeds revealed the lower value of GPx of obesitic rats when compared to negative control group (2.71±0.07 µu/mL), as shown in Table (9).

In relation to MDA, it could be observed that the mean value of positive control group was significantly higher than negative control group (7.62±0.59 & 2.82±0.48 nmol/mL, respectively). The best result found in Pumpkin & Flax seeds group which closed to normal rats values. Sunflower & Flax seeds revealed the higher value for MDA of obesitic rats when compared to negative control group (4.36±0.52 nmol/mL), as shown in Table (9).

Table (8): Effect of different plant seeds on kidney functions of obese and treated rat groups (n= 6 rats)

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Negative control	32 ±5.22^{bc}	0.65±0.104^{b}	1.73 ±0.22^{b}
Positive control	37.5 ±5.54^{ab}	1.29±0.44^{a}	1.83 ±0.39^{b}
Flaxseed	41.33 ± 5.20^{a}	0.83±0.09^{b}	1.87 ±0.55^{b}
Sunflower seeds	40.67± 6.05^{a}	0.72 ±0.22^{b}	1.8 ±0.29^{b}
Pumpkin seeds	31± 3.09^{bc}	0.75 ±0.16^{b}	2.03 ±0.26^{ab}
Pumpkin & Sunflower seeds	26.5 ± 4.59^{c}	0.71 ±0.15^{b}	2.33 ±0.36^{a}
Pumpkin & Flax seeds	28.67± 7.03^{c}	0.77±0.16^{b}	1.9 ±0.18^{b}
Sunflower & Flax seeds	30.83± 8.06^{bc}	0.85 ±0.09^{b}	2.13 ±0.27^{ab}
Mixed seeds	37.33 ± 3.87^{ab}	0.92 ±0.16^{b}	2.35 ±0.16^{a}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at $p \leq 0.05$ using Duncan Range Multiple test, while those with similar letters are non−significantly different.

Table (9): Effect of different plant seeds on antioxidant enzyme levels (SOD, CAT & GPx) and lipid peroxidation as MDA of obese treated rat groups (n= 6 rats)

Groups	GPx (µu/mL)	MDA (nmol/mL)	SOD (U/mL)	Catalase (U/L)
Negative control	3.94 ±0.21^{a}	2.82 ±0.48^{b}	988 ±123.09^{ab}	85.33 ±6.56^{b}
Positive control	2.37±1.59^{c}	7.62 ±0.59^{a}	460.83 ±44.62^{d}	50 ±21.61^{d}
Flaxseed	3.63±1.36^{ab}	3.92 ±1.03^{ab}	920.17 ±52.58^{ab}	76.33 ±7.20^{bc}
Sunflower seeds	2.71±0.07^{bc}	3.74 ±0.74^{b}	816.83 ±95.25^{c}	64.50 ±5.13^{c}
Pumpkin seeds	3.48±0.32^{ab}	3.51 ±0.56^{b}	898.17±52.66^{bc}	73.67 ±12.01^{bc}
Pumpkin & Sunflower seeds	3.41±0.63^{ab}	4.11 ±0.97^{b}	911 ±13.04^{b}	75.33 ±9.42^{bc}
Pumpkin & Flax seeds	3.93±.82^{a}	3.46 ±0.33^{b}	968.17±73.08 ^{ab}	78.33±13.84^{bc}
Sunflower & Flax seeds	3.93±0.55^{a}	4.36 ±0.52^{b}	1005.83 ±71.75^{a}	119 ±12.68^{a}
Mixed seeds	2.93 ±0.11a^{bc}	3.56 ±0.90^{b}	935.5 ±46.07^{ab}	82 ±9.72^{b}

Values denote arithmetic means ± standard deviation of the mean. Means with different letters (a, b, c, d, etc.) in the same column differ significantly at p≤ 0.05 using Duncan Range Multiple test, while those with similar letters are non-significantly different.

In the same table show the mean values of SOD showed a significant decrease (P≤0.05) in positive control group as compared to negative control group, it was (460.83±44.62 and 988±123.09 U/mL, respectively). All treated group showed a significant increases (P≤0.05) when comparing with positive control group. Pumpkin & Flax seeds group revealed the best result for increasing SOD of obesitic rats when compared to negative control group (1005.83±71.75 U/mL, respectively), as shown in Table (9).

Calculation of Catalase results illustrated a significant decrease in control (C+) group as compared to normal rats group (50±21.61 and 85.33±6.56 U/L, respectively). All treated groups recorded significant increased (P≤0.05) values when comparing with positive control group. The best result found in Mixed seeds group which closed to normal rats values. Sunflower & Flax seeds revealed the higher value for increasing Catalase of obesitic rats when compared to negative control group (119±12.68 U/L), as shown in Table (9).

The obtained results are in agreement with Rajesha *et al.*, (2006), they found that treated with 10.0% flaxseed has shown the restoration of 95.02%, 182.31%, and 136.0% of catalase, SOD, and peroxidase. In the case of the group treated with toxin without flaxseed, the level of superoxide dismutase and the catalse value decreased 91.4% and 55.33%, respectively, in comparison with the control group. These results clearly indicate the beneficial effect of flaxseed components as an antioxidant. In accordance to the present study Shivanna *et al.,* (2013) reported that the flaxseed group had significantly

decreased monoamine oxidase A and B activity and increased superoxide dismutase activity. Lipid peroxidation was completely inhibited. Also, data agree with those of Hussien *et al*., (2016), they reported that treatment with flaxseed oil in hypercholesterolemic rats ameliorate antioxidant enzymatic decreases in liver tissue such as (CAT, SOD and GPx). Also, it agree with those of Abd El-Ghany *et al*., (2018) found that the pumpkin extract and oil rats groups showed a significant decrease in blood GPX & SOD. Moreover, data agree with those of Makni *et al*., (2010) who indicated that diet supplemented with flax and pumpkin seed mixture in the DMS group ameliorated antioxidant enzymes activities and level of reduced glutathione (GSH) in diabetic rats and significantly decreased MDA levels.

C. Histopathological changes of hearts.

Photos (1 to 9) indicated that due to obesity inducation control (+) rats revealed certain histopathological changes in particular portal infiltration with inflammatory cells (Photo 2).No histopathological changes occurred when rats fed with different kinds of seeds (Photos 3,4,5,6,7,8,9).

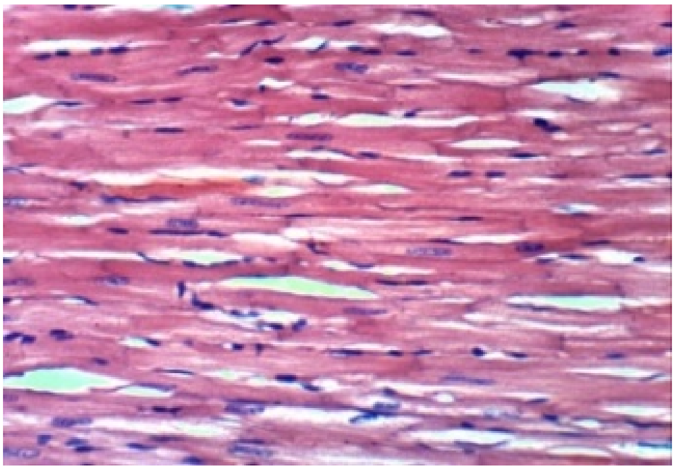

Photo (1): Heart of rat from (C-) showing the normal histological structure of cardiac myocytes (H & E X 400).

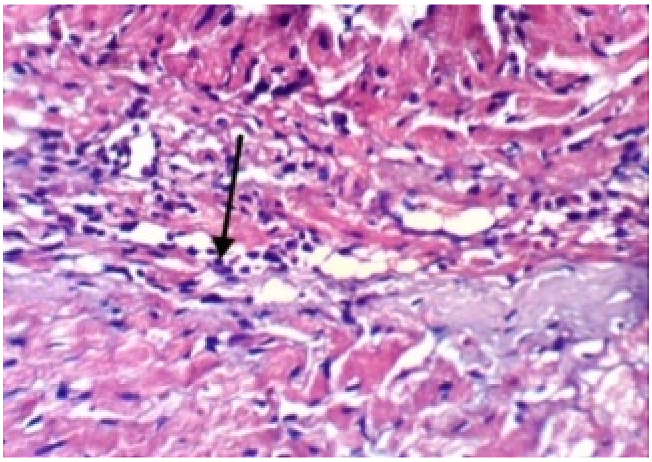

Photo (2): Heart of rat from (C+) showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

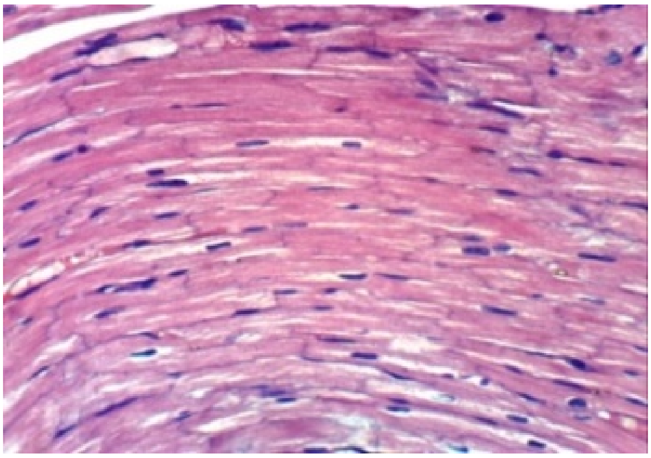

Photo (3): Heart of rat from (Pumpkin seed diet group) showing no showing nohistopathological changes (H & E X 400).

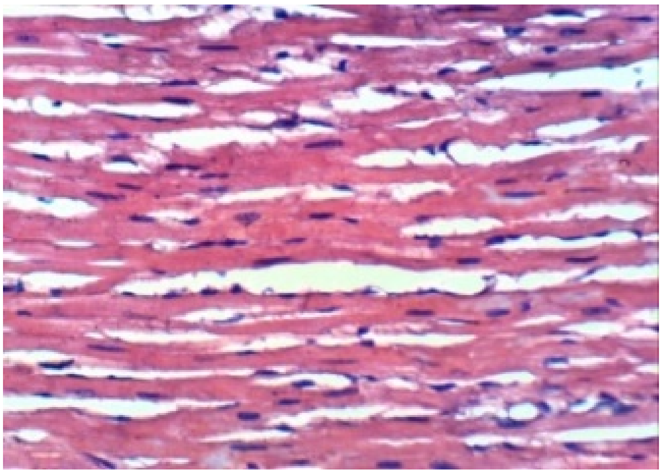

Photo (4): Heart of rat from (flaxseed diet group no histopathological changes (H & E X 400).

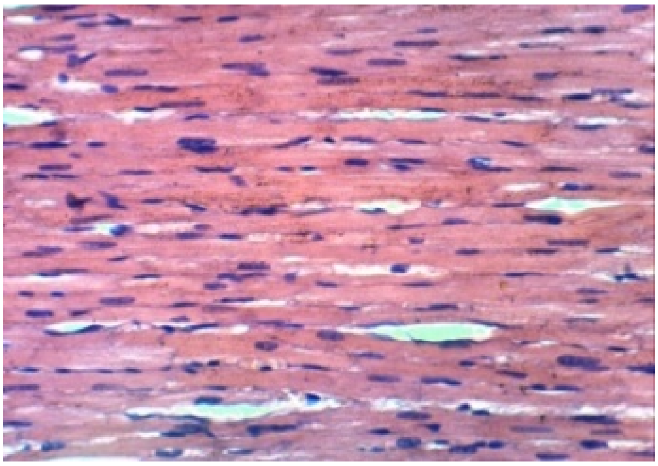

Photo (5): Heart of rat from (sunflower seed diet group) showing no seeds) histopathological changes (H & E X 400).

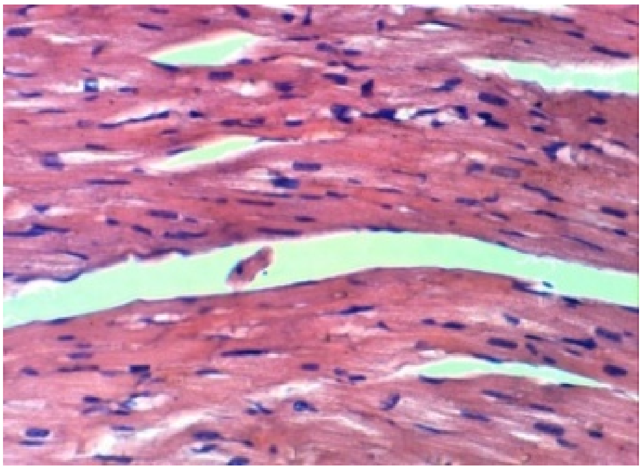

Photo (6): Heart of rat from (pumpkin and sunflower showing no histopathological changes (H & E X 400).

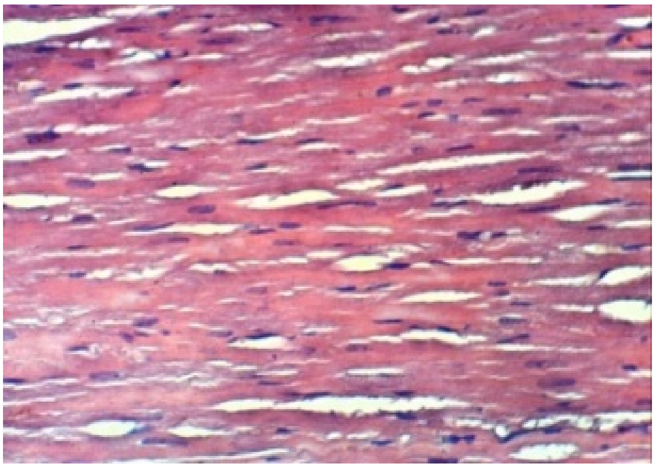

Photo (7): Heart of rat from (pumpkin and flaxseed) diet group showing no histopathological changes (H & E X 400).

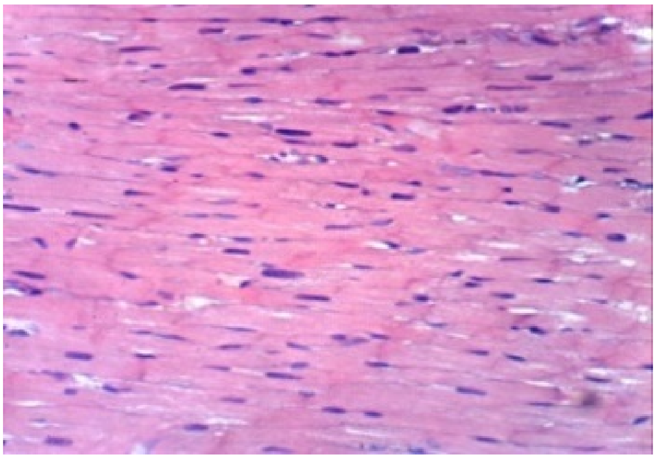

Photo (8): Heart of rat from (sunflower and flaxseed) diet group showing no histopathological changes (H & E X 400).

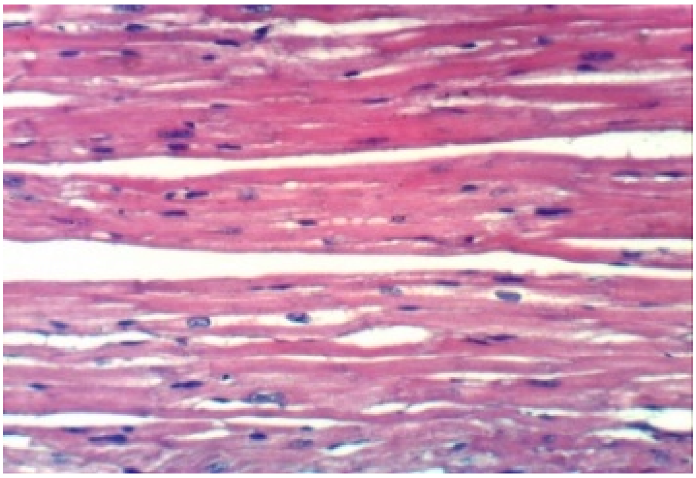

Photo (9): Heart of rat from (sunflower , pumpkin and flaxseed diet group) showing no histopathological changes (H & E X 400).

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دراسة التأثير الغذائي والعلاجي لبذور الكتان ودوار الشمس والقرع على مستوي دهون الدم وزيادة الوزن لدى الفئران

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قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلى – جامعة المنوفية

الملخص العربى

أجريت الدراسة الحالية لمعرفة تأثير مسحوق كلا من بذور الكتان ودوار الشمس والقرع فى تحسين مستوي دهون الدم و إنزيمات الكبد المرتفعة ووظائف الكلي والانزيمات المضادة للأكسدة. تم استخدام ٥٤ فأر ابيض ذكر بالغ يتراوح وزن كل منها ١٥٠±١٠ جرام وتم تقسيمهم الي مجموعتين اساسيتن مجموعه أساسية ضابطه سالبه تتكون من ٦ فئران تغذت علي الغذاء الاساسي ومجموعه ضابطه موجبه تتكون من ٤٨ فأراً تغذوا علي وجبه عاليه الدهن الحيواني لمده أربعة اسابيع لرفع دهون الدم. تم تقسيم الفئران إلي مجموعتين اساسيتين المجموعة الاولى تتكون من ٦ فئران تغذت علي الغذاء الأساسي) والثانية قسمت الى ثماني مجموعات فرعيه كالتالي: المجموعة الأولي المصابة تركت كمجموعه ضابطه موجبه. المجموعة الثانية المصابة تغذت على الغذاء المحتوى على ٥% من بذور الكتان لكل كجم من الغذاء. المجموعة الثالثة المصابة تغذت على الغذاء المحتوى على ٥% من بذور دوار الشمس لكل كجم من الغذاء. المجموعة الرابعة المصابة تغذت علي الغذاء المحتوي علي ٥% من بذور القرع لكل كجم من الغذاء. المجموعة الخامسة المصابة خليط من بذور القرع مع بذور دوار الشمس بتركيز٥%. المجموعة السادسة المصابة خليط من بذور القرع مع بذور الكتان بتركيز ٥%. المجموعة السابعة المصابة خليط من بذور دوار الشمس مع بذور الكتان بتركيز ٥%. المجموعة الثامنة المصابة خليط من بذور دوار الشمس و بذور الكتان مع بذور القرع بتركيز ٥%. وتم تقدير الكولسترول الكلى، الجلسريدات الثلاثية، الليبوبروتينات (HDL–C, LDL–C, VLDL–C، CRR, LDL/HDLمعامل تصلب الشرايين, إنزيمات الكبد(ALT, AST, ALP)، البروتين الكلى ، الألبيومين, الجلوبيولين, النسبة بين الألبيومين إلي الجلوبيولين, اليوريا, حمض اليوريك, الكرياتنين, الانزيمات المضادة للاكسدة (سوبرأكسيد ديسموتيز والكتاليز وجلوتاثيون بيروكسيديز) ومستوى مالون داى الدهيد. كذلك إجراء الفحص الهستوباثولوجى للقلب. وقد أظهرت نتائج هذه الدراسة أن تناول بذور الكتان ودوار الشمس والقرع (٥%) قد نتج عنه تحسن في دهون الدم ووظائف الكبد والكلي ومستوى الانزيمات المضادة للاكسدة (سوبرأكسيديز والكتاليز وجلوتاثيون بيروكسيديز) ومستوى مالون داى الدهيد وتحسن في تغيرات انسجه القلب. وطبقا لهذه النتائج فإنه يمكن استخدام بذور الكتان ودوار الشمس والقرع لتحسين الحالة الصحية للبدناء والمساهمة فى انقاص اوزانهم.

الكلمات المفتاحية: السمنة، دهون الدم، إنزيمات الكبد، الانزيمات المضادة للاكسدة (سوبرأكسيد ديسموتيز والكتاليز وجلوتاثيون بيروكسيديز) ومستوى مالون داى الدهيد, التغيرات الهستوباثولوجية

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