Faculty of Home Economics

Journal of Home Economics
Print ISSN: 2735-5934, Online ISSN: 2735-590X
Menoufia University, Shibin El Kom, Egypt
https://mkas.journals.ekb.eg

Menoufia University

Nutrition and Food Sciences

Article Type
Original Article

Effect of Chia (*Saliva Hispanic L.*) Seeds on Hypercholesterolemic Rats.

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DOI:*10.21608/MKAS.2022.149623.1147*

Cite as:
Helal et al., 2023, Effect of Chia (Saliva Hispanic L.) Seeds on Hypercholesterolemic Rats. J Home Econ. 33(1), 47-62.

Received: 7 Jul 2022
Accepted: 27 Sep 2022
Published: 1 Jan 2023

Printed in Menoufia University, Egypt.

Abstract:
The present study was designed to study the effect of chia seeds powder on hepatic rats. Thirty six adult male albino rats were used in this study, weighting (150±10g) were divided into six groups, six rats each. One of them was kept as a control –ve group, while the other five groups were injected by 1.5% cholesterol for 21 days to induce hypercholesterolemia disorder. One of them was kept as a control (+) group and the other four groups treated with chia seeds powder at concentrations 1.5%, 3%, 4.5% and 6% from the main diet. Body weight gain, feed intake, feed efficiency ratio, serum lipid profiles (TG, TC, LDL-c, VLDL-c and HDL-c), serum glucose, serum liver enzymes (ALT, AST and ALP) and kidney functions (creatinine, uric acid and urea levels) were determined. From the obtained results it could be concluded that feeding on chia seeds powder caused significant (P≤0.05) increase in HDL-c, but with significant (P≤0.05) decreases in body weight gain, liver functions, kidney functions, lipid profile and serum glucose as compared with control (+ve) group, which reflects the powerful nutraceutical therapeutic effect for feeding on chia seeds powder for treatment hypercholesterolemia disorder in rats. The best results recorded for 6% chia seeds powder.

***Keywords**: Cholesterol, Lipoproteins, Chia seeds, Rats, Biochemical analysis*

Introduction

Hypercholesterolemia can be defined as the presence of high plasma cholesterol levels, with normal plasma triglycerides, as a consequence of the rise of cholesterol and apolipoprotein B (apoB)-rich lipoproteins, called low-density lipoprotein (LDL) **(1).**

Cholesterol is a sterol, a sort of fat. It is one of three major classes of lipids which all animal cells utilize to construct their membranes and is thus manufactured by all animal cells. Plant cells do not manufacture cholesterol. it is also precursor of all steroid hormones, bile acid and vitamin D. cholesterol circulate in the blood stream. It is an essential molecule for the human body. Cholesterol is a molecule from which hormone and steroids are made. it is also used to maintain nerve cells between 75% and 80% of the cholesterol that circulate

in a person blood stream is made in that precursor liver. A normal or desirable cholesterol level is defined as less than 200mg of cholesterol per deciliter of blood. Blood cholesterol is considered to be border line when it in the range of 200 to 239mg/dl. Elevated cholesterol is level is 240mg/dl or above. Elevated level cholesterol is considered to be hypercholesterolemia. Since cholesterol is insoluble in water, is transported in the blood plasma within protein particles (lipoprotein). Lipoprotein are classified by their density very low density lipoprotein (VLDL), Low density lipoprotein (LDL), High density lipoprotein (HDL). All the lipoprotein carry cholesterol, but elevated level of lipoprotein other than HDL (termed non HDL cholesterol), particularly LDL-cholesterol are associated with an increased risk of atherosclerosis and coronary heart disease. In contrast higher levels of HDL cholesterol are protective [(2)].

Lipid (cholesterol and triglyceride) and lipoprotein metabolism disorders are key proven independent risk factors in the development and progression of atherosclerotic CHD. Cholesterol is necessary, but too much of it causes deposition in the blood vessels, which constricts them and leads to obstruction and, eventually, heart attack. Cholesterol accumulation in blood vessels, resulting in plaque formation and blocking blood flow. Hereditary factors, stress, smoking, obesity, and dietary cholesterol are the primary causes of elevated cholesterol levels. Cardiovascular diseases (CVDs) are the leading causes of death worldwide, accounting for around 30% of all deaths [(3)].

Chia (*Salvia hispanica L.)* belongs to the family Labiatae, originated from Mexico and northern and Guatemala. The seeds have been the part of human nutrition since 1500 BC [(3,4)].

Chia seeds is high in protein, soluble dietary fiber, antioxidants, and polyunsaturated fatty acids (PUFA), especially linolenic acid (omega3). These fibers may help to decrease LDL cholesterol and slow digestion, preventing blood sugar rises after meals and promoting a sensation of fullness [(6)].

Linolenic acid (ALA), which makes up around 60% of all fatty acids, and other polyunsaturated fatty acids are also abundant in chia seeds. Smaller amounts of linoleic, oleic, and palmitic acids are also found. Chia seeds also have higher omega-3 fatty acids than flaxseed. Additionally, the advantageous 0.3:0.35 ratio of omega-6 to omega-3 fatty acids should be emphasized [(7)].

Also, Higher dietary consumption of omega-3 fatty acids from both marine (EPA and DHA) and plant sources (ALA) is firmly linked to a lower risk of death from cardiovascular disease, which is a leading cause of death in the world today [(8)].

The goal of this study was to see how chia seeds affected rats with hypercholesterolemia.

Material and Methods

Materials

The used seeds:

Chia seeds were obtained from Imtenan Company in Cairo , Egypt.

Rats:

A total of 36 adult normal male albino rats Sprague- Dawley ,strain weighting 150±10g were obtained from Faculty of Agriculture Cairo University, Giza, Egypt.

Basal diet components:

The Basal **diet contains 10% protein (casein is the main source), 5% cellulose, 1% vitamin mixture, and 4% salt mixture.** 3%methionine and 79.5%corn starch were obtained from Technogene Chemical Co., Dokki, Egypt**.**

Chemical and kits:

Cholesterol powder and all chemical kits used in this study obtained from El-Gomhoria Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. While the animal fat obtained from a butcher shop.

Methods:

Preparation of *the dried powder of Chia seeds*:

Weighed amount of chia seeds were roasted on taws, initially for 5 minutes at low flame, so that the leaves become crisp and brittle to touch. Then the roasted chia seeds were finely grinded into fine powder to make chia seeds powder. Source of heat was gas oven. The freshly prepared chia seeds was weighed and stored at room temperature and kept in dusky stoppered glass bottles in a cool and dry location till use according to Russo, who reported that all herbs and plants are best kept in a cool, dry, and dark location to reduce oxidation of their contents [(9)]**.**

The induction of experimental hypercholesterolemia:

Hypercholesterolemia was induced in normal healthy male albino rats by addition of 1.5% cholesterol powder for 21 days.

Animals:

Thirty six adult normal male albino rats weighting 150±10g. rats were housed in wire cages under the normal laboratory condition and were fed on basal diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid loses conditions of feed, water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad-labium and checked daily.

Biological experiments:

Basel diet composition of tested rats:

The standard diet was formulated according to AIN [(10)]**,** guidelines Reeves *et al.* [(11)]. Salt mixture and vitamins mixture were prepared according to Hegested *et al.* [(12)] and Campbell, [(13)].

Experimental design:

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin El-kom. Thirty six adult male white albino, 10 weeks age, weighting (150±10 g) were used in this experiment. All rats were fed on standard diet according to American Institute of Nutrition (AIN) for 7 days for adaptation and divided into two main groups [(10)].

The first main group fed on basal diet as a control negative group (6 rats).

The second main group (Hypercholesterolemic rats) (30 rats) hypercholesterolemia was induced in normal healthy male albino rats by addition of 1.5% cholesterol powder for 21 days.

And were divided into 5 sub-groups (6 rats for each group) as the following:

Sub-group 1: Hypercholesterolemic rats fed on basal diet as a control positive group.

Sub-group 2: Hypercholesterolemic rats fed on basal diet with 1.5% of chia seeds powder.
Sub-group 3: Hypercholesterolemic rats fed on basal diet with 3% of chia seeds powder.
Sub-group 4: Hypercholesterolemic rats fed on basal diet with 4.5 % of chia seeds powder.
Sub-group 5: Hypercholesterolemic rats fed on basal diet with 6% of chia seeds powder.
During the experimental period, the body weight gain and feed intake were estimated weekly and the general behavior of rats was observed. experimental diets for (28) days according to the following:

Blood sampling:

At the end of experimental period (28) days each rat weight separately then, rats are slaughtered and collect blood samples after 12 hours of fasting. Blood samples were centrifuged at (4000 rpm) for ten minutes to separate blood serum, then kept in deep-freezer till using.

Organs:

Liver, spleen, kidney , lungs and heart removed, washed in saline solution and weight.

Biological evaluation:

During the experimental period **(**28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (B.W.G.), feed efficiency ratio (F.E.R), and relative organs weight were determined according to Chapman *et al*. [14]. Using the following equations:

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

$$\text{FI} = \text{Food consumed -food missing}$$

$$\text{F.E.R.} = \frac{\text{Grams gain in body weight(g)}}{\text{Grams feed consumed(g)}}$$

Analytical methods:

The following techniques were used for determination of different parameters in serum:
Total lipid (TL) according to Schmidt-Sommerfeld *et al*. [15], Total cholesterol (TC) according to Allain [16], Triglycerides (TG) according to Fossati & Prencipe [17] and High density lipoprotein (HDL) lipid according to Allain [16]. Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were calculated according to the methods of Lee and Nieman [18] as follows:

$$\text{VLDL}=\text{TG}/5.$$
$$\text{LDL}=\text{Total cholesterol (HDL+VLDL)}$$

Atherogenic Index of Plasma (AIP) was calculated according to Bhardwaj *et al*. [19] as follow**:**

$$\text{AI} = \log \text{TG/HDL-c}$$

Alanine amino transferase (ALT) according to Bergmeyer & Harder [20], aspartate amino transferase (AST) according to Kachmar & Moss [21] and alkaline phosphates (ALP) enzymes were measured according to and Belfield & Goldberg [22]. Serum glucose was estimated

according to Rojas *et al.* [23]**.** Urea, uric acid and creatinine levels were determined in serum according to the method described by Houot *et al.* [24]**.**

Statically analysis:

The data were statistically analyzed using a computerized costat program by one way ANOVA. The results are presented as mean± SD. Differences between treatments at (P > 0.05) were considered significant according to SAS [25]**.**

Results and Discussion

Table (1) Showed the Effect of chia seeds on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of hypercholesterolemic rats. It could be noticed that the mean values of BWG% of control (+) group was significantly lower than control (-) group, being 19.3 and 23 respectively with percent of increase 19.7 as comparing to control (+) group. All hyperchoesterolemic rats fed on chia seeds showed significant increases in mean values as compared to control (+) group. The best BWG% was recorded for group 6 (hyperchoesterolemic rats fed on chia seeds 6%) when compared to control (-) group.

Data revealed that the mean value of FI of control (+) group was lower than control (-) group, being 19.6 and 23.3 respectively. the percent of increase was 18.87 for control (-) group as compared with control(+) . All hyperchoesterolemic rats fed on chia seeds powder showed significant differences in mean values as compared to control (+) group. Rats fed on groups 4 and5 showed nonsignificant differences between them. The percent of increase were 29.08, 7.14, 18.87 and 27.55 for groups 3,4,5 and 6 respectively.

Numerically the best FER was recorded for group 6 (hyperchoesterolemic rats fed on chia seeds 6%) when compared to control (-) group.

Data revealed that the mean value of FER of control (-) group was lower than control (+) group, being 0.96 and 0.98 respectively. the percent of decrease was -2.04. All hyperchoesterolemic rats fed on chia seeds powder showed non-significant increase in mean values as compared to control (+) group. Numerically the best (F.E.R)was recorded for group6 (hyperchoesterolemic rats fed on *chia* seeds 6%) when compared to control (-) group. This result disagree with Da Silva *et al*. who found that chia seeds decreased body weight compared to the group of animals consuming the standard control diet [26]. These results are in agreement with Poudyal *et al.* reported that chia seeds increased the weight in rats fed an HFD, it also reduced the visceral adiposity index and decreased the retroperitoneal and omental fat depositions [27].

As well, these findings are in accordance with study by Creus *et al*. who found Three months of chia eating resulted in a considerable increase in rat body weight [28]. Also, these data are harmonious with Montes chani *et al*. they confirmed that long-term (13-month) ingestion of black chia causes a considerable rise in rat body weight [29]. According to Da Silva *et al*. found that The rats' weight gain could be attributed to the reduced digestion of chia protein, among other causes [26].

Data presented in table (2) showed the effect of chia seeds powder on serum triglycerides (T.G) and serum total cholesterol (T.C) levels of hypercholesterolemic rats. It could be observed that the mean value of (T.C) of control (+) group was significantly higher than control (-) group, being 141.3 and 65.00 mg/dI, respectively. with percent of decrease -

53.99% as comparing to control (+) group. All hyperchoesterolemic rats fed on chia seeds powder revealed significant decreases in mean values as compared to control (+) group. Rats fed on groups 5 and 6 showed nonsignificant differences between them. Numerically the better serum (T.C) was showed for group 6 hyperchoesterolemic rats fed on chia seeds 6%) when compared to control (-) group.

Table (1) Effect of chia seeds powder on body weight gain%, feed intake and feed efficiency ratio of hypercholesterolemic rats.

Parameters / Groups	Body weight gain	Feed intake (g/day)	Feed efficiency ratio
G1:- (-ve)	23.00 b ± 2.64	23.30 ab ± 1.52	0.96 a ± 0.115
G2:- (+ve)	19.30 c ± 1.52	19.60 b ± 2.08	0.98 a ± 0.155
G3:- 1.5% Chia seeds powder	32.30 a ± 2.08	25.30 a ± 2.08	1.28 a ± 0.168
G4:- 3% Chia seeds powder	24.00 b ± 1.00	21.00 ab ± 2.00	1.14 a ± 0.143
G5:- 4.5% Chia seeds powder	23.00 bc ± 1.00	23.30 ab ± 1.52	0.986 a ± 0.023
G6:- 6% Chia seeds powder	31.60 a ± 1.52	25.00 b ± 2.00	1.27 a ± 0.104
LSD	3.29	3.38	0.225

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

In case of (T.G) it could be noticed that the mean values of control (+) group were significantly higher than control (-) group, being 191.60 and 97.30 mg/dI, respectively. with percent of decrease -2.7% as comparing to control (+) group. All hyperchoesterolemic rats fed on chia seeds powder revealed significant decreases in mean values as compared to control (+) group. Rats fed on groups 5 (hyperchoesterolemic rats fed on chia seeds 4.5%) and 6 (hyperchoesterolemic rats fed on chia seeds 6%) showed nonsignificant differences between them. Numerically the better serum (T.G) was showed for group 6 (hyperchoesterolemic rats fed on chia seeds 6%) when compared to control (-) group. The results in this Table show hyperchoesterolemic (control +ve) resulted in significant increases (P≤0.05) in total cholesterol and triglycerides when compared to (control-ve). The use of chia seeds in hyperchoesterolemic groups improved total cholesterol and triglycerides.

These findings are consistent with Oliva *et al.* who found that the ratio of TG was considerably greater in the positive control groups than in the treatment groups given chia seeds for 21 days [30]**.** According to Fortino *et al***.** found that on a pregnant rat research, the offspring had decreased liver steatosis, hypertriglyceridemia, and hypercholesterolemia when maize oil was replaced with chia seed in a sucrose-rich diet [31].

These finding are identical to Da Silva *et al*. indicated that chia seeds reduce the levels of serum cholesterol, since it has high concentrations of dietary fiber and unsaturated omega-3 fatty acids [32]. Coelho *et al*. found that chia proteins and chia bioactive peptides can block key markers of cholesterol synthesis such as 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) [33]. Also, Creus *et al*. mentioned that Chia normalized dyslipidemia by preventing FAT/CD36 translocation, which lowered FA inflow while also

lowering M-CPT1 activity and fat storage, resulting in improved glucose oxidation in cardiac muscles [28].

Table (2): Effect of chia seeds powder on serum triglycerides (T.G) and serum total cholesterol (T.C) levels of hypercholesterolemic rats

Parameters Groups	Total cholesterol mg/dl	Triglycerides mg/dl
G1:- (-ve)	65.00 d ± 1.00	97.30 d ± 6.60
G2:- (+ve)	141.30 a ± 4.72	191.60 a ± 18.23
G3:- 1.5% Chia seeds powder	96.60 b ± 8.96	158.60 b ± 7.50
G4:- 3% Chia seeds powder	86.00 c ± 2.00	127.00 c ± 2.30
G5:- 4.5% Chia seeds powder	77.00 d ± 3.60	110.60 d ± 5.50
G6:- 6% Chia seeds powder	69.33 d ± 2.51	104.00 d ± 1.73
LSD	15.45	15.45

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Data presented in table (3) show the effect of chia seeds powder on serum lipid profile levels of hypercholesterolemic rats. It could be noticed that the mean value of high density lipoprotein (HDL-c) of control (-) group was significantly higher than control (+) group, being 34.60 and 17.00 mg/dl, respectively. with percent of increase 103.52% as comparing to control (+) group. All hyperchoesterolemic rats fed on chia seeds powder revealed significant decreases in mean values as compared to control (+) group. Rats fed on groups 3 (hyperchoesterolemic rats fed on *chia* seeds 1.5%) and 4 (hyperchoesterolemic rats fed on *chia* seeds 3%) showed nonsignificant differences between them but groups 5 (hyperchoesterolemic rats fed on *chia* seeds 4.5%) and 6 (hyperchoesterolemic rats fed on *chia* seeds 6%) showed significant differences. Numerically the better serum HDL was showed for group 6 (hyperchoesterolemic rats fed on *chia* seeds 6%) when compared to control (-) group.

It could be noticed that the mean value of low density lipoprotein(LDL-c) of control (+) group was significantly higher than control (-) group, respectively. All hyperchoesterolemic rats fed on chia seeds powder revealed significant decreases in mean values as compared to control (+) group. with percent of increase -87% as comparing to control (+) group. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group. The percent of decreases were -46.62 ,-53.25 ,-62.956 and -73.80 for groups 3 (chia seeds 1.5%), 4(chia seeds 3%), 5(chia seeds 4.5%), and 6(chia seeds 6%), respectively. Rats fed on group 6 (chia seeds 6%) recorded the best serum (LDL-c).

It could be noticed that the mean values of very-low-density lipoprotein cholesterol (VLDL-c) of control (+) group was higher than control (-) group. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group. Groups 4(chia seeds 3%) and 5(chia seeds 4.5%) indicated nonsignificant differences. The best treatment was recorded for group 6(chia seeds 6%) considering serum (VLDL-c).

The results in this Table show hyperchoesterolemic (control +ve) resulted in significant increases (P≤0.05) in LDL-c and VLDL-c ,decreases in HDL when compared to (control-ve). concentrations of HDL-c in treated groups had significantly higher compared to the positive control group. With respect to LDL-c and VLDL-c.

These results are conformed with Toscano *et al*. reported that overweight and obese people who consumed 35 g of chia flour per day had their total cholesterol and VLDL-c levels drop while their HDL-c levels rise [(34)]**.** According to Koh *et al*. found that Higher dietary consumption of omega-3 fatty acids from both marine (EPA and DHA) and plant sources (ALA) is firmly linked to a lower risk of death from cardiovascular disease, which is a leading cause of death in the world today [(35)].

These are conformed with results Da Silva *et al*. reported that Wistar rats fed both regular and thermally treated chia had higher HDL-c levels, as well as greater intestinal muscle layer hypertrophy and protein digestibility [(26)].

These results are in agreement with Da Silva *et al*. reported that in wistar rats fed a high-fat diet, ingestion of chia seed flour for 35 days lowered TC, LDL-c, and VLDL-c while increasing PPAR-protein levels [(36)]**.** As well, these findings are not in accordance with study by **Toscano *et al*.** found that 26 men and women between the ages of 45 and 55 (placebo 7; chia flour 19) After consuming 35 g of chia flour each day for 12 weeks, LDL-c levels increased [(34)].

Table (3): Effect of chia seeds powder on serum lipid profile levels of hypercholesterolemic rats.

Parameters Groups	HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
G1:- (-ve)	34.60 a ± 1.52	10.86 e ± 3.31	19.46 d ± 1.33
G2:- (+ve)	17.00 e ± 1.00	86.00 a ± 2.80	38.33 a ± 3.60
G3:- 1.5% Chia seeds powder	19.00 d ± 1.00	45.90 b ± 9.15	31.73 b ± 1.50
G4:- 3% Chia seeds powder	20.30 d ± 2.08	40.20 b ± 1.21	25.46 c ± 0.46
G5:- 4.5% Chia seeds powder	23.00 c ± 2.00	31.86 c ± 4.49	22.10 cd ± 1.10
G6:- 6% Chia seeds powder	26.00 b ± 1.70	22.53 d ± 4.36	20.80 d ± 0.346
LSD	1.84	6.621	3.41

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05). HDL-c = High density lipoprotein cholesterol. LDL-c = Low density lipoprotein cholesterol. VLDL -c = Very low density lipoprotein cholesterol.

Data presented in table 4 effect of chia seeds as powder on atherogenic index (AI) levels of hypercholesterolemic rats. It could be noticed that the mean value of AI of control (-) group was higher than control (+) group, being 3.09 and 2.82 mg/dl, respectively, showing non-significant differences between them with percent of increase 9.57% of control (-) group when compared to control (+) group. All hyperchoesterolemic rats fed on chia seeds powder revealed non-significant decreases in mean values as compared to control (+) group. The percent of decreases were -2.83,0,.35 and -3.19 of groups 3(chia seeds 1.5%), 4(chia seeds 3%), 5(chia seeds 4.5%),and6(chia seeds6%), respectively. Numerically the best treatment was recorded for group 6 (chia seeds 6%) considering serum (A.I.).

Table (4): Effect of chia seeds on (A.I.) (mg/dl)of hyperchoesterolemic rats

Groups	Atherogenic Index (AI) mg/dl
G1:- (-ve)	3.09 a ± 0.247
G2:- (+ve)	2.82 a ± 0.14
G3:- 1.5% Chia seeds powder	2.74 a ± 0.72
G4:- 3% Chia seeds powder	2.82 a ± 0.78
G5:- 4.5% Chia seeds powder	2.83 a ± 0.16
G6:- 6% Chia seeds powder	2.73 a ± 0.31
LSD	0.842

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05). AI: Atherogenic Index.

Data presented in table 5 show the effect of chia seeds powder on serum liver functions levels (ALP, AST and ALT) levels of hypercholesterolemic rats. It could be noticed that the mean value of (GOT) of control (+) group was significantly higher than control (-) group with percent of decrease -79.24% of control (-) group when compared to control (+) group. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group . The percent of decreases -13,-20.35,-38.29 and -54.49 for groups 3(chia seeds 1.5%), 4(chia seeds 3%), 5(chia seeds 4.5%),and6 (chia seeds 6%), respectively. Rats fed on groups 3 and 4 showed nonsignificant differences between them. The best treatment was observed for group 6(chia seeds 6%) when compared to control (-) group considering (GOT) activity.

It could be observed that the mean value of (GPT) of control (+) group was higher than control (-) group, being 72.00 and 47.33 U/L, respectively, showing nonsignificant differences with percent of decrease -34.26 % of control (-) group when compared to control (+) group. All hyperchoesterolemic rats fed on chia seeds powder revealed significant decreases in mean values as compared to control (+) group The percent of decreases -4.6,-15.75,-21.30and -27.77 for groups 3,4,5 and 6, respectively. Rats fed on groups 4 and 5 showed nonsignificant differences between them. Numerically the best treatment was observed for group 6 (chia seeds 6%) when compared to control (-) group.

It could be observed that the mean value of (ALP) of control (+) group was significantly higher than control (-) group, being 273.33 and 152.00 U/L, respectively, with percent of decrease - 44 % of control (-) group when compared to control (+) group. All hyperchoesterolemic rats fed on chia seeds powder revealed significant decreases in mean values as compared to control (+) group. Rats fed on groups 5 and 6 showed nonsignificant differences between them. Numerically the best treatment was observed for group 6 (chia seeds 6%) when compared to control (-) group. From the results in this table, it could be observed that, the mean value of (ALP), (AST) and (ALT) of control (+) group was significantly higher than control (-) group. All treated groups with 1.5% chia, 3% chia, 4.5% chia and 6% chia improved the mean values of ALT, AST and ALP

These results are parallel with results of Marineli Rda *et al.* they reported that the consumption of white chia seeds was found to be effective for reducing AST levels .also improves liver damage [37].

Also, These findings are consistent with the findings of Alamri, 2019 who found that the levels of ALT and AST were significantly greater in the negative and positive control groups compared to the rats fed chia seed [38]**.** According to the findings of the Da Silva *et al*. study, a high fat, high-fructose diet caused an increase in AST and ALT levels when compared to a control group. Except for the 6-week chia seed therapy, all groups treated with chia demonstrated a return to baseline in AST and ALT (liver damage markers) [39]. According to Fernandez-Martinez *et al.* the hepatoprotective effects of chia may be connected to its high content of α linolenic acid (omega-3), fiber, protein, and phenolic compounds [40].

Also Montes Chañi *et al.* mentioned that, The richest vegetal source of omega 3-fatty acids, antioxidants, and fiber is chia seed (Salvia hispanica). Although some animal studies have showed that chia could be used to lower intrahepatic fat content [29].

Table (5): Effect of chia seeds powder on serum liver functions levels (ALP, AST and ALT) levels of hypercholesterolemic rats:

Parameters / Groups	ALP U/L	AST U/L	ALT U/L
G1:- (-ve)	152.00 d ± 1.52	34.66 e ± 3.51	47.33 d ± 4.61
G2:- (+ve)	273.33 a ± 20.8	167.00 a ± 11.10	72.00 a ± 2.00
G3:- 1.5% Chia seeds powder	229.00 b ± 8.18	145.00 b ± 3.60	68.66 a ± 0.577
G4:- 3% Chia seeds powder	198.33 c ± 8.14	133.00 b ± 6.08	60.66 b ± 2.08
G5:- 4.5% Chia seeds powder	170.33 d ± 3.78	102.00 c ± 20.07	56.66 b ± 2.08
G6:- 6% Chia seeds powder	160.00 d ± 4.04	76.00 d ± 4.35	52.00 c ± 1.00
LSD	16.18	18.04	4.63

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05). ALP: Alkaline phosphatase. AST: Aspartate transaminase. ALT: Alanine aminotransferase.

Data presented in table 6 show the effect of chia seeds as powder on serum kidney functions (Urea, Creatinine and Uric Acid) levels of hypercholesterolemic rats. It could be noticed that the mean value of urea of control (+) group was higher than control (-) group, being 57.66 and 29.33 mg/dl, respectively. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group. The percent of decreases -6.93, -19.7, -27.74 and -39.29 for groups 3,4,5 and 6, respectively. Numerically the best treatment was recorded for group 6(chia seeds 6%) when compared to control (-) group of serum urea.

It could be observed that the mean value of creatinine of control (+) group was significantly higher than control (-) group, being 1.36 and 1.10 mg/dl, respectively. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group. The precedent of decreases were -4.41, -3.67, -11.02 and -14.70 for groups 3,4,5 and 6, respectively. Groups 3 and 4 indicated nonsignificant

differences. the best treatment was recorded for group 6 (chia seeds6%) when compared to control (-) group.

It could be observed that the mean value of uric acid of control (+) group was higher than control (-) group, being 4.96 and 1.86 mg/dl, respectively. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group. The percent of decreases -24.15 ,-36.29 ,-43.54 and -52.29 for groups 3,4,5 and 6, respectively. Rats fed on groups 3 and 4 showed nonsignificant differences between them. Numerically the best treatment was recorded for group 6 (chia seeds 6%) & when compared to control (-) group. These results are in agreement with Mostafa, 2021 who found that revealed treatment with CS at various concentrations resulted in a substantial (p0.05) decrease in uric acid, creatinine, and urea levels as compared to the positive control group **(41).** Also, Fayez *et al*. mentioned that omega-3 fatty acids have a preventive effect against renal impairment in rats **(42).**

Table (6): Effect of chia seeds as powder on serum kidney functions (Urea, Creatinine and Uric Acid) levels of hypercholesterolemic rats.

Parameters	Urea	Creatinine	Uric Acid
Groups	**mg/dl**	**mg/dl**	**mg/dl**
G1:- (-ve)	29.33 f ± 2.51	1.10 c ± 0.10	1.86 e ± 0.25
G2:- (+ve)	57.66 a ± 1.15	1.36 a ± .057	4.96 a ± 0.35
G3:- 1.5% Chia seeds powder	53.66 b ± 3.05	1.30 ab ± .011	3.76 b ± .15
G4:- 3% Chia seeds powder	46.66 c ± 2.08	1.31ab ± .01	3.16 c ± 0.23
G5:- 4.5% Chia seeds powder	41.66 d ± 1.52	1.21bc ± .01	2.80 c ± 0.10
G6:- 6% Chia seeds powder	35.00 e ± 3.00	1.16 c ± .057	2.366 d ± 0.115
LSD	2.63	2.63	0.386

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Data presented in table 7 show the effect of chia seeds as powder on serum glucose levels of hypercholesterolemic rats. It could be observed that the mean value of glucose of control (+) group was significantly higher than control (-) group, being 223.33 and 124.0 mg/dl, respectively. All hyperchoesterolemic rats fed on chia seeds revealed significant decreases in mean values as compared to control (+) group. The percent of decreases -22.08, -28.98, -34.92 and -38.20 for groups 3,4,5 and 6, respectively. Rats fed on groups 5 and 6 showed nonsignificant differences between them. Numerically the best treatment was observed for group 6 (chia seeds 6%) when compared to control (-) group. Our results agreed with Weickert and Pfeiffer they found intestinal lumen viscosity increased by the consumption of soluble dietary fiber, which can decrease the absorption of glucose due to the reduction in the contact between glucose and the enterocyte **(43)**.

Clinical studies also showed that incorporating chia seeds into bread decreased the blood glucose level of patients **(44)**.

These findings are consistent with those of Alamri who found that rats fed white or black chia seeds had lower fasting blood glucose levels, with no significant differences between the two [38].

Table (7): Effect of chia seeds as powder on serum glucose levels of hypercholesterolemic rats.

Parameters Groups	Glucose mg/dl
G1:- (-ve)	124.00 e ± 3.46
G2:- (+ve)	223.33 a ± 7.63
G3:- 1.5% Chia seeds powder	174.00 b ± 7.21
G4:- 3% Chia seeds powder	158.60 c ± 6.42
G5:- 4.5% Chia seeds powder	145.33 d ± 3.05
G6:- 6% Chia seeds powder	138.00 d ± 8.71
LSD	8.74

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Conclusion

In conclusion, our results showed that feeding experimental animals with chia seeds as powder caused a significant (P≤0.05) increase in HDL-c, but with a significant (P≤0.05) decrease in body weight gain, serum lipid profile and blood glucose levels. Also, a significant (P≤0.05) improvement in liver and kidney functions compared to the group control (+ve), reflecting the strong therapeutic effect of feeding on chia seeds as powder for the treatment of hypercholesterolemic rats.

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تأثير بذور الشيا علي الفئران المصابة بارتفاع الكوليسترول

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الملخص العربي:

صممت الدراسة الحالية لدراسة تأثير مسحوق بذور الشيا على الفئران المصابة بارتفاع الكوليسترول. تم استخدام ست و ثلاثون فأر من ذكور الفئران الألبينو البيضاء في هذه الدراسة, يتراوح أوزانها (150±10) وتم تقسيمها الى 6 مجموعات 6 فئران في كل مجموعة، تركت إحداها كمجموعة ضابطة سالبة، أما المجموعات الخمسة الأخرى تم تغذيتها علي 1.5% كوليسترول لمدة 21 يوم وذلك لإحداث الإصابة بارتفاع الكوليسترول و تركت إحداهم كمجموعة ضابطة موجبة, بالنسبة للأربع مجاميع الأخرى تم إضافة مسحوق بذور الشيا بنسبة 1.5 , 3 , 4.5 و 6% من الوجبة. من النتائج التي تم الحصول عليها يمكن استنتاج أن التغذية على مسحوق بذور الشيا أدت عند مستوى معنوية 5% زيادة فى البروتين الدهني عالي الكثافة وانخفاض في باقي التحاليل وذلك مقارنة بالمجموعة الضابطة الموجبة, حيث أدي العلاج بواسطة مسحوق بذور الشيا إلي خفض مستوي الدهون فى الدم و تعزيز وظائف الكلى والكبد عن طريق خفض إنزيمات ألانين أمينو ترانسفيريز , أسبرتات أمينو ترانسفيريز , ألكالين فوسفاتيز, أيضا خفض مستوي اليوريا والكرياتينين وحمض اليوريك وخفض مستوى سكر الدم. هذه النتيجة تعكس التأثير التغذوى العلاجي لمسحوق بذور الشيا على الفئران المصابة بارتفاع الكوليسترول. أفضل نتيجة سجلت لمجموعة 6% مسحوق بذور الشيا.

الكلمات المفتاحية: كوليسترول ، ليبوبروتينات, بذور الشيا ، الفئران ، التحاليل الكيميائية و الحيوية.