

THE EFFECT OF DIFFERENT LEVELS OF NIGELLA SATIVA ON BIOCHEMICAL CHANGE ON HYPERCHOLESTEROLEMIC RATS

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ABSTRACT: *Interest in medicinal plants has burgeoned due to the increased efficiency of new plant-derived drugs and the growing interest in natural products. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. The use of plants as medicines dates from the earliest years of man's evolution. Medicinal plants serve as therapeutic alternatives, safer choices. A larger number of these plants and their isolated constituents have shown beneficial therapeutic effects so we worked this study to determine the effect of different levels of nigella sativa on biochemical changes on hypercholesterolemic rats. Twenty eight white Albino rats 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 seven sub-groups, including five groups fed with different concentrations of (5%, 10%, 15%, 20% and 25%) nigella sativa 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 seven groups of four mice in each group. The results showed the best effect recorded for 20% nigella sativa which this treatment corrected completely the rise of GOT activity which was even less than control (-ve) group ,at the same time highest decrease of T. cholesterol was recorded for 10% nigella sativa also lowest LDL was recorded for (10% nigella sativa. which maximum decrease in T. lipids was found for 15% of nigella sativa. Best result in phospholipids may be that of 15%, nigella sativa diet, maximum increase of HDL was found for (15%) nigella sativa diet.*

Key words: *Nigella sativa - hypercholesterolemic, - Therapeutic nutrition*

INTRODUCTION

Black seeds, also known as nigella sativa, black cumin, kalonji seeds and haba al-barakah (Arabic phrase) have been used by people for thousands of years. Some associate black caraway with black seeds and they come from two different plants. Kalonji seeds are found in India and haba al-barakah is an Arabic word and used in the Middle East mainly. (Ali and Blunden, 2003). nigella sativa (black seeds), an annual flowering plant that grows to 20-30cm tall, is native to Asia and the Middle East. The flowers of this plant are very delicate and pale colored and white. The seeds are used in Middle Eastern cooking, such as in their local breads. The seeds are also used by

thousands for their natural healing abilities, (Banerjee *et al*, 2010). Cholesterol is a soft, waxy substance found in all parts of the body. This includes the nervous system, skin, muscle, liver, intestines, and heart. It is made by the body and also obtained from animal products in the diet. Hypercholesterolemia refers to the presence of higher than normal amounts of total cholesterol circulating in the bloodstream. Cholesterol like fatty substance (lipid) that is essential to the body as protection for the walls of the vasculature (veins and arteries) and linings of body organs, a component in the manufacture of hormones, and a factor in the digestion of consumed fats in foods. N. sativa may have some beneficial

therapeutic effects in the treatment of hyperlipidemia. However, further investigations with a larger sample size are necessary. (Dattner, 2003).

AIM OF STUDY:-

This work aims to show the probable benefit of *nigella sativa* to correct hypercholesterolemia in rats.

MATERIALS AND METHODS

1- Materials:

A- Preparation of *nigella sativa*: *nigella sativa* seeds purchased from local markets and were cleaned thoroughly by washing, dried in draft oven at 45°C for 16 h and milled by Brown Miller, Japan. The obtained powder was packed in polyethylene films and stored at 4°C until using in animal feeding.

B-Experimental animals: Twenty eight Sprague Dawley white male albino rats, weighing about 150 ± 10g were used. The animals were obtained from Helwan Experimental Animals Station. 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.

C-Used chemicals: Cholesterol powder (obtained from Morgan Co. Cairo, Egypt).

2- Methods:

A- Biological experiment

Basal diet composition of tested rats:

The basal diet in the experiment (Tables , 1-3 consisted of casein(10 %) , corn 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%) .

- Preparation of hypercholesterolemic 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).

Table (1): Composition of basal diet:

Ingredients	%
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 10g protein.
Source: (Campbell ,1963).

Abd El- meged**Table (2): Composition of salt mixture%:**

Compounds	Amount (mg)
CaCO ₃	600 mg
K ₂ HPO ₄	645 mg
Ca HPO ₄ . 2H ₂ O	150 mg
MgSO ₄ .2H ₂ O	204 mg
Nacl	334 mg
Fe (C ₆ H ₅ O ₇) ₂ 6H ₂ O	55 mg
KI	1.6 mg
MnSO ₄ .4H ₂ O	10 mg
Zncl ₂	0.5 mg
Cu SO ₄ . 5H ₂ O	0.06 mg

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

Table (3): Composition of vitamin mixture:

Vitamin	Amount
Vitamin E	10 lu
Vitamin K	0.50 lu
Vitamin A	200 lu
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 lu
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

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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 eight Sprague Dawley white male albino rats, weighing about 150 ± 10 g were used in the study. The rats were obtained from Helwan Experimental Animals Station. 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 7 groups each of 4 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 + 5% *nigella sativa*
- Group (4): Hypercholesterolemic group fed on basal diet + 10% *nigella sativa*
- Group (5): Hypercholesterolemic group fed on basal diet + 15% *nigella sativa*
- Group (6): Hypercholesterolemic group fed on basal diet + 20% *nigella sativa*

Group (7): Hypercholesterolemic group fed on basal diet + 25% *nigella sativa*

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 (37°C) 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 remainder was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20°C) until analysis. The organs (liver, kidney, heart, and spleen) were removed and washed with saline solution, weight and kept in formalin solution (10%) according to methods described by (Drury and Wallington ,1980).

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Biological evaluation:

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

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

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

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

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 lipoproteins) 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)..

3.2) Determination of Aspartate Transferase (AST):

Determination of (AST) was carried out according to the method of Henry (1974).

3.3) Determination of Total protein:

T. protein was determined by Biuret method according to the method described by (Weichselbaum, 1964).

3.4) Determination of Albumin :

Albumin was determined in serum according to the method described by Doumas and Biggs, (1971).

4- Determination of kidney function parameter:

4.1) Determination of Creatinine

Creatinine was determined according to kinetic method of (Henry, 1974),

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 with saline solution, 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 probable benefits of *nigella sativa* to correct hypercholesterolemia in rats.

hypercholesterolemia rats evaluation:

Effect of different concentration (5%,10%,15%,20% and 25%) nigella sativa on body weight gain (B. W. G.), food intake (F. I.), and food efficiency ration (F. E. R.).

Data listed in table (5) show the effect of different concentration (5%,10%,15%,20% and 25%) nigella sativa on B. W. G. , F. I. , F. E. R. , of hypercholesterolemia rats. It could be noticed that in control (-ve) normal rats body weight gain (B. W. G.) was 81.75 ± 2.50 g ,while in control (+ve) rats fed on hypercholesterolemia diet without treatment was 55.00 ± 1.47 g. These results denote that there was significant decrease in control (+ve) (B. W. G.) compared to control (-ve) rats. (5%,10%,15%,20% and 25%) *nigella sativa* showed significant B. W. G. increase compared to control (+ve) which was (62.00 ± 2.06 , 75.00 ± 2.74 , 86.00 ± 1.55 , 62.50 ± 1.91 and 66.25 ± 2.47) g respectively.

Food intake (F. I.):

It could be observed that for control (-ve) normal rats food intake (F. I.) was 16.13 ± 0.81 g.. While for control (+ve) rats fed on hypercholesterolemia diet without treatment it

was 17.75 ± 0.45 g. This result denotes there was significant increase in control (+ve) compared to control (-ve). but rats fed on (5%,10%,15%,20% and 25%) *nigella sativa* revealed significant increase in (F. I) when compared to control (+ve) which was (17.75 ± 0.85 , 20.15 ± 0.79 , 21.4 ± 0.99 , 19.7 ± 0.56 and 17.65 ± 0.55) g respectively.

Food efficiency ratio (F. E. R.):

It could be noticed that for control (-ve) normal rats food efficiency ratio (F. E. R.) was 0.18 ± 0.03 , while in control (+ve) rats fed on hypercholesterolemia diet without treatment it was 0.11 ± 0.01 . This result indicated that there was significant difference between control (-ve) and control (+ve). Rats fed on hypercholesterolemia diet and fed on(5%,10%,15%,20% and 25%) *nigella sativa* showed significant F. E. R.) increase compared to control (+ve) which were (0.12 ± 0.03 , 0.13 ± 0.01 , 0.14 ± 0.02 , 0.12 ± 0.03 ,and 0.13 ± 0.02) respectively. Similar results were reported by (Fong, 2002). and (Kider and Abeer , 2006) working on hypercholesterolemia& hyperglycemic rats fed with plant diet

Table (5): Effect of different concentration (5%,10%,15%,20% and 25%) nigella sativa on B. W. G., F. I., F. E. R. of hyperglycemic rats.

Parameters Groups	B. W. G. (g)	F. I. (g)	F. E. R.
Control (-ve)	81.75 ± 2.50^b	16.125 ± 0.81^d	0.18 ± 0.03^a
Control(+ve)	55.00 ± 1.47^f	17.75 ± 0.45^c	0.11 ± 0.01^f
5% <i>nigella sativa</i>	62.00 ± 2.06^e	17.75 ± 0.85^c	0.12 ± 0.03^d
10% <i>nigella sativa</i>	75.00 ± 2.74^c	20.15 ± 0.79^b	0.13 ± 0.01^c
15% <i>nigella sativa</i>	86.00 ± 1.55^a	21.4 ± 0.99^a	0.14 ± 0.02^b
20% of <i>nigella sativa</i>	62.50 ± 1.91^e	19.7 ± 0.56^b	0.12 ± 0.03^d
25% of <i>nigella sativa</i>	66.25 ± 2.47^d	17.65 ± 0.55^c	0.13 ± 0.02^c

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- Effect of different levels of nigella sativa on the rats organs:

Data listed in table (6) showed the effect of (5%,10%,15%,20% and 25%) nigella sativa on relative organs weight of hypercholesterolemic rats.

- Relative weight of liver:

Data of table (6) showed that for control (-ve) normal rats liver (%) was $3.68 \pm 0.3\%$ while in control (+ve) rats fed on hypercholesterolemic diet without treatment was $2.96 \pm 0.09\%$. These result denotes there was significant decrease in control (+ve) compared to control (-ve) due to possible atrophy.

Rats fed on (5%,10%,15%,20% and 25%) nigella sativa included significant increase of liver % compared to control (+ve) group, values were 3.06 ± 0.12 , 3.31 ± 0.11 , 3.36 ± 0.05 , 3.08 ± 0.06 , and 3.18 ± 0.07 respectively.

- Relative weight Heart:

Data of table (6) showed that for control (-ve) normal rats heart % was $0.35 \pm 0.03\%$ while for control (+ve) rats fed on hypercholesterolemic diet without treatment it was $0.28 \pm 0.02\%$. The obtained data indicated that there was significant decrease in control (+ve) compared to control (-ve) probably due to atrophy. Rats fed on (5%,10%,15%,20% and 25%) nigella sativa included the heart % increase compared to control (+ve) group which showed 0.34 ± 0.05 , 0.30 ± 0.03 , 0.34 ± 0.02 , 0.33 ± 0.01 and $0.33 \pm 0.02\%$ respectively. These results were in agreement with reported by (Ahmed and Reham, 2007); feeding of hypercholesterolemic rats on certain plants ameliorated the heart weight % occurred to ailment.

- Relative weight Spleen:

Data recorded in table (6) revealed that in control (-ve) normal rats spleen % was $0.44 \pm 0.05\%$, while for control (+ve) rats fed on hypercholesterolemic diet without

treatment showed $0.47 \pm 0.02\%$, indicated possible inflammation; any how it could be observed that there were significant increase in control (+ve) compared to control (-ve). Rats fed on hypercholesterolemic diet and fed on (5%,10%,15%,20% and 25%) nigella sativa indicated significant decrease of spleen compared to control (+ve) group which were 0.43 ± 0.09 , 0.35 ± 0.02 , 0.46 ± 0.19 , 0.43 ± 0.05 , $0.44 \pm 0.09\%$ respectively. The best result of treatments was recorded for rats fed on hypercholesterolemic diet and fed on 10%, nigella sativa compared to control (+ve) group which were 0.35 ± 0.02 , and $0.47 \pm 0.02\%$ respectively. according to (Ahmed and Reham, 2007); feeding of hypercholesterolemic rats on certain plants decreased the spleen weight % rise due to ailment.

- Relative weight Kidneys:

Data of table (6) revealed that in control (-) normal rats kidneys % was $0.73 \pm 0.06\%$ while for control (+ve) rats fed on hypercholesterolemic diet without treatment was $0.53 \pm 0.02\%$. The obtained data stated that there were significant decrease in control (+) compared to control (-) due to possible atrophy. Rats fed on hypercholesterolemic diet and fed on (5%,10%,15%,20% and 25%) nigella sativa included significant increase of relative kidneys weight compared to control (+ve) which were 0.57 ± 0.03 , 0.62 ± 0.02 , 0.61 ± 0.02 , 0.56 ± 0.03 , and 0.62 ± 0.04 respectively. As reported by (Ahmed and Reham, 2007); feeding of hypercholesterolemic rats with certain plants ameliorated the kidney weight decrease due to hypercholesterolemia.

- Relative weight Lungs:

The results of table (6) indicated that in control (-) normal rats lungs % was $0.76 \pm 0.08\%$, while for control (+ve) rats fed on hypercholesterolemic diet without treatment it was $0.57 \pm 0.02\%$. These data reflected that there was significant decrease in control

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(+ve) compared to control (-ve) possibly due to atrophy. Rats fed on hypercholesterolic diet and fed on (5%,10%and15%,) *nigella sativa* included significant increase compared to control (+ve) which were 0.72 ± 0.03 , 0.71 ± 0.03 , and 0.65 ± 0.03 % respectively. On the other hand in hypercholesterolic rats and fed on(20% and 25%) *nigella sativa* nonsignificant changes occurred compared to control (+) group which were 0.57 ± 0.06 , 0.58 ± 0.03 and 0.57 ± 0.02 % respectively.

Effect of different levels of *nigella sativa* on the liver enzymes of rats

Data presented in table (7) show the effect of (5%,10%,15%,20% and 25%) *nigella sativa* on liver enzymes (GOT, GPT, and ALP) of hypercholesterolic rats.

- Relative GOT:

The obtained data table (7) revealed that in control (-) normal rats revealed GOT value of 48.75 ± 1.25 (U/L) while for control (+ve) rats fed on hypercholesterolic diet without treatment it was 88.00 ± 1.30 (U/L). These results denote that there was significant increase in control (+ve) compared to control (-ve) group due to inflammation. Rats fed on hypercholesterolic diet with (5%,10%,15%,20% and 25%) *nigella sativa* recorded significant decrease compared to

control (+ve) group which were 34.25 ± 1.04 , 44.25 ± 1.27 , 60.25 ± 2.29 , and 46.75 ± 3.35 (U/L) respectively. The best result recorded for 20% *nigella sativa* which was 21.25 ± 2.61 (U/L). This treatment corrected completely the rise of GOT activity which was even less than control (-ve) group, percent decrease was 56.4 % compared to control (-ve) rats.

Relative GPT:

The GPT of control (-) normal rats was 17.25 ± 0.63 (U/L), while in control (+ve) rats fed on hypercholesterolic diet without treatment it was 51.00 ± 0.92 (U/L). The obtained data reflected that there were significant differences between control (+ve) and (-ve) groups indicating liver inflammation. Rats fed on hypercholesterolic diet with (5%,10%,15%,20% and 25%) *nigella sativa* reflected significant decrease compared to control (+ve) group which were 14.25 ± 1.75 , 13.25 ± 1.71 , 17.25 ± 2.66 , and 12.5 ± 1.07 (U/L) respectively. On the other hand the best result recorded in rats fed on sweet (20%) *nigella sativa* diet which was 10.5 ± 1.20 (U/L). This treatment corrected completely the rise of GPT due to ailment, value was even less than of control (-ve) group,(percent decrease was 39.1 %); at the same time was evidently less than control (+ve) rats(percent decrease was 79.4 %).

Table (6): Effect of (5%,10%,15%,20% and 25%) *nigella sativa* on relative organs weight in hypercholesterolic rats.

Parameters Groups	Liver (%)	Heart (%)	Spleen (%)	Kidney (%)	Lungs (%)
Control (-ve)	3.68 ± 0.3^a	0.35 ± 0.03^a	0.44 ± 0.05^b	0.73 ± 0.06^a	0.76 ± 0.08^a
Control(+ve)	2.96 ± 0.09^e	0.28 ± 0.02^c	0.47 ± 0.02^a	0.53 ± 0.02^d	0.57 ± 0.02^d
5% <i>nigella sativa</i>	3.06 ± 0.12^d	0.34 ± 0.05^a	0.43 ± 0.09^b	0.57 ± 0.03^c	0.72 ± 0.03^b
10 % <i>nigella sativa</i>	3.31 ± 0.11^b	0.30 ± 0.03^b	0.35 ± 0.02^c	0.62 ± 0.02^b	0.71 ± 0.03^b
15% <i>nigella sativa</i>	3.36 ± 0.05^b	0.34 ± 0.02^a	0.46 ± 0.19^a	0.61 ± 0.02^b	0.65 ± 0.03^c
20% <i>nigella sativa</i>	3.08 ± 0.06^d	0.33 ± 0.01^a	0.43 ± 0.05^b	0.56 ± 0.03^c	0.57 ± 0.06^d
25% <i>nigella sativa</i>	3.18 ± 0.07^c	0.33 ± 0.02^a	0.44 ± 0.09^b	0.62 ± 0.04^b	0.58 ± 0.03^d

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Table (7): Effect of (5%,10%,15%,20% and 25%) nigella sativa on liver enzymes (GOT, GPT, and ALP) of hypercholesterolmic rats.

Parameters Groups	GOT (U/L)	GPT (U/L)	ALP (U/L)
Control (-ve)	48.75±1.25 ^c	17.25±0.63 ^b	260.5±7.06 ^e
Control(+ve)	88.00±1.30 ^a	51.00±0.92 ^a	390.0±2.20 ^a
5% <i>nigella sativa</i>	34.25±1.04 ^d	14.25±1.75 ^c	327.5±4.29 ^c
10% <i>nigella sativa</i>	44.25±1.27 ^c	13.25±1.71 ^c	350.5±3.12 ^b
15% <i>nigella sativa</i>	60.25±2.29 ^b	17.25±2.66 ^b	359.5±3.34 ^b
20% <i>nigella sativa</i>	21.25±2.61 ^e	10.5±1.20 ^d	350.00±2.38 ^b
25% <i>nigella sativa</i>	46.75±3.35 ^c	12.5±1.07 ^d	300.5±4.70 ^d

- Relative ALP:

The obtained data revealed that in control (-) normal rats ALP activity was 260.5 ± 7.06 (U/L) while in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 390.0 ± 2.20 (U/L) indicating possible inflammation of liver. Also it could be observed that there were significant increase in control (+ve) to about 50% that of control (-ve). Rats fed on hypercholesterolmic diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* recorded significant decrease compared to control (+ve) group, values were 327.5 ± 4.29, 350.5 ± 3.12, 359.5 ± 3.34, 350.00 ± 2.38, and 300.5 ± 4.70 (U/L) respectively. (Mohammad Aziz Dollah *et al*, 2013) found that there was no significant change in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) between treatment groups. Histopathological study showed very minimal and mild changes in fatty degeneration in normal and high doses of *nigella sativa* treated group. Inflammation and necrosis were absent

Effect of (5%,10%,15%,20% and 25%) *nigella sativa* on (T. protein, Albumin, Globulin, and Alb/Glob) in hypercholesterolmic rats

Data present in table (8) show the effect of (5%,10%,15%,20% and 25%) *nigella sativa* on (T. protein, Albumin, Globulin, and Alb/Glob) of hypercholesterolmic rats.

- T. protein:

It could be noticed that for control (-ve) normal rats T. protein was 10.9±0.56 (g/dl). While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 9.23±0.48 (g/dl). These results denote that there were significant decrease in control (+ve) compared to control (-ve); percent increase was 15.3%. Rats fed on hypercholesterolmic diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant increase compared to control (+ve) which were 10.5 ± 0.38, 9.88 ± 0.86, 9.83 ± 0.63, 11.63±0.08, 11.08 ± 0.41(g/dl) respectively. The best result was for hypercholesterolmic rats fed on (20%) *nigella sativa* which was 11.63±0.08 (g/dl).

- Serum albumin:

Data in table (8) show that in control (-ve) normal rats albumin was 3.53 ± 0.41(g/dl). While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 2.67 ± 0.25 (g/dl). These results reflected a significant decrease in control (+ve) compared to control (-ve) groups. Rats fed

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on hypercholesterolmic diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant increase of albumin compared to control (+ve) which were 3. 55 ± 0. 42, 4. 18± 0. 68, 3. 98± 0. 33, 4. 65±0. 28,and 3. 78±0. 36 (g/dl) respectively. The best result was for hypercholesterolmic rats fed on (20%) *nigella sativa* which was 4. 65 ± 0. 28 (g/dl).

- Serum globulin:

Data in table (8) show the control (-ve) normal rats serum globulin was 5. 73 ± 0. 71(g/dl). While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 8. 20 ± 0. 31(g/dl). These results reflected a significant increase in control (+ve) compared to control (-ve) groups. Rats fed on hypercholesterolmic diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant decrease compared to control (+ve) which were 6. 95 ± 0. 33, 5. 70 ± 0. 89, 5. 85 ± 0. 33, 6. 98 ± 0. 27, and 7. 30 ± 0. 06 (g/dl) respectively.

- Alb/Glob ratio:

Data table (8) show the control (-ve) normal rats Alb/Glob was 0. 68 ± 0. 18, While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 0. 32 ± 0. 02 These results indicated that there were a significant decrease in control (+ve) compared to control (-ve)

groups. Rats fed on hypercholesterolmic diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant increase compared to control (+ve) which were 0. 57 ± 0. 11, 0. 83 ± 0. 27, 0. 68 ± 0. 03, 0. 75 ± 0. 16, 0. 53 ± 0. 03 respectively.

Effect of different levels of *nigella sativa* on kidney function:

Data presented in table (9) show the effect of (5%,10%,15%,20% and 25%) *nigella sativa* on kidney function (urea, creatinine and U. acid) of hypercholesterolmic rats.

- Serum Urea:

It could be observed table (9) that in control (-ve) normal rats urea was 25. 58±1. 14 (mg/ dl). While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 60. 00±1. 69 (mg/dl). These obtained data reflected that a significant increase in control (+ve) compared to control (-ve) groups. Rats fed on hypercholesterolmic diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* indicated significant decrease compared to control (+ve) which were 34. 50 ± 1. 56, 39. 17 ± 1. 17, 30. 50 ± 0. 65, 36. 50 ± 0. 65, 33. 50 ± 1. 19 (mg/dl) respectively. Other plants could correct hyperuricemia of rats inflicted with hypercholesterolemia (Khider and Abeer, 2006).

Table (8): Effect of (5%,10%,15%,20% and 25%) *nigella sativa* on (T. protein, Albumin, globulin, and Alb/Glob) of hypercholesterolmic rats.

Parameters Groups	T. protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/Glob
Control (-ve)	10. 9±0. 56 ^b	3. 53±0. 41 ^d	5. 73 ±0. 71 ^c	0. 68± 0. 18 ^c
Control(+ve)	9. 23±0. 48 ^d	2. 67±0. 25 ^e	8. 20±0. 31 ^a	0. 32±0. 02 ^e
5% <i>nigella sativa</i>	10. 5±0. 38 ^b	3. 55± 0. 42 ^d	6. 95±0. 33 ^b	0. 57± 0. 11 ^d
10% <i>nigella sativa</i>	9. 88±0. 86 ^c	4. 18± 0. 68 ^b	5. 70±0. 89 ^c	0. 83±0. 27 ^a
15% <i>nigella sativa</i>	9. 83±0. 63 ^c	3. 98± 0. 33 ^c	5. 85±0. 33 ^c	0. 68±0. 03 ^c
20% <i>nigella sativa</i>	11. 63±0. 08 ^a	4. 65±0. 28 ^a	6. 98± 0. 27 ^b	0. 75±0. 16 ^b
25% <i>nigella sativa</i>	11. 08±0. 41 ^a	3. 78±0. 36 ^c	7. 30±0. 06 ^b	0. 53±0. 03 ^d

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Table (9): Effect of (5%,10%,15%,20% and 25%) nigella sativa on kidney function (Urea, creatinine, and U. acid) in hypercholesterolmic rats.

Parameters Groups	Urea (mg/dl)	Creatinine (mg/dl)	U. Acid (mg/dl)
Control (-ve)	25. 58±1. 14 ^e	0. 63±0. 05 ^b	1. 40±0. 12 ^c
Control(+ve)	60. 00±1. 69 ^a	0. 97±0. 01 ^a	2. 25±0. 07 ^a
5% <i>nigella sativa</i>	34. 50±1. 56 ^c	0. 60±0. 04 ^b	1. 09± 0. 05 ^e
10% <i>nigella sativa</i>	39. 17±1. 17 ^b	0. 70±0. 04 ^c	1. 31±0. 11 ^d
15% <i>nigella sativa</i>	30. 50± 0. 65 ^d	0. 70± 0. 07 ^c	1. 21±0. 09 ^d
20% <i>nigella sativa</i>	36. 50±0. 65 ^b	0. 63±0. 07 ^b	1. 89±0. 15 ^b
25% <i>nigella sativa</i>	33. 50±1. 19 ^c	0. 65± 0. 03 ^b	1. 98±0. 20 ^b

- Serum Creatinine:

Results of table (9) denote that in control (-ve) normal rats creatinine was 0. 63 ± 0. 05 (mg/dl). While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 0. 97 ± 0. 01 (mg/dl). These data revealed that there were significant increase in control (+ve) compared to control (-ve) groups. Rats fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant decrease in creatinine compared to control (+ve) group which were 0. 60 ± 0. 04, 0. 70 ± 0. 04, 0. 70 ± 0. 07, 0. 63 ± 0. 07, 0. 65 ± 0. 03 (mg /dl) respectively. The best results recorded in rats fed on hypercholesterolmic diet then fed in 5% *nigella sativa*. There was non-significant changes compared to control (-ve) normal rats which were 0. 63±0. 07 and 0. 63± 0. 05 (mg/dl) respectively. Similar results were reported by (El-Malah and Maysa ,2007) using broccoli for correcting creatinine of hypercholesterolmic rats. This result accepted with(Mohammad Aziz Dollah *et al* ,2012) The finding revealed that there was no significant difference in serum urea of treatment groups compared with the control group. The results showed a significant decline in serum creatinine of

high dose of *Nigella sativa* treated compared with low dose treated and control groups ($p<0.05$). Histopathological examination of kidney tissue showed normal kidney architecture with no tissue degeneration, inflammation, necrosis, and tubular dilation in all groups.

- Serum Uric. acid:

It could be observed that in control (-ve) normal rats U. acid was 1. 40 ± 0. 12 (mg/dl). While in control (+ve) rats fed on hypercholesterolmic diet without treatment it was 2. 25 ± 0. 07 (mg /dl). These result denoted the significant increase in control (+ve) compared to control (-ve) groups. Rats fed on hypercholesterolmic diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant decrease com-pared to control (+) group which were 1. 09 ± 0. 05, 1. 31 ± 0. 11, 1. 21 ± 0. 09, 1. 89 ± 0. 15, 1. 98 ± 0. 20 (mg /dl) respectively. Similar trends of change were reported by (Ahmed and Reham , 2007).

Effect of different levels of *nigella sativa* on some lipids profile:

Data presented in table (10) show the

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effect of (5%,10%,15%,20% and 25%) *nigella sativa* on some lipids profiles (T. cholesterol, T. lipids, triglyceride, and phospholipids)

- Total cholesterol (T. C):

It could be noticed that in control (-) normal rats T. cholesterol was 117.78 ± 1.66 (mg /dl). While in control (+) rats fed on hypercholesterolemia diet without treatment it was 210.0 ± 1.69 (mg /dl). The obtained data revealed that there was a significant increase of T. cholesterol in control (+) compared to control (-). Rats fed on hypercholesterolemia diet then fed (5%,10%,15%,20% and 25%) of *nigella sativa* showed significant decreases of T. cholesterol compared to that of control (+) which were 140.7 ± 1.19 , 138.75 ± 1.95 , 158.0 ± 0.92 , 136.25 ± 2.61 , and 143.25 ± 1.12 (mg /dl) respectively. Highest decrease of T. cholesterol was recorded for 10% *nigella sativa*.

- Serum T. Lipids :

The obtained data presented in table (10) indicated that in control (-) normal rats T. lipids was 440.0 ± 2.36 (mg /dl). While in control (+) rats fed on hypercholesterolemia diet without treatment it was 840.5 ± 2.22

(mg /dl). The obtained data reflected the significant increase in control (+) compared to control (-). Rats fed on hypercholesterolemia diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* reversed significant decreases of T. lipids compared to control (+) group which were 622.0 ± 1.47 , 541.0 ± 2.78 , 512.0 ± 1.26 , 545.0 ± 2.42 , 577.5 ± 2.35 (mg / dl) respectively. Maximum decrease was found for 15% of *nigella sativa*

- Serum triglyceride (T. G):

Result of table (10) showed that in control (-ve) normal rats serum triglyceride was 74.25 ± 1.11 (mg /dl). While in control (+) rats fed on hypercholesterolemia diet without treatment it was 230.5 ± 1.55 (mg/dl). These result revealed that there were significant increase in control (+ve) compared to that of the control (-ve) group. Rats fed on hypercholesterolemia diet and fed on (5%,10%,15%,20% and 25%) *nigella sativa* indicated significant decrease compared to control (+) rats which were 150.5 ± 2.22 , 122.0 ± 2.12 , 165.0 ± 2.8 , 109.75 ± 1.60 , 151.75 ± 2.62 (mg/dl) respectively. Maximum decrease was found for 20% *nigella sativa* diet.

Table (10): Effect of (5%,10%,15%,20% and 25%) *nigella sativa* on lipids profiles (T. cholesterol, T. lipids, triglyceride, and phospholipids) of hypercholesterolemia rats.

Parameters Groups	T. Cholestrol (mg/dl)	T. Lipids (mg/dl)	Triglyceride (mg/dl)	Phospholipids (mg/dl)
Control (-ve)	117.78 ± 1.66^e	440.0 ± 2.36^f	74.25 ± 1.11^f	248.25 ± 3.23^e
Control(+ve)	210.0 ± 1.69^a	840.5 ± 2.22^a	230.5 ± 1.55^a	400.0 ± 1.83^a
5% <i>nigella sativa</i>	140.7 ± 1.19^c	622.0 ± 1.47^b	150.5 ± 2.22^c	331.0 ± 3.58^b
10% <i>nigella sativa</i>	135.25 ± 1.95^d	541.0 ± 2.78^d	122.0 ± 2.12^d	303.75 ± 2.63^c
15% <i>nigella sativa</i>	158.0 ± 0.92^b	512.0 ± 1.26^e	165.0 ± 2.8^b	219.0 ± 2.15^f
20% <i>nigella sativa</i>	138.75 ± 2.61^d	545.0 ± 2.42^d	109.75 ± 1.60^e	299.0 ± 3.59^c
25% <i>nigella sativa</i>	143.25 ± 1.12^c	577.5 ± 2.35^c	151.75 ± 2.62^c	278.0 ± 3.23^d

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- Serum phospholipids:

Data present in table (10) showed that in control (-) normal rats phospholipids level was 248.25 ± 3.23 (mg/dl) While in control (+) rats fed on hypercholesterolic diet without treatment it was 400.0 ± 1.83 (mg /dl) These result cleared out that there were significant increase in control (+ve) rats compared to that of the control (-ve) group. Rats fed on hypercholesterolic diet (5%,10%,15%,20% and 25%) *nigella sativa* indicated significant decrease in phospholipids compared to control (+) rats which were 331.0 ± 3.58 , 303.75 ± 2.63 , 219.0 ± 2.15 , 299.0 ± 3.59 , and 278.0 ± 3.23 (mg /dl) respectively. Best result may be that of 15%, *nigella sativa* diet

- Effect of different levels of *nigella sativa* on cholesterol fraction:

Data present in table (11) show the effect of (5%,10%,15%,20% and 25%) *nigella sativa* on cholesterol fraction (HDL, LDL,VLDL, and VLDL+ LDL /HDL)of hypercholesterolic rats.

- Serum HDL:

It could be noticed that in control (-ve) normal rats HDL was 64.5 ± 9.97 (mg / dl). While in control (+ ve) rats fed on hypercholesterolic diet without treatment it was 45.00 ± 1.29 (mg /dl). These results denoted that there was a significant decrease in control (+ve) compared to control (-ve) groups considering the HDL level. Rats fed on hypercholesterolic diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant increases compared to control (+) group which were 51.00 ± 2.52 , 66.25 ± 1.55 , 74.25 ± 2.50 , 65.50 ± 1.18 ,and 68.50 ± 2.3 (mg /dl) respectively. Maximum increase of HDL was found for (15%) *nigella sativa* diet.

- Serum LDL:

The obtained data showed that in control (-ve) normal rats LDL was 38.43 ± 1.76 (mg /dl). While in control (+ve) rats fed on

hypercholesterolic diet without treatment it was 118.9 ± 2.79 (mg/dl). These data denoted that there was a significant increase in control (+ve) compared to control (-ve) in considering LDL level. Rats fed on hypercholesterolic diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant decreases of LDL compared to control (+) group; values were 59.6 ± 2.21 , 44.6 ± 1.96 , 50.75 ± 1.26 , 51.3 ± 2.91 , and 44.4 ± 1.69 (mg / dl) respectively. Lowest LDL was recorded for (10% and 25%) *nigella sativa* diets.

- Serum VLDL:

The results of table (11) denote that in control (-) normal rats VLDL was 14.85 ± 0.22 (mg /dl). While in control (+ve) rats fed on hypercholesterolic diet without treatment it was 46.1 ± 0.31 (mg/dl). These data denoted that there was a significant VLDL increase in control (+ve) compared to control (-ve) rats. Rats fed on hypercholesterolic diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant decreases of VLDL compared to control (+) group which were 30.1 ± 0.45 , 24.4 ± 0.43 , 33.0 ± 0.96 , 21.95 ± 0.92 ,and 30.35 ± 0.73 (mg/dl) respectively. Lowest VLDL was found for (10%) *nigella sativa* treatment.

- VLDL+LDL / HDL ratio:

Data of table (11) revealed that in control (-) normal rats VLDL+LDL / HDL was 0.83 ± 0.02 While in control (+ve) rats fed on hypercholesterolic diet without treatment it was 3.67 ± 0.15 . These data reflected that there was a significant increase in control (+ve) compared to control (-ve) considering the mentioned ratio. Rats fed on hypercholesterolic diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* showed significant decreases of VLDL+LDL / HDL ratio compared to control (+) group which were 1.76 ± 0.13 , 1.04 ± 0.24 , 1.23 ± 0.31 , 1.12 ± 0.16 ,and 1.09 ± 0.42 respectively. Minimum atherogenic index was recorded for (10%) *nigella sativa* s treatment.

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Table (11): Effect of (5%,10%,15%,20% and 25%) nigella sativa on cholesterol fraction (HDL, LDL, VLDL, and VLDL+ LDL /HDL) of hypercholesterolemic rats.

Parameters Groups	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	VLDL+LDL HDL ratio
Control (-ve)	64. 5±9. 97 ^c	38. 43±1. 76 ^e	14. 85±0. 22 ^f	0. 83±0. 02 ^d
Control(+ve)	45. 00±1. 29 ^e	118. 9±2. 79 ^a	46. 1±0. 31 ^a	3. 67±0. 15 ^a
5% <i>nigella sativa</i>	51. 00±2. 52 ^d	59. 6±2. 21 ^b	30. 1±0. 45 ^c	1. 76±0. 13 ^b
10% <i>nigella sativa</i>	66. 25±1. 55 ^b	44. 6±1. 96 ^d	24. 4±0. 43 ^d	1. 04±0. 24 ^c
15% <i>nigella sativa</i>	74. 25±2. 50 ^a	50. 75±1. 26 ^c	33. 0±0. 96 ^b	1. 23±0. 31 ^c
20% <i>nigella sativa</i>	65. 50±1. 18 ^c	51. 3±2. 91 ^c	21. 95±0. 92 ^e	1. 12±0. 16 ^c
25% <i>nigella sativa</i>	68. 50±2. 38 ^b	44. 4±1. 69 ^d	30. 35±0. 73 ^c	1. 09±0. 42 ^c

Effect of different levels of *nigella sativa* on serum glucose:

Data listed in table (12) Show the effect of (5%,10%,15%,20% and 25%) *nigella sativa* on serum glucose of hypercholesterolemic rat. .

It could be denoted that in control (-ve) normal rats glucose was 117. 75± 5. 72 (mg/dl). Meanwhile in control (+ve) rats fed on hypercholesterolemic diet without treatment it was 161. 0 ± 1. 83. These results denoted that there was a significant increase of glucose in control (+ve) compared to control (-ve) serum glucose. Rats fed on hypercholesterolemic diet then fed on (5%,10%,15%,20% and 25%) *nigella sativa* diet recorded significant decreases compared to control (+) group which were 111. 75 ± 5. 12, 125. 75 ± 6. 84, 103. 75 ± 3. 12, 103. 5 ± 5. 95, 123. 25 ± 6. 87(mg/dl) respectively. Lowest glucose level was recorded in serum of 20% *nigella sativa*. (Bamosa *et al* , 2010) they showed the effect of *Nigella sativa* on the glycemic control was assessed through measurement

of fasting blood glucose (FBG), blood glucose level 2 hours postprandially (2 hPG), and glycosylated hemoglobin (HbA1c). Serum C-peptide and changes in body weight were also measured. Insulin resistance and beta-cell function were calculated using the homeostatic model assessment (HOMA2). *Nigella sativa* at a dose of 2 gm/day caused significant reductions in FBG, 2hPG, and HbA1 without significant change in body weight. Fasting blood glucose was reduced by an average of 45, 62 and 56 mg/dl at 4, 8 and 12 weeks respectively. HbA1C was reduced by 1.52% at the end of the 12 weeks of treatment (P<0.0001). Insulin resistance calculated by HOMA2 was reduced significantly (P<0.01), while B-cell function was increased (P<0.02) at 12 weeks of treatment. The use of *Nigella sativa* in a dose of 1 gm/day also showed trends in improvement in all the measured parameters but it was not statistically significant from the baseline. However, no further increment in the beneficial response was observed with the 3 gm/day dose

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Table (12): Effect of (5%,10%,15%,20% and 25%) *nigella sativa* on glucose of hyperglycemic rats.

Groups	Parameter	Glucose (mg/dl)
Control (-ve)		117. 75 ± 2. 72 ^f
Control(+ve)		161. 0 ± 1. 83 ^a
5% <i>nigella sativa</i>		111. 75 ± 5. 12 ^d
10% <i>nigella sativa</i>		125. 75 ± 6. 84 ^b
15% <i>nigella sativa</i>		103. 75 ± 3. 12 ^c
20% of <i>nigella sativa</i>		103. 5 ± 5. 95 ^d
25% of <i>nigella sativa</i>		123. 25 ± 6. 87 ^e

RECOMMENDATIONS

1. It is suggested to use 20% of *nigella sativa* for hypercholesterolmic patients.
2. different levels of *nigella sativa*, especially that of 15 % of *nigella sativa* may be used for remedy of heart diseases.
3. 15% of *nigella sativa*, may be suggested for lowering LDL and atherogenig index levels.
4. Future studies may be suggested to evaluate the efficacy and advantage of using *nigella sativa* as extracts versus dried powder.

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تأثير المستويات المختلفة من بذور حبة البركة على التغيرات البيوكيميائية للفئران المصابة بارتفاع كولسترول الدم

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الملخص العربي

زاد الاهتمام فى الاونة الاخيرة بالنباتات الطبية نظرا لزيادة كفاءة العقاقير الجديدة المشتقة من هذه النباتات وأيضا بسبب المخاوف من الآثار الجانبية للدواء التقليدي ، و قد تم استخدامها كبديل للعلاج التقليدي في الشفاء والعلاج من الأمراض المختلفة منذ زمن بعيد ، و تعتبر هذه النباتات بمثابة بدائل علاجية وخيارا أكثر أمنا لما تحتويه من فوائد علاجية كثيرة لذلك ومن هذا المنطلق قد اقدمنا على اجراء هذه الدراسة و التى تهدف الى تحديد تأثير مستويات مختلفة من بذور حبة البركة على التغيرات البيوكيميائية على الفئران المصابة بارتفاع كولسترول الدم وقد استخدمت الدراسة (٢٨) فأر البينو ابيض و تم تقسيمهم الى مجموعتان رئيسيتين اولا المجموعة الرئيسية الاولى و هى مجموعة الفئران المصابة بارتفاع كولسترول الدم وثانيا المجموعة الرئيسية الثانية و هى مجموعة الفئران الغير مصابة وبعد ذلك تم تقسيم المجموعة الرئيسية الأولى الى ست مجاميع فرعية منهم خمس مجموعات تتغذى على تركيبات مختلفة من حبة البركة (٥% ، ١٠% ، ١٥% ، ٢٠% ، ٢٥%) ومجموعة واحدة ضابطة موجبة مصابة بالمرض ولا تتغذى على الغذاء التجريبي و المجموعة الرئيسية الثانية هى المجموعة الضابطة السالبة الغير مصابة بالمرض هذا يعني أن جميع الفئران مقسمة إلى سبع مجموعات فرعية و يوجد بكل مجموعه اربع فئران. وأظهرت النتائج أن نسبة ٢٠% من بذور حبة البركة ارتفاع معنوى نسبة انزيم GOT الكبدى وذلك عند مقارنة النتائج بالمجموعة الضابطة السالبة ولوحظ ايضا انخفاض معنوى فى نسبة الكولسترول الكلى و نسبة LDL لدى الفئران التى تغذت على نسبة ١٠% بذور حبة البركة وكذلك ارتفاع معنوى فى نسبة HDL عند نفس النسبة .

الكلمات الاسترشادية : بذور حبة البركة - ارتفاع كولسترول الدم - التغذية العلاجية