Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Potential Therapeutic Agents for the Treatment of Fatty Degeneration of Liver and Atheromatous Plaques: An Experimental Study in Rats

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Submitted: 15-02-2016 Revised: 01-04-2016 Published: 30-09-2016

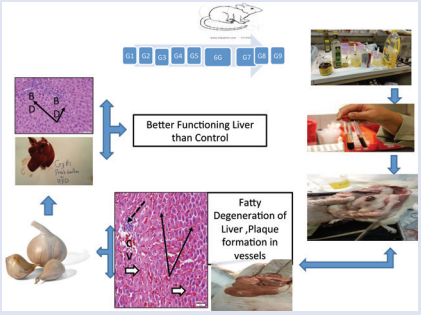
ABSTRACT

Background: Since long high fat diet (HFD) is being blamed for causing fatty degeneration of liver and formation of atheromatous plaques. At present, no proper pharmacotherapy is available for both the conditions. In this study, different substances containing monounsaturated fatty acids were used to observe their protective effects in the HFD induced damage to liver and coronary vessels. **Objectives:** To discover effective therapeutic agents for HFD induced fatty degeneration of liver and atheromatous plaques. **Materials and Methods:** The study was conducted from September 2015 to April 2016. In this study, rats were divided into nine groups according to dietary regimen. Each group comprised six rats. Saturated fat was given in the form of butter, and unsaturated fat was given in the form of corn oil, olive oil, *Nigella sativa* oil, and crushed garlic. Serum samples were taken to estimate lipid profile, liver functions, cardiac functions, and kidney functions. Visceras were removed after animal sacrifice, and histopathological examination was done. **Results and Conclusion:** During the study period, the weight of animals changed significantly in some groups. Those animals which were given crushed garlic along with high saturated fat diet, showed protection against accumulation of lipids in the hepatocytes. Olive oil and *Nigella sativa* oil were comparatively less effective.

Key words: Atheromatous plaques, fatty degeneration, garlic, high fat diet, *Nigella sativa* oil, olive oil

SUMMARY

- Consumption of Garlic ,Nigella Sativa and Olive oil significantly improved/ revised the Fatty Degeneration of liver induced by intake of High Fat Diet.
- No fat deposition was found in the liver when Garlic ,Nigella Sativa and Olive oil ,were given concomitantly with HFD.
- Hepatocytes functioned better even in comparison to control and a decrease in liver enzymes was found with use of Garlic.
- Use of Garlic ,Nigella Sativa and Olive oil, prevented the plaque formation in the vessels and decreased serum lipids.
- Beneficial effects of Garlic were significant in comparison to Nigella Sativa and Olive oil.



Abbreviations used: HFD: High Fat Diet; NS: Nigella Sativa; TQ: Thymoquinone; KFMRC: King Fahad Medical Research Center; BUN: Blood Urea Nitrogen; BNF: Buffered Neutral Formalin; G: Group

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DOI: 10.4103/0973-1296.191444

Access this article online
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INTRODUCTION

High caloric value or high fat diet is associated with insulin resistance, an increased risk of developing Type-2 Diabetes Mellitus and abnormal postprandial lipid biotransformation.[1] Accumulation of fat results from an imbalance between input–output oxidation of fatty acids, leading to impaired energy breakdown.[2] It is usually believed that diets based on saturated fatty acids such as animal fat, lead to common high-fat-diet phenotype, whereas diets containing polyunsaturated fatty acids like cod liver oil and vegetable fat produce beneficial effects on body composition, weight gain, and insulin action.[3]

Accumulation of fat in liver produces a variety of liver pathologies, ranging from fatty liver/fatty degeneration of liver, which leads to hepatic cirrhosis, hepatic failure, and hepatocellular carcinoma, in the absence of alcohol abuse and other commonly prevalent causes.[4,5]

Hepatic fatty degeneration is a reversible pathology that is characterized by large deposition of lipids in the hepatocytes, because of excessive intake of high fat diet (HFD).[6] Alteration in biotransformation of free fatty acids leads to insulin resistance and fat accumulation in the liver. Acute inflammation, oxidative stress, programmed cell death, and even phagocytosis, work as "following-hits" which lead to chronic inflammation.[7,8]

Human beings have been consuming garlic (*Allium sativum*), a member of the lily family, since time unknown. Ancient Egyptian records mentioned use of garlic as treatment for several ailments.

In recent years, research has discovered that the sulfur-containing constituents of *Allium sativum* have antimutagenic and anticarcinogenic

Cite this article as: Sangi SM. Potential therapeutic agents for the treatment of fatty degeneration of liver and atheromatous plaques: An experimental study in rats. Phcog Mag 2016;12:414-23.

effects.[9] Garlic has also been suggested to possess several therapeutic properties like reduction in serum lipids, antihyperglycemic, anticoagulant, antihypertensive, antibacterial, antifungal, and hepatoprotective.[10,11]

Administration of garlic has shown to cure hyper lipidemia in humans.[12] In the case of HFD-induced hypercholesterolemia in rats, administration of garlic (1–4%), significantly normalized serum dyslipidemia.[13,14] Administration of garlic in experimental atherosclerosis induced by an HFD in rabbits significantly reduced serum cholesterol and formation of atheromatous lesions.[15,16]

Nigella sativa usually familiar with the name of black seed is a member of Ranu nculaceae family. It is an annual, erect herb, 30–40 cm high. Seeds of *N. sativa,* also known as black cumin or kalonjis, are being used by folklore since long for the treatment of various disorders. The essential oil of *N. sativa* seed possess antioxidant properties that help in curing cardiovascular diseases.[17]

Nigella sativa constituents include 30% of fixed oil, 0.4%–0.45% of volatile oil. The volatile oil includes in itself 18.4–24% thymoquinone (TQ) and 46% monoterpenes like p-cymene and a-pinene.[18] The *Nigella sativa* oil contains 50% linoleic acid, 25% oleic acid, 12% palmitic acid, 2.84% stearic acid, 0.34% linolenic acid, and 0.35% myristic acid.[19]

Clinical and experimental research has proved that extract of *Nigella Sativa*. possesses many therapeutic effects such as immune-modulative[18] antibacterial[20] hypotensive[21] hepatoprotective[22] and antidiabetic.[23] Black seed also contains antioxidant activity.[24] It has been reported[25] that NS oil and its derivative TQ inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation.[26,27]

Olea europaea (olive) from the family, Oleaceae, familiar as olive is being cultivated since ancient times with many therapeutic properties. Mediterranean diet rich in olives has various beneficial effects, as reported in several studies.[28,29] Olive products are rich in antioxidants which can reduce oxidative stress during the pathogenesis of pathologies like acute coronary syndrome,[30,31] cancer,[32] and neurodegenerative diseases.[33] The antioxidant properties of olive products are attributed to the presence of phenolic compounds[34] that scavenge free radicals and chelate metallic ions.[28]

MATERIALS AND METHODS

This study was conducted in the Department of Clinical Pharmacy, College of Pharmacy, Northern Border University, Rafha Campus, and King Fahad Medical Research Center, King Abdul Aziz University, Jeddah, from September 2015 to April 2016.

Animals

Fifty-four healthy and active adult albino male rats, 90–120 days old and weighing 200–240 g were selected. Rats were acclimatized under environmental condition with 24 ± 3 °C, 12-h light/dark cycle and good ventilation.

Experimental design

After acclimatization for one week before use, the animals were randomly divided into nine groups, each group included six rats and was labeled as G1, G2, G3, G4 ,G5, G6, G7, G8, and G9 according to the diet given to them. Saturated fat was used in the form of butter, in a dose of 20 g/100 g (20%) of diet.[35] Unsaturated fat was used in the form of corn oil (Corolli), in the dose of 20 mL/100 g (20%) of diet.[36] Fresh crushed garlic, olive, and *Nigella sativa* were obtained from the local market. Garlic was mixed with the diet, in the dose of 6 g/100 g (6%) of diet.[37]

Group 1 animals were considered as control. They were fed normal diet. Group 2 animals were fed highly saturated fat diet (butter along with normal diet). Group 3 animals were given highly unsaturated fat diet (corn oil along with normal diet). Group 4 animals received fresh, crushed garlic, along with butter in their diet.

Group 5 animals received fresh crushed garlic, along with corn oil in their diet. Group 6 animals received highly saturated fat diet with olive oil, Group 7 animals received highly unsaturated fat diet (olive oil), Group 8 animals received highly saturated fat diet with *Nigella sativa* oil, and Group 9 received highly unsaturated fat diet (*Nigella sativa* oil).

All the study groups were given oils via oral gavage in the dose of 5mL/kg body weight.[38]

At the end of the 8th week of study, blood was taken from the animals for serum analysis and after that animals were sacrificed for histopathological examination.

Serum analysis and histopathological examination

Serum analysis and histopathological examination were performed at KFMRC, King Abdul Aziz University, Jeddah, Saudi Arabia. The blood samples were obtained in plain test tubes without anticoagulant, and blood was left to clot at room temperature. After that, serum was taken to estimate the level of triglyceride, low density lipoprotein, cholesterol, albumin, total bilirubin, and total proteins. Serum alkaline phosphatase, uric acid, blood urea nitrogen (BUN), serum creatinine, and cardiac enzyme levels were estimated.

A midline, longitudinal incision was made, extending from manubrium sterni to lower abdomen. Skin, fascia, and muscles were carefully cut and retracted to expose abdominal viscera. After exposure, liver and coronary arteries were removed from the body and were fixed in buffered neutral formalin (BNF) for 24 hours.

The liver and coronary arteries' tissues were processed for frozen sectioning. Ten micron thick sections were obtained on gelatinized glass slides and stained with oil red-O and hematoxylin to observe the fat content.[39]

Statistical analysis

Statistical Package for Social Science (SPSS) version 20 for windows program was applied to analyze the present data. The data was expressed as means +/– standard deviation (SD). Comparison of variables between groups was performed using one-way analysis of variance (ANOVA). Statistical significances were considered at P-value <0.05.

RESULTS

In the 1st week, body weights in G2, G5, G6, and G9 were significantly higher than G1 (P = 0.0001, P = 0.0001, P = 0.0001, P = 0.012, and P = 0.0001). In the 2nd week, body weights in G2, G5, G6, and G9 were significantly higher than G1 (P = 0.0001, P = 0.040, P = 0.0001, P = 0.018, and P = 0.0001). In the 3rd week, body weights in G2 and G6 were significantly higher than G1 (P = 0.0001 and P = 0.020). In the 4th week, body weights in G2, G3, and G6 were significantly higher than G1 (P = 0.002, P = 0.013, and P = 0.029). In the 5th week, body weights in G2, G3, and G6 were significantly higher than G1 (P = 0.001, P = 0.019, and P = 0.023). In the 6th week, body weights in G2 and G6 were significantly higher than G1 (P = 0.002 and P = 0.040). In the 7th week, body weights in G2 and G6 were significantly higher than G1 (P = 0.001 and P = 0.027). In the 7th, 8th, 9th, and 10th weeks, body weights in G2 were significantly higher than G1 (P = 0.002, P = 0.002, P= 0.004 and P = 0.001, respectively) [Table 1 and Figure 1].

In the 2nd week, the percentage change in body weight in G3 was significantly lower than G1 (P = 0.041). In the 3rd week, the percentage change in body weights in G3, G6, G7, and G9 was significantly lower than G1 (P = 0.004, P = 0.001, P = 0.017 and P = 0.0001). In the 4th week,

the percentage change in body weights in G3, G4, G5, G6, and G9 was significantly lower than G1 ($P = 0.001$, $P = 0.004$, $P = 0.018$, $P = 0.007$, and $P = 0.0001$). In the 5th week, the percentage change in body weights in G3, G4, G5, and G9 was significantly lower than G1 ($P = 0.010$, $P = 0.015$, $P = 0.030$, and $P = 0.004$). In the 6th week, the percentage change in body weights in G3, G4, G5, and G9 was significantly lower than G1 ($P = 0.010$, $P = 0.015$, $P = 0.030$, and $P = 0.004$). In the 7th and 10th weeks, the percentage change in body weights in G9 was significantly lower than G1 ($P = 0.019$, and $P = 0.030$). In the 9th week, the percentage change in body weights in G3 and G9 was significantly lower than G1 ($P = 0.029$ and $P = 0.012$) [Table 2 and Figure 2].

Table 3 and Figure 3 showed the mean of food intake in different weeks in different groups.

Table 4 and Figure 4 showed that serum level of triglyceride in G6 and G7 groups was significantly lower than G1 ($P= 0.012$ and $P= 0.039$).

Table 5 and Figure 5 showed that serum level of albumin in G5 group was significantly higher than G1 ($P= 0.030$). Serum total bilirubin was significantly higher in G3, G4, and G6 compared to G1 ($P= 0.041$ for all). Serum alkaline phosphatase in G6 and G8 was significantly lower than G1 ($P = 0.021$ and $P = 0.016$).

Table 6 and Figure 6 showed insignificant difference between cardiac enzymes level (cardiac troponin and Gamma-Glutamyl Transferase) in different groups versus control.

Table 7 and Figure 7 showed that serum level of uric acid in G7 group was significantly higher than G1 ($P = 0.049$). Serum creatinine was

Table 1: Comparison of body weights (grams) in different studied groups.

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
G1 (control group)	194.75 ± 3.59	205.75 ± 5.75	228.50 ± 6.40	245.50 ± 9.03	258.50 ± 7.85	276.25 ± 14.52	280.00 ± 9.63	289.25 ± 20.27	306.50 ± 31.51	310.00 41.35
G2 (Butter group)	240.00 ± 8.68	261.50 ± 15.50	277.00 ± 19.24	287.00 ± 22.32	315.25 ± 19.00	327.00 ± 19.61	345.25 ± 21.09	355.75 ± 24.74	371.50 ± 27.93	382.75 ± 29.51
Significance	$^1P = 0.0001$	$^1P = 0.0001$	$^1P = 0.0001$	$^1P = 0.002$	$^1P = 0.001$	$^1P = 0.002$	$^1P = 0.001$	$^1P = 0.002$	$^1P = 0.004$	$^1P = 0.001$
G3 (Butter and Garlic Juice group)	202.25 ± 2.22	203.25 ± 4.92	214.00 ± 12.08	214.00 ± 20.93	221.00 ± 33.42	256.00 ± 10.68	267.00 ± 18.51	270.00 ± 19.61	272.50 ± 15.67	282.75 ± 9.84
Significance	$^1P = 0.098$	$^1P = 0.722$	$^1P = 0.150$	$^1P = 0.013$	$^1P = 0.019$	$^1P = 0.177$	$^1P = 0.458$	$^1P = 0.342$	$^1P = 0.111$	$^1P = 0.186$
G4 (Butter and olive oil group)	201.25 ± 5.97	215.25 ± 5.68	225.00 ± 9.02	225.75 ± 17.15	237.00 ± 25.39	260.75 ± 22.44	275.50 ± 25.63	282.75 ± 36.40	306.33 ± 19.14	308.67 ± 17.21
Significance	$^1P = 0.149$	$^1P = 0.183$	$^1P = 0.724$	$^1P = 0.109$	$^1P = 0.164$	$^1P = 0.298$	$^1P = 0.796$	$^1P = 0.746$	$^1P = 0.994$	$^1P = 0.951$
G5 (Butter and *Nigella sativa* oil group)	216.25 ± 6.50	220.75 ± 7.50	237.25 ± 22.53	249.25 ± 19.55	258.75 ± 22.77	277.50 ± 26.15	283.25 ± 31.79	298.50 ± 33.04	307.00 ± 32.40	332.50 ± 23.06
Significance	$^1P = 0.0001$	$^1P = 0.040$	$^1P = 0.380$	$^1P = 0.755$	$^1P = 0.987$	$^1P = 0.932$	$^1P = 0.852$	$^1P = 0.646$	$^1P = 0.981$	$^1P = 0.272$
G6 (Corn oil group)	240.50 ± 2.08	249.50 ± 6.95	252.75 ± 10.21	273.00 ± 9.06	294.75 ± 12.61	307.75 ± 18.14	320.25 ± 21.65	324.25 ± 30.45	336.25 ± 37.38	345.50 ± 32.54
Significance	$^1P = 0.0001$	$^1P = 0.0001$	$^1P = 0.020$	$^1P = 0.029$	$^1P = 0.023$	$^1P = 0.040$	$^1P = 0.027$	$^1P = 0.090$	$^1P = 0.161$	$^1P = 0.088$
G7 (Garlic Juice group)	196.55 ± 10.24	204.75 ± 12.84	214.50 ± 13.99	232.50 ± 19.69	250.25 ± 16.34	268.50 ± 18.86	268.50 ± 31.08	280.75 ± 30.93	288.25 ± 25.24	291.75 ± 23.87
Significance	$^1P = 0.684$	$^1P = 0.887$	$^1P = 0.164$	$^1P = 0.285$	$^1P = 0.588$	$^1P = 0.600$	$^1P = 0.511$	$^1P = 0.673$	$^1P = 0.385$	$^1P = 0.371$
G8 (Olive oil group)	206.50 ± 6.86	223.25 ± 10.53	241.75 ± 14.75	265.00 ± 14.38	284.25 ± 22.87	298.00 ± 21.69	306.25 ± 24.23	323.50 ± 20.98	337.25 ± 30.35	344.75 ± 30.97
Significance	$^1P = 0.012$	$^1P = 0.018$	$^1P = 0.187$	$^1P = 0.113$	$^1P = 0.098$	$^1P = 0.148$	$^1P = 0.140$	$^1P = 0.097$	$^1P = 0.148$	$^1P = 0.095$
G9 (*Nigella sativa* group)	231.75 ± 4.19	240.00 ± 13.59	234.50 ± 7.72	250.50 ± 13.48	266.50 ± 20.50	277.25 ± 28.08	285.50 ± 28.10	302.25 ± 31.22	302.75 ± 33.36	316.25 ± 31.06
Significance	$^1P = 0.0001$	$^1P = 0.0001$	$^1P = 0.545$	$^1P = 0.678$	$^1P = 0.599$	$^1P = 0.946$	$^1P = 0.752$	$^1P = 0.519$	$^1P = 0.857$	$^1P = 0.758$

Data are expressed as mean+/- standard deviation. ^1P: significance versus G1

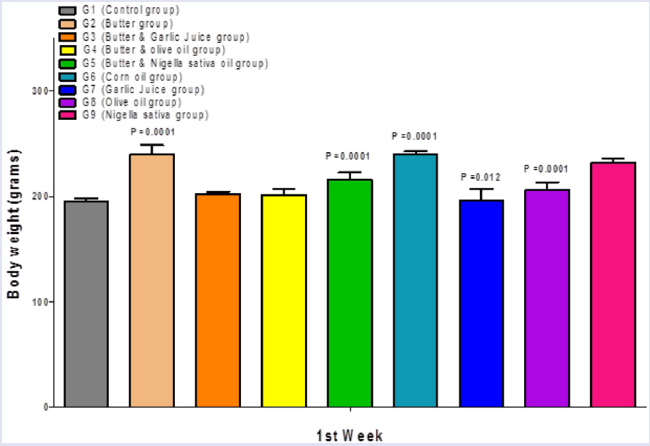

Figure 1: Comparison of body weights(gms) in different studied groups

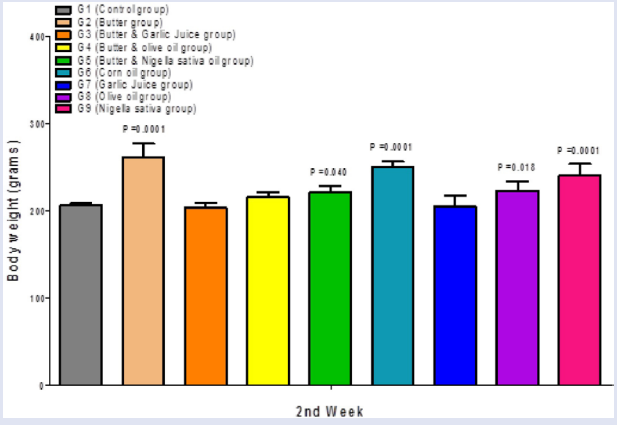

Figure 2: Comparison of percentage changes in body weight (%) in different studied groups

significantly lower in G9 compared to G1 ($P = 0.024$). Serum alkaline phosphatase in G3 was significantly higher than G1 ($P = 0.002$).

DISCUSSION

Food plays an essential role in the maintenance of normal physiological conditions in the body. Several food contents have either beneficial/therapeutic or harmful effects. In different parts of the world, different types of foods are taken, as in the subcontinent more spices are taken or in the middle east Mediterranean diet is taken. In this study, beneficial and harmful effects of commonly used food items and spices were studied in animals. It is a well-known fact that accumulation of fats, especially cholesterol in different vital organs, produces reversible and irreversible damage. Intake of saturated fatty acids leads to deposition of fat in the liver leading to fatty degeneration, which results in the development of irreversible cirrhosis of liver and deposition of cholesterol causes atheromatous plaque formation in the vessels which may lead to myocardial infarction or stroke.[40,41] In this study, effects of unsaturated

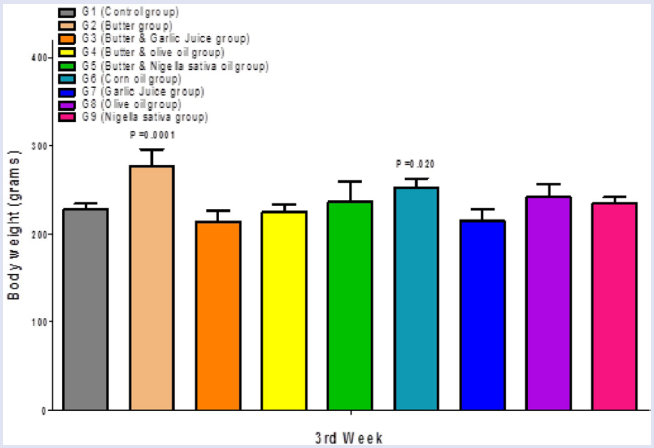

Figure 3: Mean food intake (grams) in different studied groups

Table 2: Comparison of percentage changes in body weights (%) in different studied groups.

Groups	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
G1 (control group)	5.66 ± 1.20	17.34 ± 2.87	26.06 ± 3.78	32.79 ± 5.61	41.96 ± 9.52	43.86 ± 7.22	48.70 ± 13.16	57.63 ± 19.13	59.51 ± 24.39
G2 (Butter group)	8.93 ± 4.15	15.34 ± 4.55	19.48 ± 5.92	31.30 ± 4.72	36.20 ± 4.96	43.82 ± 6.22	48.15 ± 6.74	54.68 ± 7.46	59.37 ± 8.26
Significance	$^1P = 0.104$	$^1P = 0.540$	$^1P = 0.139$	$^1P = 0.796$	$^1P = 0.388$	$^1P = 0.996$	$^1P = 0.954$	$^1P = 0.768$	$^1P = 0.989$
G3 (Butter and Garlic Juice group)	1.48 ± 0.70	7.30 ± 3.35	10.50 ± 2.54	16.93 ± 3.07	26.60 ± 5.77	32.05 ± 9.73	33.52 ± 10.04	34.78 ± 8.59	39.82 ± 5.38
Significance	$^1\mathbf{P = 0.041}$	$^1\mathbf{P = 0.004}$	$^1\mathbf{P = 0.001}$	$^1\mathbf{P = 0.010}$	$^1\mathbf{P = 0.027}$	$^1P = 0.163$	$^1P = 0.123$	$^1\mathbf{P = 0.029}$	$^1P = 0.060$
G4 (Butter and olive oil group)	7.00 ± 3.31	11.92 ± 6.72	12.29 ± 9.89	18.01 ± 14.89	2970 ± 12.56	37.06 ± 14.44	40.59 ± 18.84	51.45 ± 14.02	52.58 ± 13.12
Significance	$^1P = 0.495$	$^1P = 0.105$	$^1\mathbf{P = 0.004}$	$^1\mathbf{P = 0.015}$	$^1P = 0.072$	$^1P = 0.416$	$^1P = 0.402$	$^1P = 0.569$	$^1P = 0.527$
G5 (Butter and *Nigella sativa* oil group)	3.47 ± 2.73	11.09 ± 6.94	15.24 ± 8.13	19.67 ± 10.35	28.42 ± 12.94	31.14 ± 15.97	38.18 ± 16.45	42.11 ± 16.28	54.02 ± 13.12
Significance	$^1P = 0.269$	$^1P = 0.063$	$^1\mathbf{P = 0.018}$	$^1\mathbf{P = 0.030}$	$^1\mathbf{P = 0.049}$	$^1P = 0.134$	$^1P = 0.279$	$^1P = 0.130$	$^1P = 0.588$
G6 (Corn oil group)	3.74 ± 2.51	5.09 ± 3.94	13.51 ± 3.62	22.55 ± 4.90	27.95 ± 7.21	33.15 ± 8.71	34.81 ± 12.42	39.79 ± 15.24	43.64 ± 13.20
Significance	$^1P = 0.331$	$^1\mathbf{P = 0.001}$	$^1\mathbf{P = 0.007}$	$^1P = 0.084$	$^1\mathbf{P = 0.042}$	$^1P = 0.204$	$^1P = 0.156$	$^1P = 0.084$	$^1P = 0.125$
G7 (Garlic Juice group)	4.15 ± 2.61	9.12 ± 3.67	18.23 ± 6.39	27.31 ± 4.44	36.53 ± 3.62	36.76 ± 15.53	43.00 ± 15.51	46.79 ± 12.32	48.63 ± 12.47
Significance	$^1P = 0.443$	$^1\mathbf{P = 0.017}$	$^1P = 0.081$	$^1P = 0.346$	$^1P = 0.415$	$^1P = 0.396$	$^1P = 0.555$	$^1P = 0.284$	$^1P = 0.287$
G8 (Olive oil group)	8.08 ± 2.21	17.02 ± 4.65	28.34 ± 5.71	37.53 ± 7.70	44.25 ± 8.08	48.24 ± 9.42	56.69 ± 9.26	63.33 ± 13.65	66.94 ± 13.69
Significance	$^1P = 0.223$	$^1P = 0.923$	$^1P = 0.601$	$^1P = 0.415$	$^1P = 0.729$	$^1P = 0.599$	$^1P = 0.409$	$^1P = 0.571$	$^1P = 0.465$
G9 (*Nigella sativa* group)	6.83 ± 3.50	1.82 ± 1.81	8.09 ± 5.38	15.06 ± 9.68	19.72 ± 13.05	23.27 ± 12.92	30.52 ± 14.44	30.76 ± 15.61	36.58 ± 14.77
Significance	$^1P = 0.551$	$^1\mathbf{P = 0.0001}$	$^1\mathbf{P = 0.0001}$	$^1\mathbf{P = 0.004}$	$^1\mathbf{P = 0.002}$	$^1\mathbf{P = 0.019}$	$^1P = 0.067$	$^1\mathbf{P = 0.012}$	$^1\mathbf{P = 0.030}$

Data are expressed as mean+/- standard deviation. ^1P: significance versus G1. Percentage change in body weight (%) = Body weight – initial body weight/initial body weight X 100. ^1P: significance versus control group)

Table 3: Mean food intake (grams) in different studied groups.

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week
G1 (control group)	20.80	20.00	22.72	17.00	25.10	25.00	28.50	19.28	29.97	22.60
G2 (Butter group)	17.20	18.60	10.60	18.00	14.60	15.50	14.50	12.90	18.30	16.20
G3 (Butter and Garlic Juice group)	5.40	8.20	6.44	11.00	8.80	12.60	11.50	10.60	18.50	22.80
G4 (Butter and olive oil group)	10.70	12.30	9.80	13.00	10.10	13.30	13.80	10.60	17.90	17.60
G5 (Butter and *Nigella sativa* oil group)	9.20	5.50	16.00	14.00	10.30	11.60	10.30	10.40	14.14	17.63
G6 (Corn oil group)	15.60	15.00	13.52	17.00	16.30	15.60	15.30	11.90	17.11	13.50
G7 (Garlic Juice group)	11.30	16.30	16.00	23.00	20.10	19.20	19.00	17.70	24.25	20.90
G8 (Olive oil group)	14.70	18.70	16.00	22.00	19.60	19.10	18.60	17.05	24.25	18.97
G9 (*Nigella sativa* group)	14.70	10.40	13.88	18.00	14.50	16.40	16.60	16.60	21.20	19.50

and saturated fatty acid diet contents were observed on the kidney and results of many studies have shown that intake of high fat diet results in deposition of fat in kidney parenchyma leading to impairment in the function of the kidney.[42]

In the present study, compared to control (G1) as shown in Figure 8, hepatic steatosis with evident deposition of lipid droplets within hepatocytes was observed in most animals fed on high fat diet (animal butter). Individual variation was observed among animals regarding scoring of amount of lipid deposition and its distribution in both central and peripheral parts of lobules. In Figure 9 a and b, these results are consistent with the previous studies.[43]

The animals which were administrated whole crushed garlic with HFD (G3) showed marked protection and hepatocytes in all examined animals looked normal with absence of any features of lipid deposition, Figure 10a and b. At the same time, garlic prevented deposition of cholesterol and formation of plaques in the coronary vessels.[44] Results of this study are in conformity with the previous studies regarding hepatoprotective effects and cholesterol reduction.[44-46] On the other hand, livers of animals, which were administrated olive oil, in two animals out of six, no protection was observed Figure 11. In previous studies, it was found that active ingredient of olive, i.e. oleoropien has been very effective in revising the Carbon Tetra Chloride. induced hepatotoxicity in comparison to Thymoquinone, the active ingredient of *Nigella sativa*.[47] In this study, both of these substances prevented the deposition of fat in the liver, but efficacy was low in comparison to garlic.

Olive oil and *Nigella sativa* oil were also found effective in reducing the serum cholesterol levels; this effect is similar to the findings of previous studies.[48,49] This study also discovered that the substances used in the study were very much effective in reducing the incidence of atheromatous plaque formation.

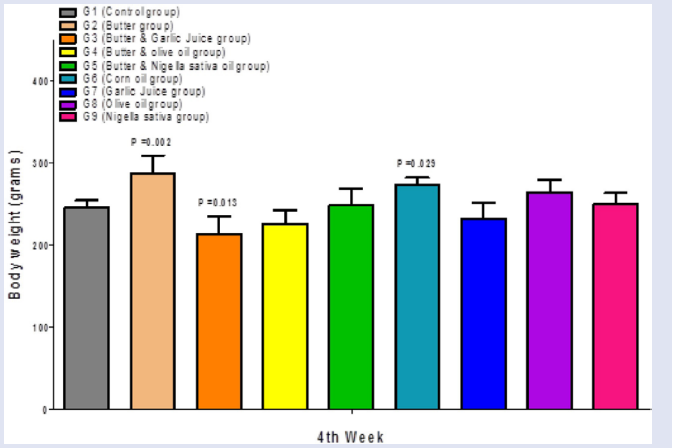

Figure 4: Comparison of lipid profile in different studied groups

Table 4: Comparison of lipid profile in different studied groups.

Groups	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Low density lipoprotein cholesterol(U/L)
G1 (Control group)	1.38 ± 0.11	0.89 ± 0.60	327.50 ± 266.16
G2 (Butter group)	1.30 ± 0.00	0.47 ± 0.46	592.00 ± 471.30
Significance	$^1P = 0.531$	$^1P = 0.087$	$^1P = 0.230$
G3 (Butter and Garlic Juice group)	1.49 ± 0.21	0.52 ± 0.21	507.75 ± 312.79
Significance	$^1P = 0.405$	$^1P = 0.128$	$^1P = 0.410$
G4 (Butter and olive oil group)	1.40 ± 0.13	0.45 ± 0.23	561.75 ± 372.66
Significance	$^1P = 0.909$	$^1P = 0.073$	$^1P = 0.286$
G5 (Butter and *Nigella sativa* oil group)	1.40 ± 0.20	0.49 ± 0.21	592.25 ± 372.65
Significance	$^1P = 0.894$	$^1P = 0.098$	$^1P = 0.229$
G6 (Corn oil group)	1.36 ± 0.12	0.25 ± 0.06	519.50 ± 43.04
Significance	$^1P = 0.864$	$^1\mathbf{P = 0.012}$	$^1P = 0.380$
G7 (Garlic Juice group)	1.43 ± 0.25	0.38 ± 0.16	690.50 ± 254.62
Significance	$^1P = 0.732$	$^1\mathbf{P = 0.039}$	$^1P = 0.103$
G8 (Olive oil group)	1.60 ± 0.34	0.64 ± 0.13	588.25 ± 142.53
Significance	$^1P = 0.114$	$^1P = 0.297$	$^1P = 0.236$
G9 (*Nigella sativa* group)	1.30 ± 0.00	0.56 ± 0.52	758.25 ± 278.77
Significance	$^1P = 0.531$	$^1P = 0.170$	$^1P = 0.055$

Data are expressed as mean+/- standard deviation. ^{1}P: Significance versus G1 (Control group).

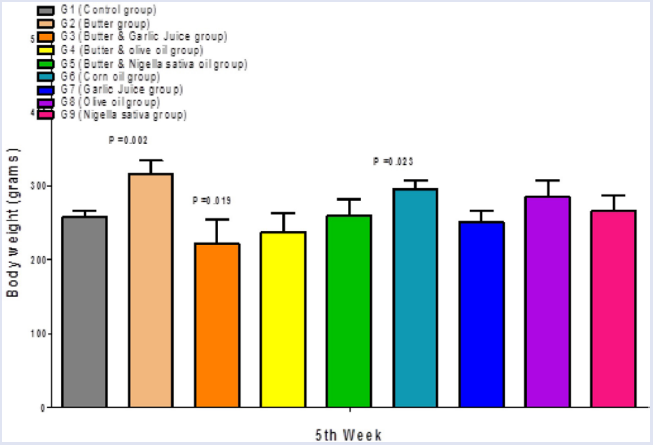

Figure 5: Compariosn of liver functions in different studied groups

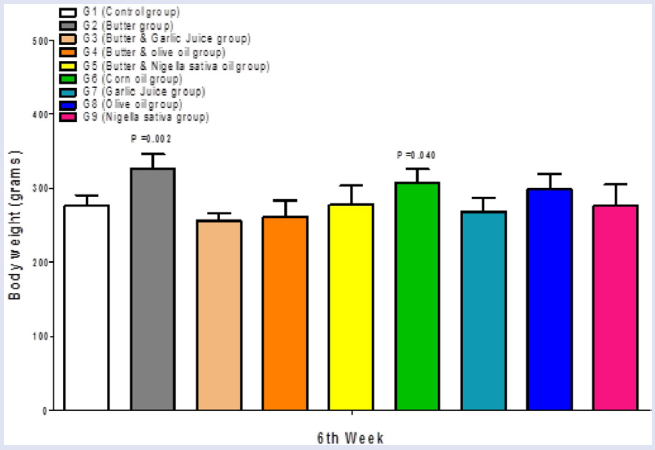

Figure 6: Comparison of heart functions in different studied groups

Table 5: Comparison of liver functions in different studied groups.

Groups	Albumin(g/L)	Total bilirubin(µmol/L)	Total proteins(g/L)	Alkaline phosphatase (U/L)
G1 (control group)	11.30 ± 0.95	2.75 ± 0.50	64.55 ± 3.13	237.75 ± 43.04
G2 (Butter group)	11.04 ± 0.03	3.25 ± 0.50	62.33 ± 4.26	201.75 ± 23.39
Significance	$^1P = 0.727$	$^1P = 0.293$	$^1P = 0.403$	$^1P = 0.463$
G3 (Butter and Garlic Juice group)	11.28 ± 1.08	3.75 ± 0.50	63.48 ± 7.32	147.75 ± 37.16
Significance	$^1P = 0.974$	$^1\mathbf{P = 0.041}$	$^1P = 0.685$	$^1P = 0.074$
G4 (Butter and olive oil group)	12.25 ± 0.97	3.75 ± 0.96	67.58 ± 2.33	264.25 ± 104.09
Significance	$^1P = 0.217$	$^1\mathbf{P = 0.041}$	$^1P = 0.258$	$^1P = 0.588$
G5 (Butter and *Nigella sativa* oil group)	13.03 ± 0.56	3.00 ± 0.82	65.43 ± 0.25	274.00 ± 141.92
Significance	$^1\mathbf{P = 0.030}$	$^1P = 0.596$	$^1P = 0.741$	$^1P = 0.460$
G6 (Corn oil group)	11.60 ± 0.73	3.75 ± 0.50	64.98 ± 1.03	119.50 ± 10.75
Significance	$^1P = 0.693$	$^1\mathbf{P = 0.041}$	$^1P = 0.872$	$^1\mathbf{P = 0.021}$
G7 (Garlic Juice group)	10.23 ± 1.88	3.50 ± 0.58	64.45 ± 3.02	148.75 ± 67.44
Significance	$^1P = 0.164$	$^1P = 0.120$	$^1P = 0.970$	$^1P = 0.077$
G8 (Olive oil group)	11.15 ± 1.05	3.00 ± 0.82	67.43 ± 3.88	114.00 ± 17.68
Significance	$^1P = 0.843$	$^1P = 0.596$	$^1P = 0.282$	$^1\mathbf{P = 0.016}$
G9 (*Nigella sativa* group)	10.45 ± 1.30	2.50 ± 0.58	61.03 ± 3.35	200.75 ± 49.07
Significance	$^1P = 0.268$	$^1P = 0.596$	$^1P = 0.190$	$^1P = 0.451$

Data are expressed as mean+/- standard deviation. ^1P: Significance versus G1 (Control group).

Table 6: Comparison of heart functions in different studied groups.

Groups	Cardiac troponin I (ng/ml)	GGT(U/L)
G1 (control group)	0.03 ± 0.01	3.00 ± 0.00
G2 (Butter group)	0.02 ± 0.00	3.00 ± 0.00
Significance	$^1P = 0.573$	$^1P = 1.000$
G3 (Butter and Garlic Juice group)	0.02 ± 0.00	3.00 ± 0.00
Significance	$^1P = 0.573$	$^1P = 1.000$
G4 (Butter and olive oil group)	0.05 ± 0.04	3.00 ± 0.00
Significance	$^1P = 0.168$	$^1P = 1.000$
G5 (Butter and *Nigella sativa* oil group)	0.04 ± 0.03	3.00 ± 0.00
Significance	$^1P = 0.387$	$^1P = 1.000$
G6 (Corn oil group)	0.02 ± 0.01	3.00 ± 0.00
Significance	$^1P = 0.386$	$^1P = 1.000$
G7 (Garlic Juice group)	0.04 ± 0.04	3.00 ± 0.00
Significance	$^1P = 0.562$	$^1P = 1.000$
G8 (Olive oil group)	0.02 ± 0.00	3.00 ± 0.00
Significance	$^1P = 0.573$	$^1P = 1.000$
G9 (*Nigella sativa* group)	0.04 ± 0.02	3.00 ± 0.00
Significance	$^1P = 0.460$	$^1P = 1.000$

Data are expressed as mean+/- standard deviation. ^1P: Significance versus G1 (Control group).

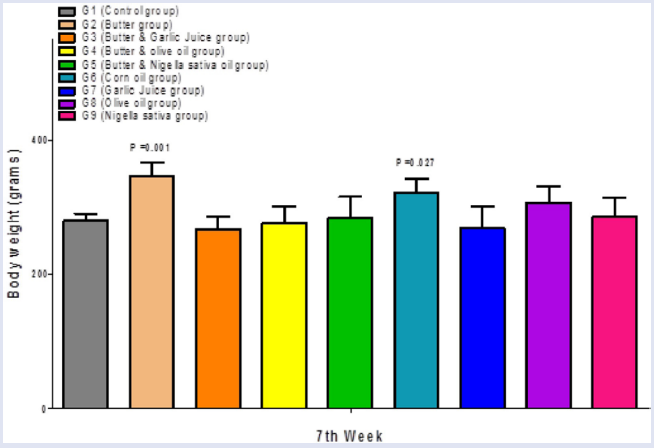

Figure 7: Comparison of kidney funtions in different studied groups

Table 7: Comparison of Kidney functions in different studied groups.

Groups	Uric acid (µmol/L)	Creatinine (µmol/L)	Blood urea nitrogen (mmol/L)
G1 (control group)	67.50 ± 28.36	47.50 ± 6.14	6.65 ± 0.75
G2 (Butter group)	80.25 ± 24.41	46.75 ± 3.77	5.54 ± 1.71
Significance	$^1P = 0.433$	$^1P = 0.859$	$^1P = 0.216$
G3 (Butter and Garlic Juice group)	70.50 ± 13.10	43.50 ± 2.52	9.75 ± 1.70
Significance	$^1P = 0.853$	$^1P = 0.347$	$^1\mathbf{P = 0.002}$
G4 (Butter and olive oil group)	80.00 ± 25.70	42.00 ± 1.73	5.90 ± 1.70
Significance	$^1P = 0.442$	$^1P = 0.234$	$^1P = 0.435$
G5 (Butter and *Nigella sativa* oil group)	69.75 ± 7.80	43.75 ± 5.25	8.38 ± 1.33
Significance	$^1P = 0.889$	$^1P = 0.377$	$^1P = 0.060$
G6 (Corn oil group)	80.00 ± 13.24	46.75 ± 7.14	6.90 ± 1.26
Significance	$^1P = 0.442$	$^1P = 0.859$	$^1P = 0.778$
G7 (Garlic Juice group)	100.50 ± 29.19	42.75 ± 12.37	7.75 ± 0.51
Significance	$^1\mathbf{P = 0.049}$	$^1P = 0.266$	$^1P = 0.221$
G8 (Olive oil group)	83.75 ± 34.48	51.75 ± 0.96	8.18 ± 1.11
Significance	$^1P = 0.320$	$^1P = 0.318$	$^1P = 0.094$
G9 (*Nigella sativa* group)	65.25 ± 10.53	37.50 ± 2.38	6.45 ± 0.45
Significance	$^1P = 0.889$	$^1\mathbf{P = 0.024}$	$^1P = 0.821$

Data are expressed as mean+/- standard deviation. ^1P: Significance versus G1 (Control group).

The serum analysis results correlate with the histopathological observations, along with preventing deposition of fat in the coronary vessels and liver [Figures 12–17]. Garlic was found more effective than the other substances used in the study.[50] No significant increase was found in cardiac enzymes in all the groups including HFD group as these enzymes are released when there is damage to the cardiac muscle.

CONCLUSION

These results of this study show that garlic is superior in protection from HFD-induced hepatic steatosis, as well as deposition of cholesterol in

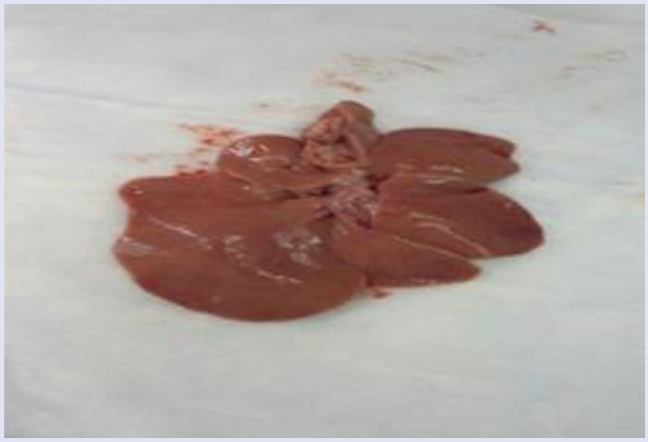

Figure 8: High Fat diet induced fatty degenration of liver

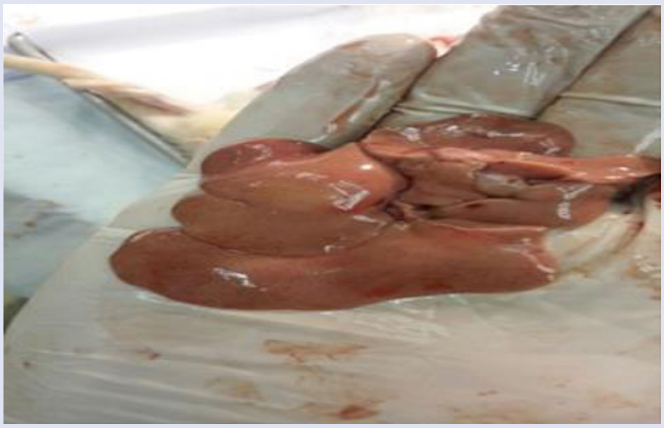

Figure 9a: Fat deposition in different parts of liver

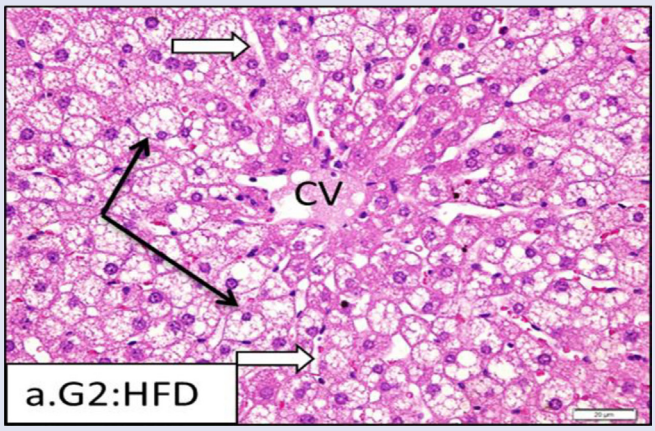

Figure 9b: High fat diet showing swollen hepatocytes with marked lipid deposition result in cytoplasmic vacuolation with small dark degenerated nuclei (black arrows). B. Similar finding at portal area region. (H & E Stain)

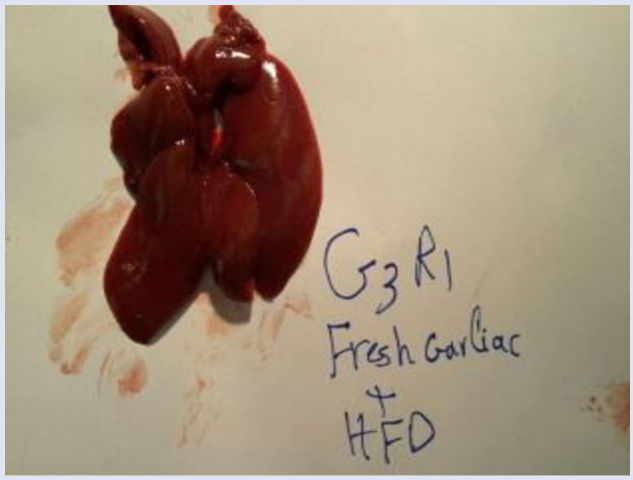

Figure 10a: Garlic + high fat diet showing protection and reversal of fatty liver to normal liver parenchyma

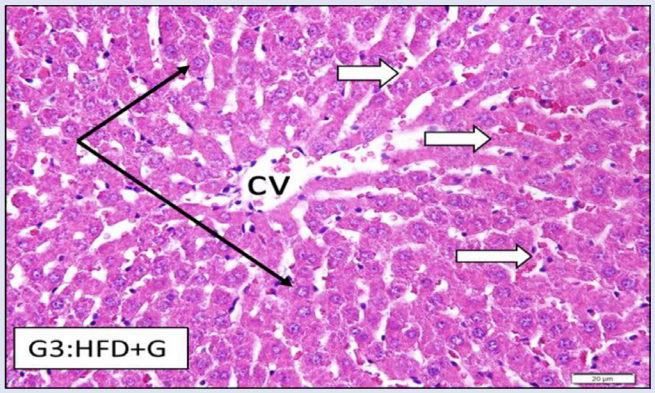

Figure 10b: Garlic + High fat diet section in G3: (HFD+G) showing marked protection against HFD induced changes, No deposition of lipids was seen. Hepatocytes looked normal (thin black arrows) around the central vein (CV) with normal hepatic sinusoids, some showed slight congestion. (H&E Stain)

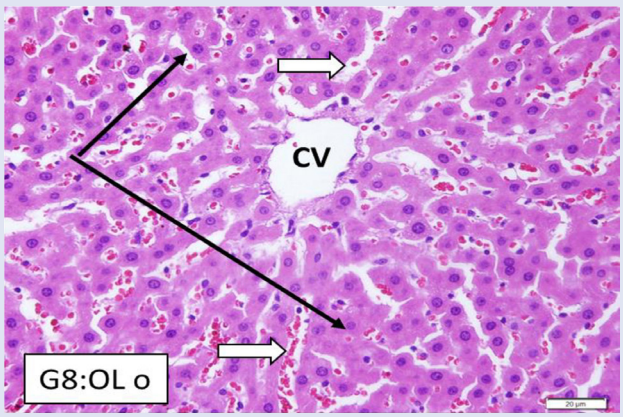

Figure 11: Group 8 olive oil result in slight dilatation of blood sinusoids(white arrows), hepatocytes looked less in size but have active auchromatic nuclei (black arrows). (H & E Stain)

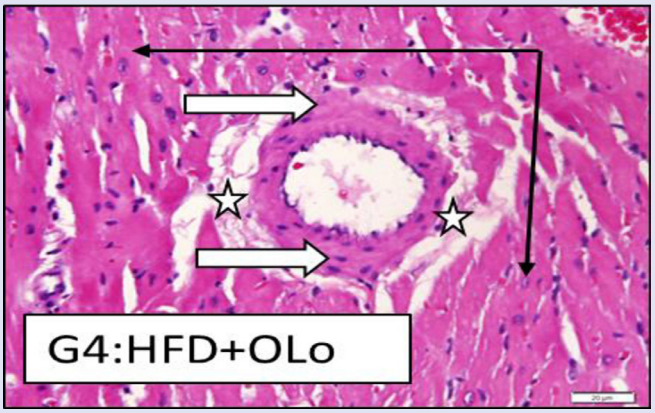

Figure 12: G4: :high fat diet with Olive Oil, Coronary artery showed only focal thickness (white arrows). Fine perivascular fibrosis (Stars) muscles are of normal appearance (black arrows). (H & E Stain)

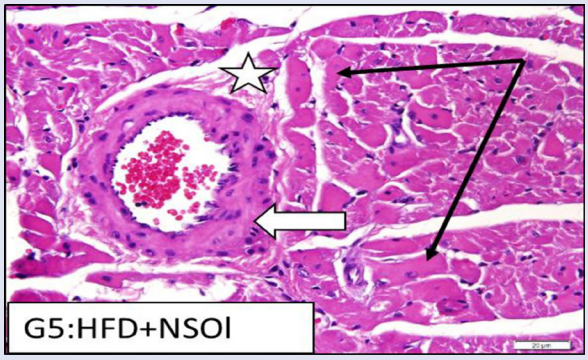

Figure 13: G4 High fat diet with Nigella sativa oil provided marked protection against coronary artery changes by HFD. only focal thickening with tiny lipid droplets within arrterial wall muscles (white arrows) were observed. Nearby muscles showed slight hypertrophy (thin black arrows), slight perivascular fibrisis (star). (H &E Stain X400).

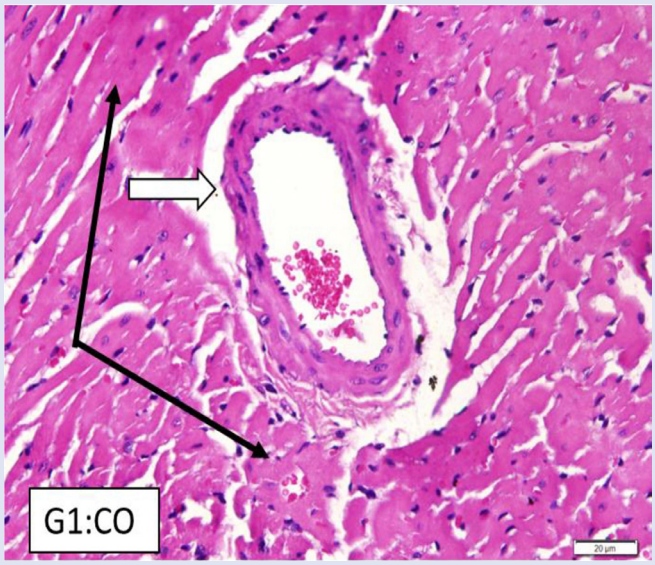

Figure 14: Control group showing normal cardiac fibers (black arrows) in the left ventricle, coronary artery showed normal wall thickness (white arrows)

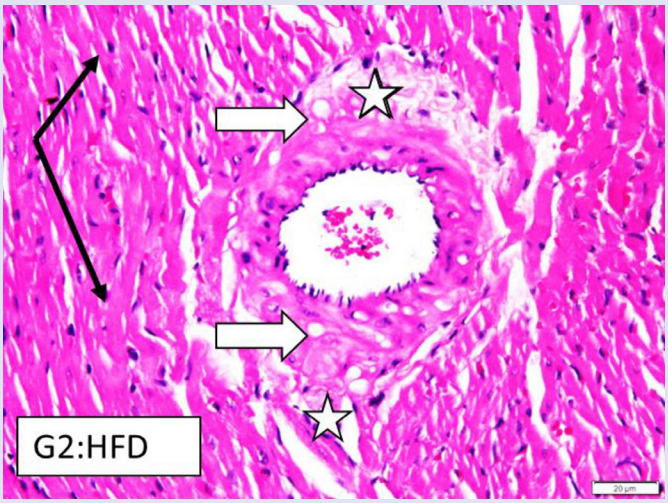

Figure 15: High fat diet showing marked thickening of coronary artery with presence of tiny fat droplets within the muscular elements (white arrows). Notice perivascular fibrosis (stars), Nearby cardiac muscles looked hypertrohied (black arrows). (H& E Stain)

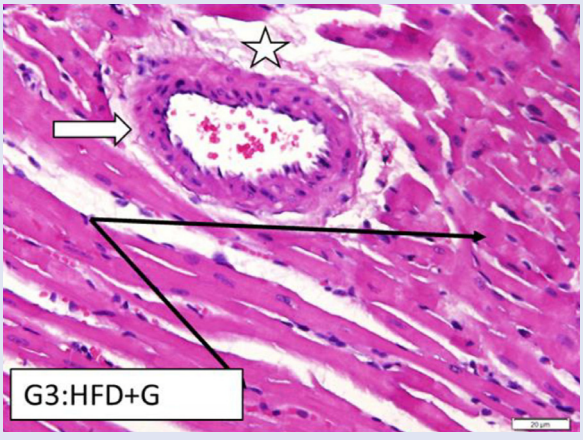

Figure 16: High fat diet with garlic showing preservation of coronary wall thickness (white arrow), Nearby cardiac muscles looked of normal size (black arrows). (H & E stain)

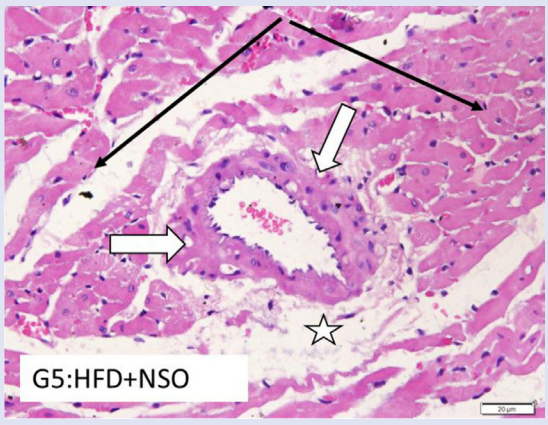

Figure 17: High fat dient with Nigella sativa oil, the coronary showed irregular wall thickening with presence of lipid within muscular elements (white arrows), Nearby muscles showed degenerated dark nuclei or atrophy (black arrows). (H& E Stain)

the coronary vessels, followed by olive oil and then *Nigella sativa* oil. It is highly recommended that garlic (*Allium Sativum*) should be used with the pharmacotherapy to prevent and treat diet-induced fatty degeneration of liver and for prevention of deposition of cholesterol in the coronary vessels. However, it is concluded that further studies should be conducted in humans with large sample size to confirm the above-mentioned therapeutic effects.

Acknowledgement

The authors wish to acknowledge the approval and the support of this research study by the grant no. 3-7-1436-5 from the Deanship of Scientific Research in Northern Border University, Arar, K.S.A.

Financial support and sponsorship

Deanship of Scientific Research, Northern Border University, Saudi Arabia.

Conflicts of interest

There are no conflicts of interest.

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