Effect of grape seed, ginger and mustard oils on lipids profile in male rats

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Abstract:

Hyperlipidemia is a major risk factor for development and progression of CVD. This study evaluates the potential effect of grapeseed, ginger and mustard oils (5% and 10%) on serum lipid, liver, renal function tests (SGPT, SGOT, creatinine, uric acid and urea) and antioxidant enzymes (catalase and superoxide dismutase) of high fat diet rats. Forty-eight male rats divided into 8 groups; G1 fed standard diet (negative control), seven groups fed hyperlipidemic, for 6 weeks, one kept as positive control fed hypercholesterolemic diet, six groups fed hypercholesteremic diet with tested oils. Total cholesterol, triglycerides, high, low, very low-density lipoproteins, liver, renal function tests and antioxidant enzyme activity measured. Hypercholesterolemic rats showed a significant elevation in TC (99.66 ± 0.88), TG (60.33 ±4.33),

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LDL-c (57.00 ± 0.57), VLDL-c (12.00 ± 0.57) and reduction in serum HDL-c (20.66 ± 0.88) compared with negative control (76.00 ±0.57, 42.00 ±3.51, 40.33 ± 1.45, 8.40 ± 0.66 and 30.66 ± 0.88 respectively). Also, liver and renal function tests showed significant elevation and antioxidant enzyme showed significant reduction. Tested oils with different concentrations were effective in reducing lipids, restoring antioxidant enzymes activity and HDL-c. Histopathological examination of liver, renal, heart and aorta of hypercholesterolemic rats showed vacuolation of some cells, intramuscular hemorrhage and edema, inflammatory cell infiltration. Tested oils preserved normal structure of liver, kidney, heart and aorta. This study confirmed the health benefits of tested oils in modulation of lipid profile, kidney, liver function tests and antioxidant enzymes activity. Also modulate histopathological changes of examined tissues.

Keywords: Vegetables, Fats, Catalase, Tissues

Introduction

Dietary fats and oils are known to affect plasma lipid profile and play a vital role in the development of atherosclerosis, heart disease and cancer which are the main causes of death (Mokhtar et al., 2016). The prevalence of hyperlipidemia has dramatically increased worldwide due to a modern lifestyle and an increase of consumption of a high-fat diet (Adisakwattana et al., 2010). Hyperlipidemia has a quite clear role in the etiology of coronary heart disease. For instance, the type of fatty acids in diet has a ma-

jor role in cardiovascular health (Kaseb and Biregani 2016). Therefore, the health benefits of vegetable oils (high in mono- and polyunsaturated fatty acids) in relation to risk factors for cardiovascular disease (CVD) is of considerable interests to be determined (Kim et al., 2010).

Grape seed oil is natural oil that was extracted from the seed of Vitis vinifira. It is rich in unsaturated fatty acids such as oleic and linoleic acid as compared with other oily seeds. It has been reported that the grape seed oil had antioxidant properties, affected the lipid metabolism of rats and led to decreasing serum and liver triglyceride concentrations (Jaffer 2017). The ginger oil has a very good antibacterial, antifungal property therefore, prevents food borne diseases. Ginger is also reported to prevent rancidity, thereby increasing the shelf life of lipid containing foods. The phytochemicals in ginger oil also possess free radical scavenging, antioxidant and anti-peroxidative effects. These properties are attributed to the plethora of biologically active compounds present in the fresh as well dried ginger oils. The antioxidant and lipid peroxidation inhibition properties of ginger prevent peroxidative damage, indicating the benefits of ginger in prevention of microbial food spoilage, free radical-induced damage and rancidity (Jakribettu et al., 2016). It is used as an anti-inflammatory, antiemetic, anti-tumor, analgesic, anti-hemorrhagic, neuronal cell protective, anti-rheumatic ,antifungal and antibacterial agent (Mesomo

et al., 2013).Ginger oil is used in many diseases as anti-inflammatory and antioxidant modulator (Gabr et al., 2017).

Mustard oil has low saturated fat as compared to other cooking oils. It consists of fatty acids (oleic, erucic and linoleic acid). In human, animal and in vitro studies, dietary mustard products (seed or leaf) decreased lipid peroxidation and increased the activity of glutathione S-transferase, superoxide dismutase, and catalase against chromosomal damage and oxidative stress induced by gamma-radiation or cancer (El-Shenawy et al., 2014). Mustard oil consumption prevents dyslipidemia, coronary artery diseases, atherosclerosis and colon cancer (Malik et al., 2011). As mustard oil contains two essential fatty acids (EFA) in appreciable amount and natural antioxidant, tocopherol, in significant amount, it could be a potential raw material to produce a low calorie, healthful edible oil (Dhara et al., 2013).

The study aimed to investigate the effect of grape seed, ginger and mustard oils on lipid profile, antioxidant enzymes activity, liver, renal function tests and histopathological examination of some tissues in hyperlipidemic rats.

Material and Methods

Materials:

- Grape seed, ginger, mustard oils obtained from local market, Cairo, Egypt.

- Normal male albino rats (48) of Sprague Dawley strain weighted 100±5g were obtained from the laboratory animal colony Agricultural, research, center, Egypt.
- All Chemical were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

Methods:

Biological experiment

Diets

- Standard diet:

Standard diet was prepared from fine ingredients per 100g. according to (AIN 1993).

It composed of Casein (14 gm), Sunflower oil (10 gm), DL-methionine (0.3gm), Choline chloride (0.2 gm), Minerals mixture (4 gm), Vitamins mixture (1 gm), Fiber (cellulose, 4 gm), Corn starch (Up to 100%).

- Hyperlipidemic / hypercholesterolemic diet:

Hyperlipidemic / hypercholesterolemic diet was prepared from fine ingredients per 100g according to (Rashwan 1994). The diet had the following composition:

Fat 20% (sunflower 10% + sheep tallow 10%), sugar 10%, salt mixture 4%, vitamin mixture 1%, choline chloride 0.2%, cholesterol powder 1%, casein 14%, DL-mithionine 0.3% and corn starch up to 100 gram.

- Experimental diets: -

Experimental diets were prepared from standard diet with a replacement oils (grape seed, ginger, and mustard) in the ratio of 5% and 10%.

Experimental design:

Forty-eight adult male albino rats Sprague Dawely strain weighting 100±5g 10 weeks were used. The rats were divided into eight groups (6 per each) with similar weight. One of them was negative control fed standard diet while the other groups fed Hyperlipidemic and hypercholesterolemic diet for 3 weeks then fed experimental diets (Hyperlipidemic / hypercholesterolemic diet containing 5% and 10% from grape seed, ginger and mustard oils) for three weeks. Food and water were provided ad-libitum for all groups as follow:

Group (1) negative control: fed standard diet.

Group (2) positive control: Fed on hyperlipidemic and hypercholesterolemic diet

Group (3) Fed on hyperlipidemic and hypercholesterolemic diet + 5% grape seed oil.

Group (4) Fed on hyperlipidemic hypercholesterolemic diet + 10% grape seed oil.

Group (5) Fed on hyperlipidemic hypercholesterolemic diet + 5% ginger oil.

Group (6) Fed on hyperlipidemic and hypercholesterolemic diet + 10% ginger oil.

Group (7) Fed on hyperlipidemic and hypercholesterolemic diet + 5% mustard oil.
Group (8) Fed on hyperlipidemic and hypercholesterolemic + 10% mustard oil.

At the end of experiment, all rats were sacrificed under ether anesthesia and blood samples were collected from hepatic portal vein. Serum was separated by centrifugation of blood at 3000 RPM for 15 minutes at room temperature then was kept in quite fit plastic vial and stored at -18^0 c until analysis. The relative weight of liver, kidney, and heart were calculated according to Carleton (1976) was removed and washed in saline solution, then dried by filter paper and weighed. Apart of liver were fixed in 10% formalin solution then were subjected to histological examination.

Biological evaluation:

The duration of the study was 8 weeks. Feed intake recorded daily and body weight measured once a week. The total body weight gain and feed intake during the experimental period calculated. Feed efficiency ratio calculated at the end of experiment as follows:

BWG (%) = (Final Weight - Initial Weight x100/ Initial Weight

Biochemical analysis:

Triglycerides, total cholesterol, antioxidant enzymes, (HDL-c, LDL-c, VLDL-c), (ALT, AST) and (urea, uric acid and creatinine)

according to (fossati and principe 1982), (Tomas 1992), (Aebi 1984), (Lee and Nieman 1996), (Reitman and Frankel 1957), (Young 2001).

Statistical analysis:

The data were expressed as mean ± SD then subjected to statistical analysis by SPSS (1998).

Result and Discussion

Food intake and body weight gain

Data in Tables (1 and 2): showed the effects of some levels (5% and 10%) from grapeseed, ginger and mustard oils on body weight, weight gain, weight gain percent, food intake and food efficiency ratio of experimental rats.

The present study found that for positive control group there was a significant ($p \leq 0.05$) increase when compared with negative control. All experimental groups with (5%and 10% tested oils) there was significant ($p \leq 0.05$) decrease when compared with positive control group. Moreover, on comparing the experimental groups, mustard oil 5% has the lowest value while grapeseed oil 5% has the highest value. These results were supported by Saham et al. (2015) who found that the rat group that feed on high cholesterol diet gave the highest values for the body weight gain when compared to the rat feed on the basal diet. While the rats fed on diet supplemented with ginger oil was close to that fed on the basal diet. Also, our results supported by Eissa et al. (2017) who showed for high fat diet rats a significant increase in body weight gain

when compared to the negative control group. However, high fat diet group treated with ginger oil showed significant reduction in body weight when compared to high fat diet group only. These results can be explained by; ginger oil reduces the cholesterol level by stimulation of cholesterol conversion to bile acids and salts then excretion with bile, it inhibits intestinal absorption of cholesterol. It helps releasing the fats from the cells, decreasing in cells size, preventing the body from producing fat cells. This leads to weight loss (Onu and Aja 2011). As regard grapeseed oil effect on these variables or results were in contrast to Kim et al. (2010) who reported that grapeseed oil effect on body weight was non-significant.

Parameters / Groups	Weight gain (gm)	Weight gain (%)
Negative control (G 1)	61.33±2.87c	53.13±2.10c
Positive control (G 2)	106.33±5.75b	93.04±6.76b
Grape seed oil 5% (G 3)	87.17±7.20ad	42.91±6.21ad
Grape seed oil 10% (G 4)	84.50±5.63a	42.25±7.51a
Ginger oil 5% (G 5)	83.50±4.04a	41.92±6.11a
Ginger oil 10% (G 6)	79.50±3.82a	40.76±4.88a

Mustard oil 5% (G 7)	74.67±8.31ad	39.33±6.09ad
Mustard oil 10% (G 8)	78.50±4.83a	40.46±7.63a

Table (1): Effect of grape seed, ginger and mustard oils on experimental rats's weight gain(gm) and weight gain (%).

Values are expressed as mean ± SD. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Table (2): Food intake and Food Efficiency Ratio of experimental rats.

Parameters / Groups	*Food intake*	Food Efficiency Ratio
Negative control (G1)	17.7±1.7a	3.46±1.7c
Positive control (G 2)	22.18±1.8b	4.79±3.19c
Grape seed oil 5% (G 3)	20.37±3.3ab	4.28±2.18c
Grape seed oil 10% (G 4)	20.63±2.8ab	4.09±2.0c
Ginger oil 5% (G 5)	19.15±1.3a	4.36±3.1c
Ginger oil 10% (G 6)	19.5±1.3a	4.07±2.85c
Mustard oil 5% (G7)	19.73±3.8ab	3.78±2.19c
Mustard oil 10% (G 8)	19.0±1.9a	4.13±2.54c

Values are expressed as mean ± SD. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Lipid profiles

Data in Tables (3 and 4) showed the effects of some levels (5% and 10%) of grapeseed, ginger and mustard oils on serum cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C and AI of experimental rats.

It was found that these variables increased significantly ($P \leq 0,01$) in the high fat diet group as compared to the negative control group while except HDL-C was significantly($P \leq 0.05$) decreased while all treated groups with experimental oils showed significant ($P \leq 0.05$) decrease in these variables except HDL-C was increased as compared to positive control group. The highest decrease in these variables except HDL-C which was increased recorded for the group treated with mustard oil 5%, grapeseed 5% and ginger oil 5%.

Hypercholesteremia is a risk factor for the development and progression of atherosclerosis and cardiovascular diseases. A high cholesterol diet is a major environmental contributor to unbalanced lipid metabolism and associated with an increase in prevalence of coronary heart disease (Libby 2008).The tested oils (grapeseed, ginger, mustard) are rich in some essential fatty acids and some bioactive compounds such as sulfur peptides that provide a healthy effect in fat metabolism and in scavenging of free

radicals. These effects are strongly indeed this time especially in some developing countries that suffering from several diseases that caused by eating unbalanced diet that rich in saturated fats (Seham et al., 2015).

The results of the present study in tables (3 and 4) indicated that serum TC, TG, HDL-c, LDL-c and VLDL-c in positive control elevated significantly ($P \leq 0.05$) as compared with negative control, while HDL-c level significantly ($P \leq 0.05$) reduced. This results were in agreement with (Laleye et al., 2007), (Bolanle 2011) and (Eissa et al., 2017) who reported that the rat group (HFD) showed high level of serum cholesterol. These results may be due to eating of foods rich in saturated fats and cholesterol. Treatment with ginger oil showed significant ($p \leq 0.05$) decrease in the level of TC, TG, LDL-c, VLDL-c compared with (HFD)and a significant increase in HDL-c levels. This is in accordance with Eissa et al., (2017). These effects may be due to ginger oil increases the pancreatic lipase activity, which plays a vital role in fat digestion Also, may be due to reduction in the absorption of the dietary fat by the active components in ginger oil (Eissa et al., 2017).

The diet containing GSO decreased significantly ($p<0.05$) Serum TC, TG, LDL-c, VLDL-c levels, and increased serum HDL-c ($p < 0.05$). These results compatible with the results of Chung et al., (2003) who reported that grape seed oil (GSO) have important role for improvement of serum lipid profile. Moreover, the study of Nash (2004) reported that grape seed oil may increase HDL and

reduce LDL-c, thus it helping to prevent heart disease. Furthermore, GSO improve the lipid ratios: atherogenic coefficient (AC) cardiac risk ratio (CRR), LDL-c to HDL-c ratio and atherogenic index of plasma (AIP) compared with the positive control group.

Table (3): Effect of grape seed, ginger and mustard oils on serum cholesterol and triglyceride of experimental rats(mg/dl).

Parameters / Groups	Cholesterol	Triglyceride
Negative control (G1)	76.00 ±0.57cd	42.00 ±3.51cd
Positive control (G 2)	99.66 ± 0.88b	60.33 ±4.33b
Grape seed oil 5% (G 3)	75.33 ±1.45cd	42.00 ±1.52cd
Grape seed oil 10% (G 4)	82.33 ±1.20a	49.00 ±1.00a
Ginger oil 5% (G 5)	76.66 ± 2.02c	43.66 ± 0.66e
Ginger oil 10% (G 6)	81.66 ±1.20a	54.00 ±2.30f
Mustard oil 5% (G7)	74.00 ± 1.52e	40.33 ±1.45cd
Mustard oil 10% (G 8)	80.00 ±1.52f	54.66 ±4.33f

Values are expressed as mean ± SD. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Table (4): Effect of grape seed, ginger, and mustard oils on serum lipoproteins and atherogenic index (LDL-c/ HDL-c ratio) of experimental rats(mg/dl).

Parameters / Groups	HDL-c	LDL-c	VLDL-c	AI
Negative control (G 1)	30.66 ± 0.88c	40.33 ± 1.45c	8.40 ± 0.66d	1.31±1.64 ab
Positive control (G 2)	20.66 ± 0.88b	57.00 ± 0.57b	12.00 ± 0.57a	2.76±0.64 a
Grape seed oil 5% (G 3)	30.00 ±1.15c	35.00 ± 1.15a	8.40 ± 0.33de	1.17±1.00 b
Grape seed oil 10% (G 4)	26.33 ±1.76a	46.66 ± 0.33d	9.80 ± 0.33b	1.77±0.19 b
Ginger oil 5% (G 5)	29.66 ±0.66cd	39.66 ±2.33c	8.70 ± 0.33d	1.34±3.53 a
Ginger oil 10% (G 6)	35.33 ± 2.60e	54.00 ±1.73e	10.80 ± 0.33ef	1.53±0.66 b
Mustard oil 5% (G 7)	30.00± 0.57cd	32.33 ±0.88f	8.06 ± 0.33e	1.08±1.54 b
Mustard oil 10% (G 8)	28.66 ± 1.66f	42.66 ±2.02a	10.93 ± 0.88f	1.49±1.22 b

Values are expressed as mean ± SD. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Liver enzymes

Effect of grape seed, ginger, and mustard oils on liver enzymes of experimental rats.

Data in Table (5) showed that the mean value of alanine amino transferase (ALT), aspartate amino transferase (AST) of high fat diet rats positive control group were 34.66± 0.66 and 89.33 ± 0.33 respectively increased significantly ($P \leq 0.05$) as compared to the negative control group 21.00 ± 0.10 and 64.66 ± 6.96 respectively. While treating rats with some levels (5% and 10%) grape seed, ginger, and mustard oils reduced significantly ($P \leq 0.05$) serum ALT, AST enzyme as compared to the positive control group. Treating rats with 5% grape seed, ginger, and mustard oils showed the best results in serum ALT, AST enzyme than 10%. Treating rats with 5% ginger, mustard and grape seed oils decreased the mean value of serum ALT by about (47.11%, 43.27% and 42.29%) respectively than that of (+ve) control group. The highest decrease in serum ALT enzyme recorded for the group which treated with 5% ginger oil followed by 5% mustard oil and 5% grape seed oil, respectively. Treating rats with 5% mustard oil, 10% ginger oil, 10% mustard oil, 5% grape seed oil, 5% ginger oil and 10% grape seed oil decreased the mean value of serum AST by about 50.37%, 36.19%, 34.33%, 32.83%, 27.23% and 17.91%, respectively than that of positive control group. The lowest decrease in serum AST enzyme recorded for the group which treated with 10% grape seed oil.

Liver is known to be the main site for biosynthesis and metabolism of number of molecules. Any damage to it leads to abnormalities in normal physiological functions of body. Excess intake of a diet rich in fatty acids deteriorates the normal functioning of hepatocytes. High-fat diet induces changes in the normal physiology of hepatocytes, by depositing fat inside them, as it exerts pressure on cell linings and makes them leaky (Faran et al., 2019). AST and ALT are cytosolic enzymes which are highly concentrated in the liver and are only found in significant quantities in the serum when the cell membrane becomes leaky and even completely ruptured. This evidence helps understand our findings of the high serum AST and ALT levels in the HFD group (Faran et al., 2019).

These results are in agreement with Bolanle (2011) and Faran et al. (2019) who reported that a significant increase was observed in the activity of liver enzymes (AST, ALT) of hypercholesteremic rats when compared with normal control. While a significant decrease was observed when comparing the hypercholesteremic rats with the group of rats treated with ginger 5%, 10% level. These results may be due to the hypercholesteremic diet caused deposition of fats in the hepatocytes which may lead to damage of the cells and hence leakage. The decrease in activity observed in these enzymes after treatment could have been due to recovery of the organ from the nutritional insult imposed by the hypercholesteremic diet (Eissa et al., 2017). Moreover, ginger oil boosted the

healing process of HFD-ruptured cell membranes and helped lower serum concentrations of AST, ALT as ginger oil has highly hepatoprotective agent (Faran et al., 2019). Also, grapeseed oil caused significant decrease in serum (AST and ALT) when compared with the positive control. these results was supported by Mokhtar et al. (2016) and may be explained by grapeseed oil has hepatoprotective properties as it contains antioxidant which prevent liver damage by oxidative stress (Maheswari and Rao 2005).

Table (5): Effect of grape seed, ginger and mustard oils on liver enzymes of experimental rats.

Parameters / Groups	ALT(U/L) [SGPT]	AST(U/L) [SGOT]
Negative control (G 1)	21.00 ± 0.10df	64.66 ± 6.96cde
Positive control (G 2)	34.66± 0.66b	89.33 ± 0.33a
Grape seed oil 5% (G 3)	20.00 ± 1.00a	60.00 ± 1.00ce
Grape seed oil 10% (G 4)	23.66 ± 1.33fe	73.33± 3.33d
Ginger oil 5% (G 5)	18.33 ± 1.33c	65.00± 7.00e
Ginger oil 10% (G	23.66 ± 3.52df	57.00± 5.77c

6)		
Mustard oil 5% (G 7)	19.66 ± 1.33ace	44.33± 1.76b
Mustard oil 10% (G 8)	20.66 ± 2.33cd	58.66± 3.75ce

Values are expressed as mean ± SD. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Antioxidant enzymes activity

Effect of grape seed, ginger, and mustard oils on antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) of experimental rats.

Data in Table (6) showed that the mean value of CAT and SOD of positive control significantly ($P \leq 0.05$) decreased than negative control. While treating rats with some levels (5% and 10) grape seed, ginger, and mustard oils caused significant ($P \leq 0.01$) increase in serum CAT and SOD enzymes as compared to positive control group. Treating rats with all experimental oils showed significant best results in serum SOD enzyme as compared to the negative control group.

Also, treating rats with 5% grapeseed oil followed by 5% mustard oil and 10% ginger oil increase the mean value of serum SOD enzyme by about 72.74%, 71.29% and 64.02% respectively than that of positive control group. The lowest increase in serum SOD enzyme recorded for the group which treated with 5% ginger oil and

10% grape seed oil. While highest value was in 5% grape seed oil and 5% mustard oil.

Treating rats with 5% mustard oil followed by 10% mustard oil and 5% grape seed oil increase the mean value of serum CAT enzyme by about 16.72%, 13.87% and 13.66%, respectively than that of positive control group. The lowest increase in serum CAT enzyme recorded for the group which treated with 5% and 10% ginger oil. While, highest value was in 5% and 10% mustard oil group.

The present results of CAT were in harmony with Seham et al. (2015) and Farahat et al. (2017) who reported that treatment with ginger oil results in significant elevation of CAT when compared with the positive control(role of the high cholesterol level in the activation of the lipid oxidation rate and the production of the free radicals that led to consumption of antioxidant enzyme for scavenging these free radicals on rat group that fed on the high cholesterol diet). Therefore, in the treated group with ginger oil the antioxidant enzymes (CAT and SOD) significantly increased when compared to the positive control. This may explained by ginger oil has antioxidant activity; scavenge free radical as it contains some natural antioxidant compounds (Sakr 2007). Therefore, it protects the cells against the oxidative damage (Seham et al., 2015). Also, it helps in slowing the lipid peroxidation processes. This leads to uplifting the activity of the antioxidant enzyme. Moreover, the antioxidant activity of ginger oil resembles that of vitamin C in low-

ering lipid peroxidation. This leads to impacting serum catalase and SOD enzyme concentrations (Faran et al., 2019).

As regard mustard oil, our results was similar to Benson and Devi (2009) who found that the mustard oil has a beneficial effect in reducing the free radicals, as it contains ω-3 fatty acids, therefore it protects antioxidant activity. This leads to prevention of lipid peroxidation. Therefore, it provides protective mechanisms against the CVD.

Table (6): Effect of grape seed, ginger, and mustard oils on anti-oxidant enzymes Superoxide dismutase (SOD) and Catalase (CAT) of experimental rats.

Parameters / Groups	SOD(U/L)	CAT(U/L)
Negative control (G 1)	374.71±0.04a	838.33 ±29.45def
Positive control (G 2)	274.84±0.01b	807.33 ±17.14a
Grape seed oil 5% (G 3)	474.78±0.03c	917.66 ±12.46b
Grape seed oil 10% (G 4)	420.67±0.01d	913.33 ±11.39bc
Ginger oil 5% (G 5)	400.74±0.01c	845.00 ±20.38e
Ginger oil 10% (G 6)	450.81±0.01e	894.66 ±30.24c
Mustard oil 5% (G 7)	470.78±0.05e	942.33 ±21.42c
Mustard oil 10% (G 8)	430.78±0.02f	919.33 ±30.33c

Values are expressed as mean ± SD. Means with different super-script letters in the same column are significantly different at $p \leq 0.05$.

Renal biomarkers

Effect of grape seed, ginger, and mustard oils on kidney function of experimental

rats(mg/dl).

Data in Table (7) revealed that the mean value of serum creatinine, and urea increased significantly ($P \leq 0.05$) in positive control group. However, the increase in uric acid was non-significant as compared to negative control group. The mean values ± SD of serum creatinine, uric acid, and urea were (0.72 ±0.05, 2.30 ±0.11 and 54.33 ±1.45) respectively for the positive control group, while negative control rats recorded (0.58 ±0.01, 2.23 ±0.13 and 43.33 ±1.76) respectively. On the other hand, all treated groups recorded significant ($P \leq 0.01$) decrease serum creatinine as compared to the positive control group. Treating high fat diet rats with 5% mustard oil recorded the best results in these parameters, followed by groups treated with 10% mustard oil and 10% ginger oil, respectively.

Furthermore, serum uric acid decreased significantly ($P \leq 0.05$) in 5% ginger oil, 5% mustard oil, and 10% mustard oil, while non-significant in 5%, 10% grape seed oil and 10% ginger oil as compared to the positive control group. Moreover, the best results recorded in groups treated with 10% mustard oil, 5% grape seed oil, (5% mustard and ginger oils) [13%, 8.6%, 6%] respectively than that of positive control group.

Moreover, serum urea of all groups treated with some levels (5% and 10%) grape seed, ginger, and mustard oils decreased significantly ($P \leq 0.01$) as compared to positive control group (Table 7). Treating high fat diet rats with 5% and 10% ginger oil, 5% and 10% mustard oil, 5% and10%grape seed oil, decreased serum urea by about 44.17%, 16.56%, 38.65%, 32.52%, 27.60%, and 21.47%, and respectively than that of positive control group.

Chronic dietary lipid intake leads to excess deposition of saturated fats inside the cells of various organs. Lipids are deposited in the kidney at a high level, leading to significant alterations in renal subcellular structures (renal cortex). Enormous adiposity leads towards dilation of large, localized blood vessels, and subcapsular adipocyte accumulation causes glomerular atrophy and necrosis, resulting in poor filtration of molecules and, consequently, raising their plasma levels. Vasculature abnormalities and nephropathy, owing to a high intake of saturated fatty acids has been reported. Hypercholesterolemia, hypertriglyceridemia, low levels of HDL-C, and high concentrations of apolipoprotein-B are known as major risk factors in the development of chronic kidney disease (CKD). Moreover, in the presence of these abnormalities, kidney disease progresses more rapidly. Such nephrotoxic and abnormal conditions lead to malfunctioning of kidneys and cause an imbalance in concentrations of small molecules and electrolytes inside and outside the cells(Faran et al., 2019).

The present study as showed in Table (7) serum creatinine, uric acid and urea were increased in positive control group than negative control group but in treated groups the result showed improving of these parameters this results in harmony with results of Faran et al. (2019) who reported that high fat diet group showed comparatively higher levels of serum creatinine, blood urea. Whereas the levels of these biochemical parameters in groups treated with ginger oil were quite lower compared to the HFD group. The reason behind this is the direct action of these natural therapeutic agents in remediation of injured cell lines and damaged organs. Also, ginger oil has been shown to normalize levels of these biomarkers and heal the damaged site. It contains polyphenols and flavonoids, which provide antioxidant and nephroprotective effects and help regulate the normal functioning of nephrons. This prevents elevations in blood urea and serum creatinine levels; hence, it normalized the levels of these biomarkers as compared to HFD group. Ginger oil is widely known as a blood cleaner when it comes to elevated levels of blood urea and serum creatinine; hence, it keeps normal concentrations of these biological compounds by eliminating them when their concentration goes higher in diseased conditions (Faran et al., 2019).

Table (7): Effect of grape seed, ginger, and mustard oils on kidney function of experimental rats (mg/dl).

Parameters / Groups	Creatinine	Uric acid	Urea
Negative control (G 1)	0.58 ±0.01cde	2.23 ±0.13abcd	43.33 ±1.76cd
Positive control (G 2)	0.72 ±0.05b	2.30 ±0.11ab	54.33 ±1.45a
Grape seed oil 5% (G 3)	0.60 ±0.03d	2.10 ± 0.57abce	39.33 ±1.45b
Grape seed oil 10% (G 4)	0.65 ±0.04a	2.20 ± 0.20bd	42.66 ± .880cd
Ginger oil 5% (G 5)	0.65 ±0.03f	2.16 ± 0.08cde	30.33 ± .880f
Ginger oil 10% (G 6)	0.59 ±0.01e	2.20 ± 0.05bc	45.33 ± .880d
Mustard oil 5% (G 7)	0.54 ±0.02e	2.16 ±0.08ce	33.33 ±1.76e
Mustard oil 10% (G 8)	0.57 ±0.02c	2.00 ± 0.01f	36.66 ± .880b

Values are expressed as mean ± SD. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Histopathological examination

Aorta

Microscopically examination of aorta of rats from group 1 revealed no histopathological changes (Photo.1). In contrary, aorta of rats from group 2 showed vacuolation of cells of tunica media (photo. 2 and 3). Meanwhile, aorta of rats from groups 3, 4 and 5 revealed vacuolation some cells of the tunica media (photo. 4, 5 and 6) Moreover, aorta of rats from group 7 and some examined sections from group 8 revealed no histopathological changes (photo. 5 and 6), whereas other sections from group 8 showed vacuolation some cells of the tunica media.

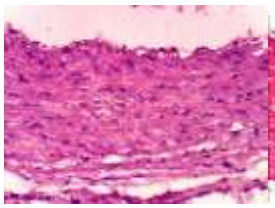


Photo. (1): Aorta of rat from group 1 showing no histopathological changes (H & E X 400)

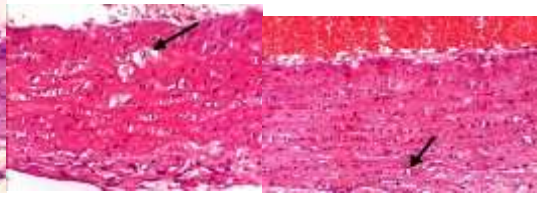


Photo. (2): Aorta of rat from group 2 showing vacuolation of the tunica media (H & E X 400).

Photo. (3): Aorta of rat from group 3, 4, 5 & 6 showing vacuolation some cells of the tunica media (H & E X 400).

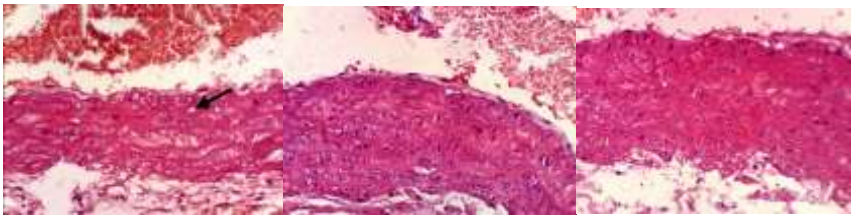


Photo. (4): Aorta of rat from group 6 showing no histopathological changes (H & E X 400).

Photo. (5): Aorta of rat from group7 & 8 showing no histopathological changes (H & E X 400).

Photo. (6): Aorta of rat from group 8 showing vacuolation some cells of the tunica media (H & E X 400).

Heart

Microscopically, heart of rat from group 1 revealed the normal histological structure of cardiac myocytes (photo.7). as well as inflammatory cells infiltration in between the cardiac myocytes (Photo.8). Moreover, heart of rats from group 3 revealed intermuscular edema. few inflammatory cells infiltration in between the cardiac myocytes (Photo.9) and vacuolation of the sarcoplasm of the cardiac myocytes (photo.10). Some examined sections from groups 7 and 8 revealed no histopathological changes (Photo.11), whereas, other sections from those groups showed intermuscular edema (Photo.12).

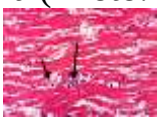


Photo. (7): Heart of rat from group 1 showing the normal histological structure ofcardiac myocytes (H & E X 400).

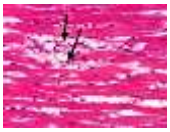


Photo. (8): Heart of rat from group 2 showing intermuscular edema and inflammatory cell infiltration in between the cardiac myocytes (H & E X 400).

Photo. (9): Heart of rat from group 3 showing intermuscular edema and few inflammatory cells infiltration in between the cardiac myocytes (H & E X 400).

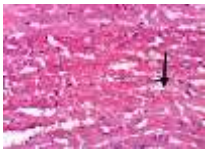


Photo (10): Heart of rat from group 3 showing vacuolation of the sarcoplasm of the cardiac myocytes (H & E X 400).

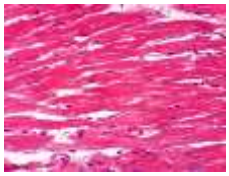


Photo (11): Heart of rat from group 4 showing no histopathological changes (H & E X 400).

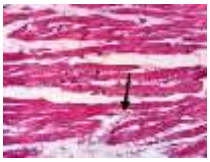


Photo. (12): Heart of rat from group 5 & 6 showing intermuscular edema (H & E X 400).

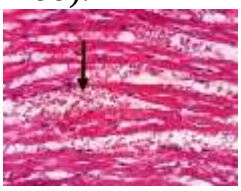


Photo. (13): Heart of rat from group 6 showing congestion of myocardial blood vessels (H & E X 400).

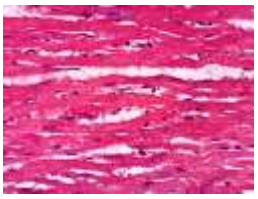


Photo. (14): Heart of rat from group 7 & 8 showing no histopathological changes (H & E X 400).

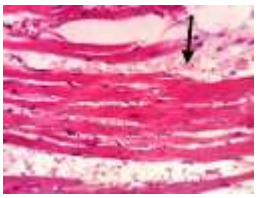


Photo. (15): Heart of rat from group 7 & 8 showing intermuscular edema (H & E X 400).

kidneys

Microscopically, kidneys of rats from group 1 revealed the normal histological structure of renal parenchyma (Photo.16). In contrary, Kidneys of rats from group 2 showed cytoplasmic vacuolization of epithelial lining renal tubules (Photo.17) and protein cast in the lumen of renal tubules (Photo.18). Meanwhile, kidneys of rats from groups 3 & 4 revealed normal histological structure (Photo.19). However, some sections from group 5 revealed cytoplasmic vacuolization of epithelial lining renal tubules and protein cast in the lumen of some renal tubules (Photo.20), whereas, other sections from this group showed no histopathological alterations (Photo.21). However, kidneys of rats from group 6 revealed normal histological structure (Photo.21).

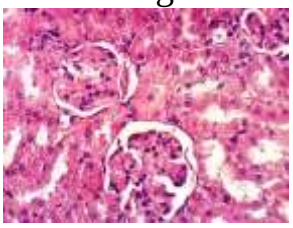


Photo. (16): Kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).

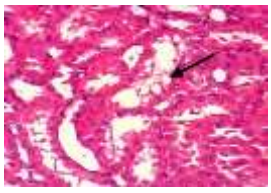


Photo. (17): Kidney of rat from group 2 showing cytoplasmic vacuolization of epithelial lining renal tubules (H & E X 400).

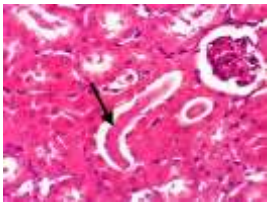
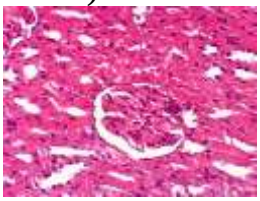


Photo. (18): Kidney of rat from group 2 showing protein cast in the lumen of renal tubules (H & E X 400).



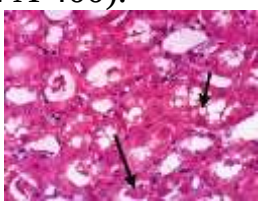


Photo. (19): Kidney of rat from group 3& 4 showing no histopathological alterations (H & E X 400).

Photo. (20): Kidney of rat from group 5 showing cytoplasmic vacuolization of epithelial lining renal tubules and protein cast in the lumen of some renal tubules (H & E X 400).

Photo. (21): Kidney of rat from group 5 & 6 showing no histopathological alterations (H & E X 400).

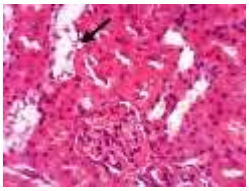


Photo. (22): Kidney of rat from group 7 showing cytoplasmic vacuolization of epithelial lining renal tubules (H & E X 400)

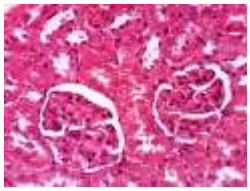


Photo. (23): Kidney of rat from group 8 showing no histopathological alterations (H & E X 400).

Liver

Microscopically, liver of rats from group 1 revealed the normal histological structure of hepatic lobule from central vein and normal hepatocyte (Photo.24).. In contrary, liver of rats from group 2 revealed hydropic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration (Photo.25). Examined sections from group 3 showed cytoplasmic vac-

uolization of some hepatocytes and fibroplasia in the portal triad around the bile duct (Photo. 26).

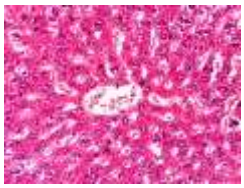


Photo. (24): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).

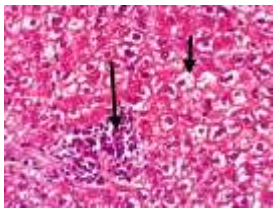


Photo. (25): Liver of rat from group 2 showing hydropic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).

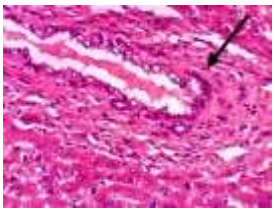


Photo. (26): Liver of rat from group 3 showing fibroplasia in the portal triad around the bile duct (H & E X 400).

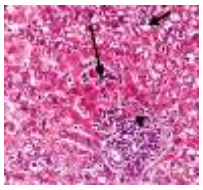


Photo. (27): Liver of rat from group 4 showing hydropic degeneration of hepatocytes, focal hepatic necrosis associated with inflammatory cells infiltration and portal inflammatory cells infiltration (H & E X 400).

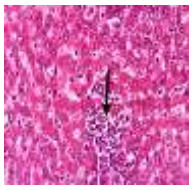


Photo. (28): Liver of rat from group 5 showing focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).

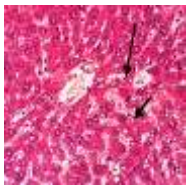


Photo. (29): Liver of rat from group 6 showing cytoplasmic vacuolization of some hepatocytes and Kupffer cells activation (H & E X 400).

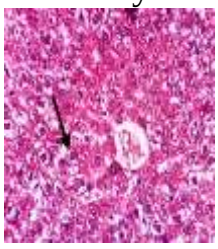


Photo. (30): Liver of rat from group 7 showing hydropic degeneration of hepatocytes (H & E X 400).

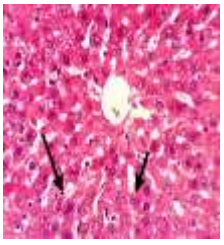


Photo. (31): Liver of rat from group 8 showing slight cytoplasmic vacuolization of some hepatocytes and Kupffer cells activation (H & E X 400).

The present study was in harmony with Kwok et al. (2010) and Mokhtar et al. (2016) who reported that there were histopathological changes in the liver associated with high fat diet, due to attenuated levels of antioxidant enzymes. However, the grape seed oil (GSO)decreased these changes slightly. Moreover, the present results of heart and aorta showed the ability of GSO to protect heart and liver against hypercholesterolemia.

Also, present study was in agreement with Faran et al. (2019) who showed ginger oil improved histological features of the liver and preserved kidney structures. This can be attributed to the renoprotective effects of active compounds (flavonoids) found in ginger oil.

Conclusion

This study demonstrated that the grape seed oil improved blood lipids and atherogenic lipid profile in hypercholesterolemic rats. This effect may be attributed to the high percentage of free fatty acids and antioxidant activity of GSO. Therefore, the GSO effective to decrease cardiovascular risk. Also, ginger oil exerts hypocholesterolemia effect in high fat diet rats. This is promising in protection against CVD. Moreover, it could be an effective against hyperlipidemia and associated complications. It has modulatory effects on antioxidant status as it enhances catalase activity.

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الملخص العربي :-

تأثير زيوت بذرة العنب والزنجبيل والخردل على دهون الدم في ذكور الفئران

تعتبر زيادة دهون الدم العامل الأكبر في حدوث وتطور امراض الاوعية الدموية القلبية وتصلب الشرايين لذلك فان الهدف من هذه الدراسة هو تقييم تأثير زيوت الزنجبيل و بذر العنب و الخردل على دهون الدم ووظائف الكبد والكلى وانزيمات مضادات الاكسدة (الكتاليز والسوبر أوكسيد دسميوتيز) على فئران التجارب .وقد شملت هذه الدراسة على ٤٨ فار مقسمة الى ثمان مجموعات المجموعة الأولى تناولت غذاء قياسي ،المجموعة الثانية تناولت غذاء غنى بالدهون والكوليسترول (مجموعة ضابطة إيجابية) المجموعة الثالثة تناولت بالإضافة لغذاء المجموعة الثانية زيت بذر العنب ٥% المجموعة الرابعة نفس الغذاء مثل الضابطة الإيجابية مضافا الية زيت بذر العنب ١٠%المجموعة الخامسة نفس غذاء المجموعة الضابطة الإيجابية مضافا الية زيت الزنجبيل ٥% المجموعة السادسة نفس الغذاء مضافا الية زيت الزنجبيل ١٠% المجموعة السابعة تناولت نفس الغذاء مضافا الية زيت الخردل ٥% المجموعة الثامنة تناولت نفس الغذاء مضافا الية زيت الخردل ١٠% ان الزيوت المستخدمة في البحث بتركيزات مختلفة لها تأثير ذات قيمة إحصائية على دهون الدم (الكوليستيرول ؛٣٣،٧٥ ±٤٥،١ ، ٧٤ ±٥٢،١ و ٦٦،٧٦ ± ٠٢،٢ علي عكس ٦٦،٩٩ ± ٨٨،٠) و(الدهون الثلاثية؛ ٤٢ ±٥٢،١ ،٣٣،٤٠ ±٤٥،١ و ٦٦،٤٣ ± ٦٦،٠ علي عكس ٣٣،٦٠± ٣٣،٤) وكذلك على انزيمات مضادات الاكسدة والكوليسترول عالي الكثافة(٣٠ ± ١٥،١ ، ٦٦،٢٩ ± ٦٦،٠ و ٣٠ ± ٥٧،٠ علي عكس ٦٦،٢٠±٨٨،٠) وأيضا لها تأثير إيجابي علي وظائف الكبد والكلي . ان الدراسة الهستوباثولوجية للكبد والقلب والأورطي والكلى في المجموعة الى تناولت غذاء غنى بالدهون تسبب في حدوث تجويفات في الخلايا ونزيف داخل العضلات في الاوعية الدموية بالأعضاء محل الدراسة وتورم وظهور التهابات في الخلايا .وان استخدام الزيوت محل الدراسة قضى على هذه التغيرات الهستوباثولوجية وأعاد الأعضاء محل الدراسة الى حالتها الطبيعية ونستخلص من هذه الدراسة ان الزيوت محل البحث لها فوائد صحية في ضبط دهون الدم ووظائف الكلى والكبد وانزيمات مضادات الاكسدة ولذالك فأنها تلعب دورا هاما في الحماية من الذبحة الصدرية وتصلب الشرايين وضبط التغيرات الهستوباثولوجية للقلب والكبد والكلى والأورطي.