Eissa et al, Afr J Tradit Complement Altern Med., (2017) 14 (4): 188-200
https://doi.org/10.21010/ajtcam.v14i4.22.

POSSIBLE HYPOCHOLESTEROLEMIC EFFECT OF GINGER AND ROSEMARY OILS IN RATS

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Abstract

Background: Hypercholesterolemia is a major risk factor for development of atherosclerosis. The present study was conducted to evaluate the potential effect of ginger oil alone or combined with rosemary oil as hypocholesterolemic agent in rats fed high fat diet.
Materials and methods: Healthy female *albino* rats (n=80) weighting about (150-180 g) were included in this study divided into two equal groups; Group (I): were fed on the basal diet. Group (I) were divided into 4 subgroups each 10: Group (Ia): negative control. Group (Ib): Rats received *i.p* 2.5 g/Kg b.w of ginger oil. Group (Ic): rats received *i.p* 2.5 g/Kg b.w of rosemary oil. Group (Id): Rats received *i.p* 5 g/Kg b.w mixture of ginger oil and rosemary oil (1:1). The second main groups; Group (II): high fat diet (HFD) were fed on the basal diet plus cholesterol (1%), bile salt (0.25%) and animal fat (15%) to induce hypercholesterolemia for six weeks. Group (II) was divided into 4subgroups: Group (IIa): HFD. Group (IIb): HFD were treated with *i.p* 2.5 g/Kg b.w ginger oil. Group (IIc): (n=10) HFD were treated with *i.p* 2.5 g/Kg b.w rosemary oil. Group (IId): (n=10) HFD were treated with *i.p* 5 g/Kg b.w mixture of oils.
Results: It was found that HFD rats showed a significant elevation in glucose, total cholesterol, triglyceride, GOT, GPT, alkaline phosphatase and a reduction in serum HDL-c compared with negative control. Treatment with ginger oil, rosemary oil and their mixture modulated the elevation of these parameters. Histopathological examination of the liver tissue of HFD rats showed a lipid deposition and macrophage infiltration and stenosis of hepatic vein. Treatment with mixture oils preserves normal structure of liver.
Conclusion: It was concluded that, hypocholesterolemic effect was related to the active oil content as Rosemary oil contain - α-pinene, Camphor, cineole, borneol and Ginger oil contain Linalool, Terpineol ,Borneol , Eucalyptol.

Keywords: Ginger Oil, Rosemary Oil, Rats, Hypocholesterolemia.

Introduction

Hypercholesterolemia is a lipoprotein metabolic syndrome known as high serum LDL and blood cholesterol. High blood cholesterol is the most risk factors for the development of cardiovascular diseases, such as atherosclerosis and its complications, acute myocardial infarction, or high blood pressure [Gerhardt and Gallo, 1998]. The modern lifestyle, with a continuous ingestion of high quantity of saturated fats and cholesterol and little physical activity, directly related to hypercholesterolemia and cardiovascular diseases [Wu et al., 2012] . In blood, cholesterol is carried by lipoproteins, including HDL, LDL, intermediate density lipoproteins (IDL), VLDL, chylomicron remnants, and triglycerides; these lipoproteins vary not only in density but also in their function. Elevated LDL-c in the blood stream increases the risk of heart disease [Costet, 2010]. HDL is considered to be beneficial as higher levels also been associated with decreased risk of negative cardiovascular actions [Ridker et al., 2010]. VLDL is similar to LDL-c that it contains mostly fat and not much protein; it is a lipoprotein that transport cholesterol from the liver to organs and tissues, and are also associated with atherosclerosis and heart disease [Sundaram and Yao, 2010]. Genetically predisposing to hypercholesterolemia usually involves alterations in lipoprotein transport and metabolism, leading to atherosclerosis. It was found that cholesterol alter the vascular structure and function because it builds within the lining of the vascular wall and can interfere with endothelial function causing lesions, plaques, occlusion, and emboli; along with a decrease in healing, recovery [Roy et al., 2009].

The abnormal cholesterol levels occur as a result of an unhealthy lifestyle including eating a diet rich in fats and other factors such as overweight, drink large amounts of alcohol and lack of exercise [Kelly ,2010]. Dietary factors like

ingestion high amounts of saturated fats and cholesterol constantly are related directly to hypercholesterolemia and can be lead to atherosclerosis. Furthermore, weight loss can help lower LDL and total cholesterol levels and consequent raise the HDL-c levels in the body. In addition, it has also been shown regular exercise reduces LDL-c and increase HDL-c levels. Other factors include diabetes and thyroid gland diseases have also been reported to cause a rise in cholesterol levels [Tamer et al., 2011]. Other diseases that may increase cholesterol levels include polycystic ovary disorder and kidney disease. Liver disease hypercholesterolemia has been reported to be caused by decreased excretion of cholesterol in the bile. Moreover, in nephritic syndrome, the general synthetic pathway for albumin and cholesterol cause low oncotic pressure eventually leading to improved cholesterol synthesis [Rohilla et al., 2012]. Other modifying factors in the development of hypercholesterolemia are gender and age. Genetic alterations are also one factor that leads to hypercholesterolemia, as in familial hypercholesterolemia (FH) that is one of the most common human genetic diseases. Homozygous FH patients have inherited allelic mutations in the gene specifying the LDL receptor placed on the surface of the cell. FH is linked with increased the risk of premature ischemic heart disorders [Castilla-Guerra et al., 2009].

Ginger (*Zingiber officinale* Rosc.), a monocotyledon belonging to tropical and subtropical family Zingiberaceae, is a common additive in a number of commercial foods and beverages originated in South-East Asia and introduced to many parts of the globe as a rich source of compounds of phytomedicinal interest. Many active ingredients are present in ginger such as terpenes and oleoresin that called ginger oil. Ginger also constitutes volatile oils and non-volatile pungent components oleoresin [Rahmani, 2014]. Moreover, phytochemical reports have shown that the major components of ginger are Gingerol, Shagaols, Zingerone and Paradol. This perennial plant with thick tuberous pungent aromatic roots or rhizomes has been cultivated for thousands of years for use as a spice and for herbal medicinal purposes includes anti-arthritic, anti-inflammatory, hypolipidaemic , anti-nausea properties and antimicrobial potential, as its role in the treatment of infectious diseases and it has beneficial effects to cancer prevention . Many researchers have been made to clarify ginger's effect on lipid profile [total cholesterol (TC), LDL, HDL, triglycerides (TG) [Akram et al., 2011].

Rosemary (*Rosmarinus officinalis* L.) is one of the most widely consumed worldwide as an ornamental and aromatic shrub, originally grows in southern Europe. Rosemary in the form of herb and oil is used as spice and flavoring agents in the preparation of foods to get the desired flavor, high antioxidant activity and anti-viral agent recently. It was discovered that rosemary can also be used in the treatment or prevention of inflammatory disorders, liver toxicity and renal toxicity. Oral administration of rosemary leaf extract leads to significant reductions in blood TG levels, TC, LDL-c and elevated HDL-c [Al Jamal, 2014].

Materials and Methods

Chemicals and kits

Enzymatic kits for glucose, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), serum glucose, alkaline phosphatase (ALP) were obtained from Human Gesellschaft for Biochemical and Diagnostica mbH, Germany. GPT, GOT and total protein kits were purchased from Spinreact, Spain.

Rosemary and ginger oil were obtained from Pharmacy, Jeddah, Saudi Arabia. With purity, 99%

Experimental design

Anesthetic method and animal handling were approved in accordance with the ethical guidelines of Medical Ethics Committee of the King Abdulaziz University. Healthy Female *albino* rats (n=80 rats) weighing about (150-180 g) were obtained from the animal experimental unit at King Fahd Center for Medical Research (KFCMR), King Abdul-Aziz University. All animals were allowed to one week to acclimatize in animal housing conditions before being used for the study. The experimental rats were housed in standard laboratory conditions at a temperature of (25º± 2 °C), relative humidity (50-55%) with a 12-hour dark/light cycle. All animals fed standard nutritionally balanced diet and drinking water *ad libitum*. Standard nutritionally balanced diet was obtained from KFMRC, the diet consists of the following ingredients; protein 20.0%, fat 4.0 %, fiber 5.0 %, vitamin mix 1.0%, mineral mix 3.50%, choline chloride 0.25%, the remained formula up to 100% corn starch, and its energy equals 2850 kcal/kg. The diet manufactured by Grain Silos and Flour Mills Organization, KSA**.** After the adaption period, rats were divided into the rats were weighed and randomly divided into two main groups (Normal diet group and High fat diet group) as shown:

The Normal diet group was divided into 4 subgroups (n=10):

- Group (Ia). (10 rats) Rats were fed on standard diet and served as a negative control group.
- Group (Ib). Rats were given 2.5g/Kg/day bw ginger oil.
- Group (Ic). Rats were given 2.5g/Kg/day bw rosemary oil.
- Group (Id). Rats were given 5g/Kg/day bw mixture ginger oil and rosemary oil (1:1).

The High fat diet group (II) was fed on the basal diet plus cholesterol (1%), bile salt (0.25%) and animal fat (15%) to induce hypercholesterolemia (Shinnick, Ink and Marlett, 1990) for six weeks. The High fat diet group was divided into 4 subgroups (n=10):

- Group (IIa). Rats fed high fat diet (HFD).
- Group (IIb). Rats fed HFD were given 2.5 g/Kg bw ginger oil /day.
- Group (IIc). Rats fed HFD were given 2.5 g/kg bw rosemary oil /day.
- Group (IId). Rats fed HFD were given 5 g/kg/day bw mixture ginger oil and rosemary oil (1:1).

Statistical Analysis:

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed with one-factor analysis of variance (ANOVA) using MegaStat. The statistical significance difference was considered when ($P< 0.05$, $P < 0.01$ and $P< 0.001$).

Results

Results obtained showed that, non-significant changes in body weight were detected in rats fed normal diet groups when compared with the negative control group. On the other hand, for high fat group HFD (control positive), showed a significant increase in body weight was detected when compared to the negative control groups ($p > 0.0008$). However, after six weeks of treatment of HFD groups with ginger oil, rosemary oil and mix, results showed significant reduction in body weight in all HFD treated groups compared to HFD group Table 1 and Figure 1.

Table1: Initial and final body weight (g), in all studied groups (Mean ± SD).

Experimental groups / Variables	Control (-ve) (Ia)	Ginger oil (Ib)	Rosemary oil (Ic)	Ginger & Rosemary oils (Id)	HFD (+ve) (IIa)	HFD + Ginger oil (IIb)	HFD + Rosemary oil (IIc)	HFD +Ginger oil & Rosemary oil (IId)
Body weight (initial) (g)	172.50±12.82	170.00±14.14	171.11±11.67	167.14±7.56	181.00.2±9.94	174.50±10.66	175.00±11.18	172.50±7.17
Body weight (final) (g)	255.86±14.96	250.00±18.89	254.43±20.35	241.50±13.34	287.50±27.93	257.38±15.45	257.50±13.83	250.78±15.63
P-value	---- ---- ----	0.518 [a] ---- ----	0.884 [a] ---- ----	0.116 [a] ---- ----	0.0008 [a***] ---- ----	0.873 [a] 0.003 [b**] ----	0.856 [a] 0.002 [b**] 0.986 [c]	0.583 [a] 0.0002 [b***] ----

[a:]Comparison between p-value of normal diet group (Ia) (control -ve) and p-value of different groups.
[b] Comparison between p-value of high fat diet group (IIa) (control +ve) and p-value of HFD treated groups.
[c] Comparison between p-value of HFD treated with ginger oil and p-value of HFD treated with rosemary oil.
([*] $P< 0.05$, [**] $P < 0.01$ and [***] $P< 0.001$)

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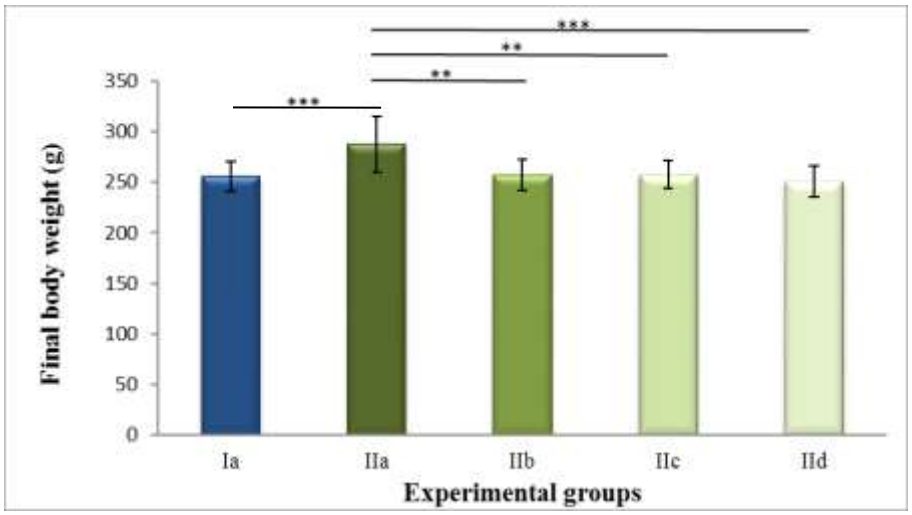

Figure 1: Final body weight (g) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

Data in Table 2 showed glucose and total protein levels. It was found that, a slightly increase in glucose level of normal diet groups treated with ginger oil, rosemary oil or combined of ginger and rosemary oil as compared with negative control and non-significant changes in total protein level in all normal diet groups when compared to the control negative group. On the other hand, HFD group showed a significant increase in glucose level when compared to negative control group, also showed no significant change in total protein levels when compared with negative control group. Administration of ginger oil, rosemary oil or their combined to HFD showed slight reduction in the levels of glucose and total protein compared to control group. There were values showed non- significant differences in all HFD treated groups when compared with HFD group except HFD treated with ginger oil group that showed a significant decrease in glucose level Figure 2.

Table 2: Serum glucose (mg/dl) and Serum total protein (mg/dl) levels of different groups.

Experimental groups / Variables	Control (-ve) (Ia)	Ginger oil (Ib)	Rosemary oil (Ic)	Ginger & Rosemary oils (Id)	HFD (+ve) (IIa)	HFD +Ginger oil (IIb)	HFD + Rosemary oil (IIc)	HFD + Ginger oil & Rosemary oil (IId)
Glucose (mg/dl)	115.77±10.17	130.06±42.40	128.68±20.90	118.60±31.20	150.58±22.80	116.31±8.55	129.15±7.79	133.44±22.07
P-value	---- ---- ----	0.259 [a] ---- ----	0.292 [a] ---- ----	0.807 [a] ---- ----	0.006 [a**] ---- ----	0.963 [a] 0.003 [b**] ----	0.309 [a] 0.096 [b] 0.293 [c]	0.345 [a] 0.082 [b] ----
TP (mg/dl)	8.29±0.84	8.83±0.99	8.29±0.68	8.30±1.60	8.84±1.41	7.71±2.03	8.59±1.75	8.14±1.12
P-value	---- ---- ----	0.382 [a] ---- ----	0.992 [a] ---- ----	0.990 [a] ---- ----	0.377 [a] ---- ----	0.353 [a] 0.131 [b] ----	0.626 [a] 0.736 [b] 0.314 [c]	0.846 [a] 0.573 [b] ----

TP: total protein.
[a:]Comparison between p-value of normal diet group (Ia) (control -ve) and p-value of different groups.
[b] Comparison between p-value of high fat diet group (IIa) (control +ve) and p-value of HFD treated groups.
[c] Comparison between p-value of HFD treated with ginger oil and p-value of HFD treated with rosemary oil.
(* P< 0.05, ** P < 0.01 and *** P< 0.001).

https://doi.org/10.21010/ajtcam.v14i4.22.

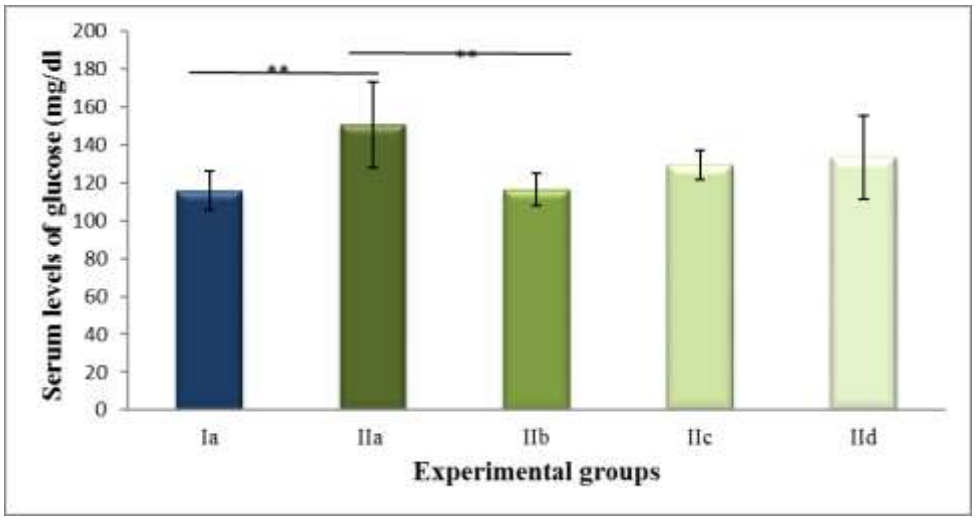

Figure 2: Serum glucose levels (mg/dl) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

Data in Table 3 depicts the level of serum total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) in all normal diet groups. There is no significant influence detected in TC, HDL, LDL and TG compared with control negative. For HFD, It was found that a significant elevation in TC and LDL as compared with negative control, also the result showed a significant reduction in HDL level when compared with negative control accompanied with no significant change in TG level when compared with control negative. Administration of ginger oil, rosemary oil or combined showed slight decrease in the level of LDL and TG, but showed a significant decrease in TC levels compared with HFD group and a significant increase in HDL levels compared with HFD group Figures (3,4).

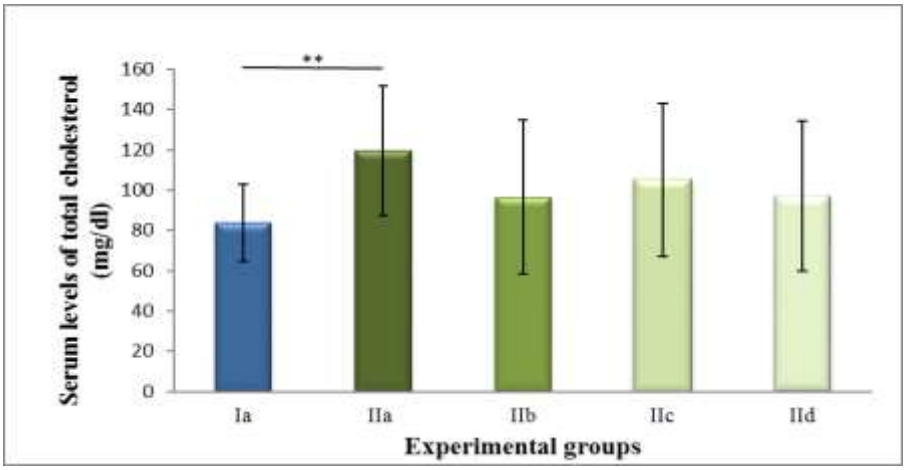

Figure 3: Serum total cholesterol (TC) levels (mg/dl) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

https://doi.org/10.21010/ajtcam.v14i4.22.

Table 3: Serum lipid profile levels (mg/dl) of different groups.

Experimental groups / Variables	Control (-ve) (Ia)	Ginger oil (Ib)	Rosemary oil (Ic)	Ginger oil & Rosemary oil (Id)	HFD (+ve) (IIa)	HFD + Ginger oil (IIb)	HFD + Rosemary oil (IIc)	HFD +Ginger oil & Rosemary oil (IId)
TC (mg/dl)	83.80±19.23	81.52±10.13	82.77±17.10	78.42±20.12	119.68±32.20	96.52±38.10	104.98±38.11	97.15±37.16
P-Value	---- ---- ----	0.872[a] ---- ----	0.938[a] ---- ----	0.686[a] ---- ----	0.009[a**] ---- ----	0.340[a] 0.164[b] ----	0.114[a] 0.373[b] 0.627[c]	0.329[a] 0.187[b] ----
HDL-c (mg/dl)	26.20±6.90	26.49±7.48	26.95±15.79	26.57±5.39	17.86±5.67	31.95±6.62	33.60±15.95	35.78±10.78
P-Value	---- ---- ----	0.956 [a] ---- ----	0.695 [a] ---- ----	0.945 [a] ---- ----	0.125 [a] ---- ----	0.275 [a] 0.007 [b**] ----	0.161 [a] 0.003 [b**] 0.736 [c]	0.078 [a] 0.001 [b**] ----
LDL-c (mg/dl)	4.83±1.18	4.07±2.48	4.67±0.94	4.14±1.79	12.65±3.96	11.41±4.01	10.47±2.88	10.51±4.62
P-Value	---- ---- ----	0.676 [a] ---- ----	0.747 [a] ---- ----	0.699[a] ---- ----	0.0001 [a***] ---- ----	0.0009 [a***] 0.565[b] ----	0.004 [a**] 0.315 [b] 0.625[c]	0.002[a**] 0.296 [b] ----
TG (mg/dl)	86.97± 23.71	125.27±49.20	106.52±33.32	96.90±26.57	130.26±65.97	105.13±34.68	112.48±32.03	118.99±59.25
P-Value	---- ---- ----	0.096[a] ---- ----	0.375 [a] ---- ----	0.651 [a] ---- ----	0.041 [a*] ---- ----	0.385 [a] 0.268[b] ----	0.223 [a] 0.432[b] 0.628[c]	0.137 [a] 0.627[b] ----

TC: total cholesterol. HDL: high density lipoprotein. LDL: low density lipoprotein. TG: triglyceride.
[a:] Comparison between p-value of normal diet group (Ia) (control -ve) and p-value of different groups.
[b] Comparison between p-value of high fat diet group (IIa) (control +ve) and p-value of HFD treated groups.
[c] Comparison between p-value of HFD treated with ginger oil and p-value of HFD treated with rosemary oil.
([*] P< 0.05, [**] P < 0.01 and [***] P< 0.001).

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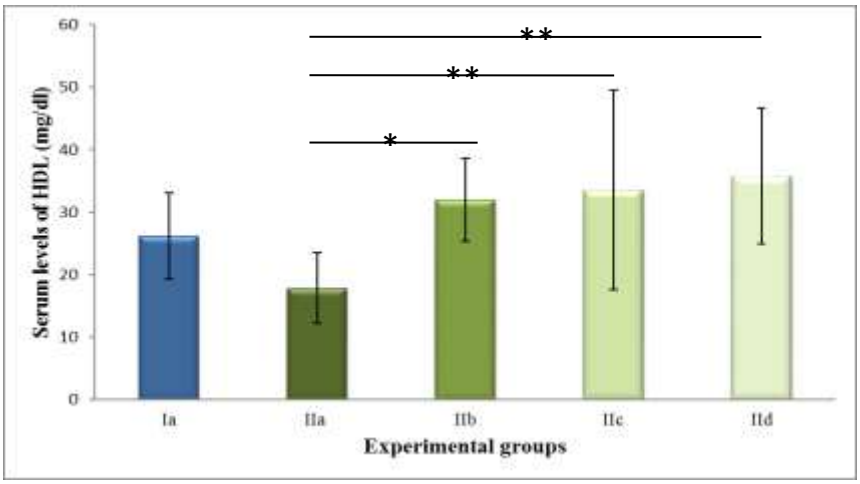

Figure 4: Serum high density lipoprotein (HDL) levels (mg/dl) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

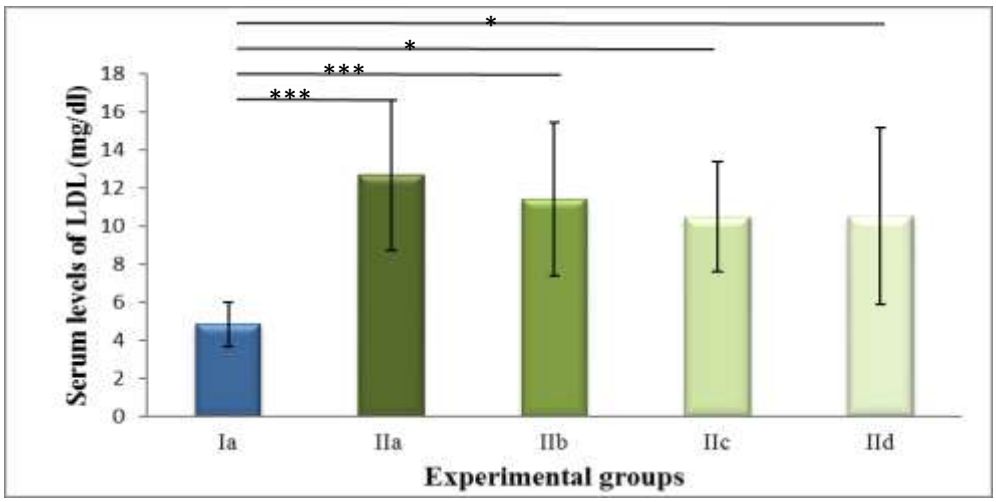

Figure 5: Serum low density lipoprotein (LDL) levels (mg/dl) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

Table 4 showed the serum levels of aspartate aminotransferase GOT and alkaline phosphatase ALP. It was found that there was showed a slight reduction in the activity of normal diet treated groups compared to control negative. However, significant decreases in GPT level in all normal diet treated groups when compared with control negative group. The result also showed serum GOT, GPT and ALP in HFD groups it revealed that, no significant changes in serum GOT and GPT level accompanied with a very highly significant elevation in serum ALP as compared with negative control group (P> 0.0003). Rats treated with either ginger oil or rosemary oil or their combined showed decrease in the level of GOT and there was no significant changes detected when compared with control negative group as well as when compared with HFD group. Also, it was found that a significant reduction in GPT levels in treated groups (HFD treated with rosemary oil and HFD treated with mix of ginger and rosemary oils) when compared with control negative (P>0.001; P>0.0002) and showed a very highly significant when compared with HFD group (P>0.0002; P>0.000) Figure 7. There was a significant decrease in ALP in all treated groups compared to control negative (P> 0.0001; P> 0.0001; P> 0.003), were there no significant changes in all treated groups compared with HFD group except group (HFD treated with mix of ginger and rosemary oils) that showed a significant reduction (P>0.009) compared with HFD Figure 8.

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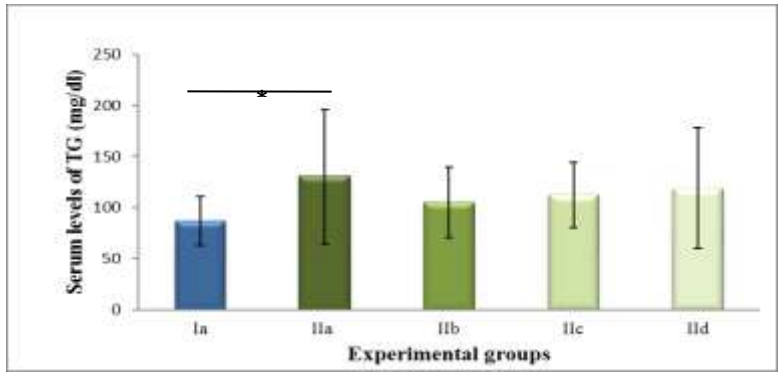

Figure 6: Serum triglyceride (TG) levels (mg/dl) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

Table 4: Serum enzymes (aspartate aminotransferase GOT, alanin amiotransferase GPT alkaline phosphatase ALP) levels (U/L) of different groups.
GOT: aspartate aminotransferase. GPT: alanin amiotransferase. ALP: alkaline phosphatase.
[a:] Comparison between p-value of normal diet group (Ia) (control -ve) and p-value of different groups.

Experimental groups / Variables	Control (-ve) (Ia)	Ginger oil (Ib)	Rosemary oil (Ic)	Ginger oil & Rosemary oil (Id)	HFD (+ve) (IIa)	HFD + Ginger oil (IIb)	HFD + Rosemary oil(IIc)	HFD + Ginger oil & Rosemary oil (IId)
GOT (U/L)	82.25±45.58	77.35±21.94	74.79±8.15	75.37±25.23	95.20±23.35	79.80±31.96	67.67±16.89	79.22±16.97
P-Value	---- ---- ----	0.767 [a] ---- ----	0.653 [a] ---- ----	0.678 [a] ---- ----	0.437 [a] ---- ----	0.882 [a] 0.308 [b] ----	0.382 [a] 0.078 [b] 0.474 [c]	0.855 [a] 0.291 [b] ----
GPT (U/L)	64.97±15.86	50.21±12.45	43.45±10.37	42.07±5.72	69.95±18.88	59.16±7.63	45.01±14.01	40.08±7.55
P-Value	---- ---- ----	0.017 [a*] ---- ----	0.0007 [a**] ---- ----	0.0002 [a***] ---- ----	0.400 [a] ---- ----	0.338 [a] 0.087 [b] ----	0.001 [a**] 0.0002 [b***] 0.016 [c*]	0.0002 [a***] 0.000[b***] ----
ALP |(U/L)	207.04±30.32	188.21±92.69	197.70±56.61	173.69±20.66	470.35±96.45	419.62±127.85	431.22±129.42	404.16±183.69
P-Value	---- ---- ----	0.772 [a] ---- ----	0.871 [a] ---- ----	0.576 [a] ---- ----	0.000 [a***] ---- ----	0.0003 [a***] 0.412[b] ----	0.0002 [a***] 0.537[b] 0.847[c]	0.0008 [a***] 0.299[b] ----

[b] Comparison between p-value of high fat diet group (IIa) (control +ve) and p-value of HFD treated groups.
[c] Comparison between p-value of HFD treated with ginger oil and p-value of HFD treated with rosemary oil.
(* P< 0.05, ** P < 0.01 and *** P< 0.001).

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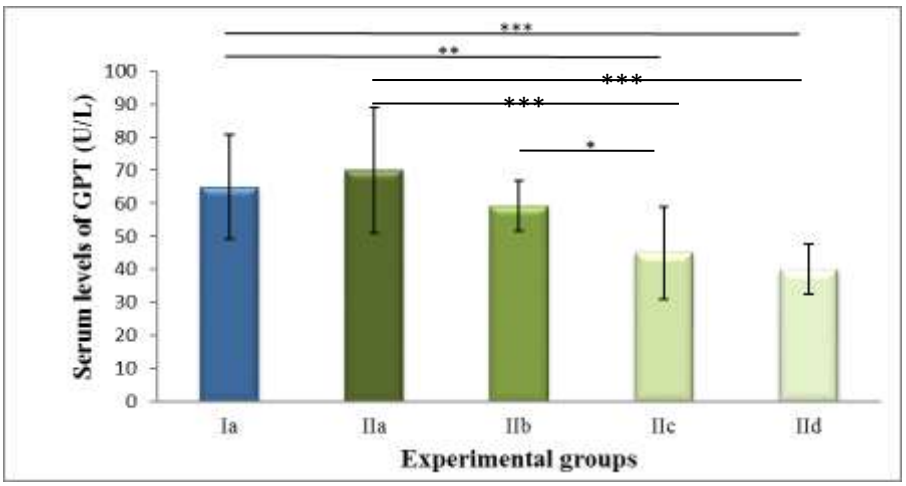

Figure 7: Serum alanin aminotransferase (GPT) levels (U/L) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

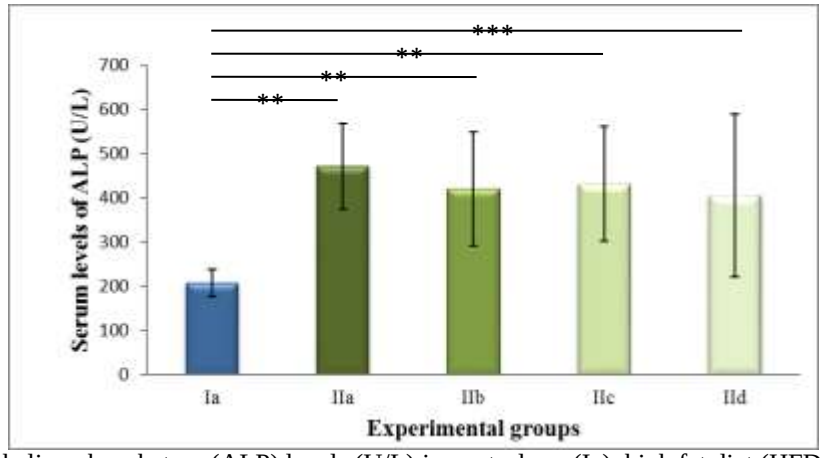

Figure 8: Serum alkaline phosphatase (ALP) levels (U/L) in control -ve (Ia), high fat diet (HFD) (IIa), HFD treated with ginger oil (IIb), rosemary oil (IIc) and mixture (IId). Each value expressed as mean and standard deviation ± SD of each rat groups (n=10). Statistically significant differences determined by one way ANOVA (* P< 0.05, ** P < 0.01 and *** P< 0.001).

Histological examination

In the present study, in negative control rat [Figure 9 (A)] showed that, the liver lobules identified by central veins (CV) and portal regions containing branched portal vessels (PV) and (BD). Administration of ginger oil in a dose of (2.5 g/kg) resulted in a marked improvement of hepatocytes histological features. The cells have acidophilic vacuoles with no vacuoles. The nuclei looked larger and more active (vesicular or euchromatic). Blood sinusoids still showed normal appearance [Figure 9 (B)]. Administration of rosemary oil in a dose of (2.5 g/kg) by gastric tube also was found to improve histological features of liver parenchyma both hepatocytes and blood sinusoids looked more healthy compared to control hepatocytes showed regular cords and vesicular active nuclei [Figure 9 (C)]. Administration of a mixture of ginger oil and rosemary oil in a dose of [5 g/kg (1:1)] result in similar improvements of liver hepatocytes and sinusoids observed in (normal diet group treated with ginger oil) and (normal diet group treated with rosemary oil) [Figure 9 (D)].

Histological examination of rat liver of HFD group showed marked alteration of rat hepatocytes. There was swelling of cells results in compression and disappearance of sinusoids. Cells showed marked cytoplasmic vacuolation (unstained). Nuclei looked deformed. Numerous apoptotic cells were observed (dark shrunken acidophilic cytoplasm, will dark small pyknotic nuclei [Figure 10 (A)]. Administration of ginger oil in a dose of (2.5 g/kg) protects the liver against changes induced by HFD, no swelling no vacuolation no change of nucleolus. The hepatocyte cell cords and blood sinusoid around central vein looked normal and similar to control. Only few cells still showed tiny lipid droplets inside cytoplasm [Figure 10 (B)]. Administration of rosemary oil in a dose of (2.5 g/kg) via oral rote also was found to

https://doi.org/10.21010/ajtcam.v14i4.22.

exert profound protective effect against HFD observed in nontreated groups both hepatocytes and blood sinusoids looked similar to control [Figure 10 (C)] except of few cells still showed lipid droplets. Administration of a mixture of ginger oil and rosemary oil markedly provide protection against lipid accumulation, hepatocyte swelling and inflammatory changes observed in non-protected groups. Scattered few cells still showed tiny lipid droplets [Figure 10 (D)].

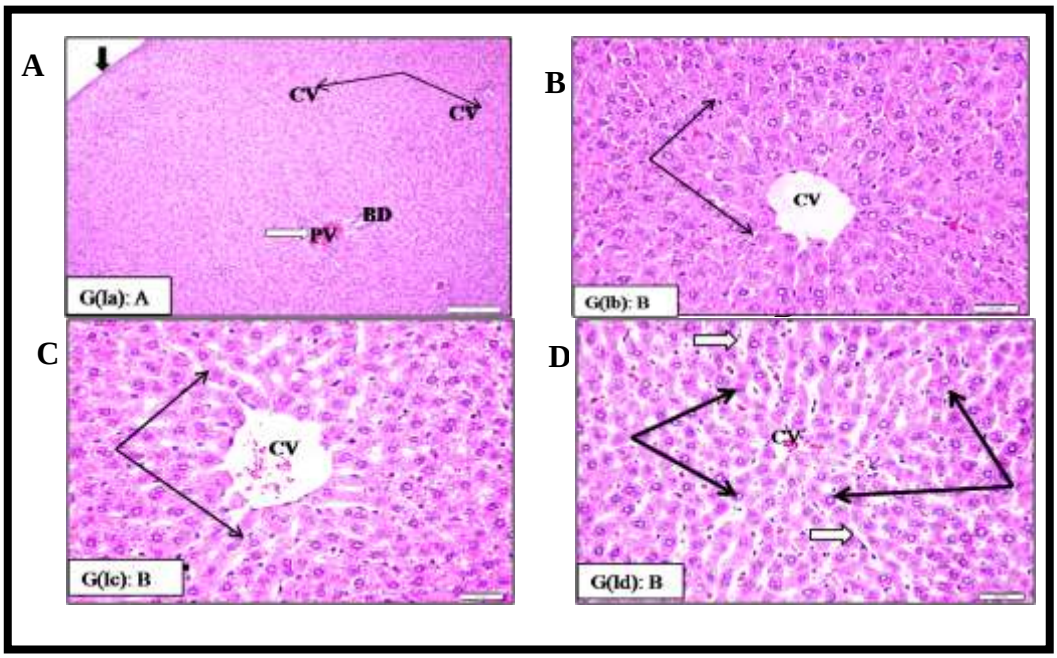

Figure 9: Sections in control rat liver showing: **A.** capsule (thick black arrow), central veins CV (thin arrows) and portal area (white arrow) the later showed branches of portal vein (PV) and bile duct (BD). **B.** liver of group Ib (ginger oil) showing normal central vein (CV) and healthy hepatocyte cell cords (arrows). The cells have more homogenous acidophilic cytoplasm and active euchromatic nuclei. **C.** liver of group Ic (rosemary oil) showing normal central vein (CV) and healthy hepatocyte cell cords (arrows). The cells have normal cytoplasm and active nuclei. **D.** liver of group Id (mixture of ginger and rosemary oils) showing: normal central vein (CV) and healthy hepatocyte cell cords (black arrows). The cells have normal cytoplasm and active euchromatic nuclei.

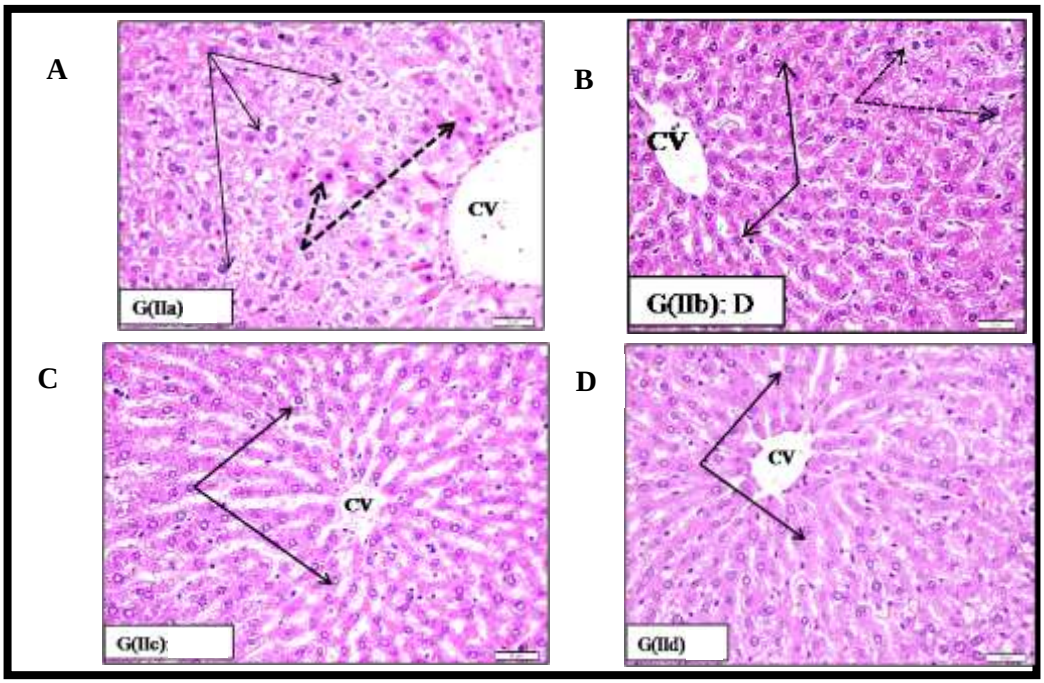

Figure 10: Sections in rat liver group IIa (HFD) showing: **A.** Hepatocytes near the central vein (CV) with lipid deposition (black arrows). Other cells (dotted arrows) are small dark with small deeply stained nuclei (signs of apoptosis). **B.** Sections from tow rats of group IIb (HFD+ ginger oil) showing: most hepatocytes looked normal (black arrows) but still some animals showed lipid deposition within hepatocytes (dotted arrows). **C.** liver of group IIc (HFD + rosemary oil) showing marked improvement of changes seen in non- treated group both in hepatocytes (black arrows)

near central vein region (CV). **D.** liver of group IId (HFD +ginger oil and Rosemary oil) showing marked improvement of changes seen in non-treated group in hepatocytes (black arrows) near central vein region (CV).
(H&E stain X20 & 40)

Discussion

Hypercholesterolemia is a metabolic disorder characterized by high serum levels of cholesterol and LDL-c [Akinyemi et al., 2015]. High blood cholesterol is a risk factor for cardiovascular diseases (CVD). In general, more than 200 mg/dl of cholesterol levels or >180 mg/dl of blood triglyceride levels regarded as hyperlipidemia. The present study was undertaken to evaluate the possible impact of ginger and rosemary oils on normal and hypercholesterolemia rats. The results obtained showed a significant increase in body weight in HFD rats compared to negative control. The previous study of who reported that rats fed with HFD showed a significant increase in body weight. Moreover, Amin and Nagy [2009] reported that feeding rats on HFD significantly increased the body weight as compared with the rats fed on normal diet. These are accordance with our result and the significant increase may be due to fat deposition. Treatment with ginger oil, rosemary oil or their combined showed no significant changes in body weight were detected in normal diet treated groups when compared with the negative control group. However, for high fat group HFD, a significant increase in body weight was detected when compared to the negative control group. However, HFD treated groups showed reduction in body weight to normal level when compared to both control and HFD group. This may be due to the inhibition of intestinal absorption by the active components of the compounds [Spiegelman, 2001].

It was found that, HFD showed a significant increase in glucose level when compared to negative control group, also showed no significant change in total protein levels when compared with negative control group. Administration of ginger oil, rosemary oil or their combined to HFD showed a reduction in the levels of glucose and total protein compared to control group. The effect of ginger oil alone is better than rosemary oil alone or combined. Our results regarding blood glucose were agree with, Sakr [2007] who reported that ginger oil was found to decrease blood glucose in adult male rats. The hypoglycemic effect was attributed to their content of rosemary oil as α-pinene, Camphor, cineole, borneol and Ginger oil contain Linalool, Terpineol, Borneol, Eucalyptol [Al-Attar and Zari, 2007].

In the present study, serum TC, HDL-c, LDL-c and TG in HFD were significant elevated as compared with negative control and a significant reduction in HDL-c level when compared with negative control accompanied with no significant change in TG level when compared with control negative. Previous study of Bolanle [2011], reported that rats fed hypercholesterolemia diets showed a significant increase ($P < 0.05$) in the TC and LDL-c compared to the control or baseline values. Also, these results are in agreement with [Laleye, et al., 2007], who reported that all the rat groups fed high lipid cholesterol feed (HLCF) showed high level of serum cholesterol. This is accordance with our result and a significant elevation in serum cholesterol level may be due to the eating of foods that are rich in saturated fats and contains high level of cholesterol. Treatment with ginger oil, rosemary oil and combined showed decrease in the level of LDL and TG, but showed a significant decrease in TC levels compared with HFD group and a significant increase in HDL levels compared with HFD group. These effects may be due to increase in the pancreatic and intestine lipase occurred; lipase is the other key factor which plays a vital role in fat digestion when ginger was ingestion. The reduction in lipid levels observed after the consumption of rosemary has been suggested in other studies to be caused by a reduction in the absorption of dietary fat supported by an increase in fecal fat excretion. The reduction in lipid profile was attributed to the active components Rosemary oil contain - α-pinene, Camphor, cineole, borneol and Ginger oil contain Linalool, Terpineol , Borneol , Eucalyptol.

Alkaline phosphatase (ALP) is a membrane bound enzyme while AST and ALT are cytosolic enzymes which are highly concentrated in the liver and kidney and are only found in significant quantities in the serum when the cell membrane becomes leaky and even completely ruptured. Increase the concentration of these enzymes in the liver may cause different diseases such as hypercholesterolemia, diabetic mellitus and chronic hepatitis. In this study, serum levels of aspartate aminotransferase AST|, ALT and ALP showed a significant reduction in normal diet treated with mixture oils. HFD revealed that, showed a very highly significant elevation in serum ALP as compared with negative control group (P> 0.0003). Rats treated with either ginger oil or rosemary oil or their combined showed decrease in the level of AST and there was no significant changes detected when compared with control negative group as well as when compared with HFD group. Also, it was found that a significant reduction in ALT levels in all treated groups IIc and IId when compared with control negative (P>0.001; P>0.0002) and showed a very highly significant decrease when compared with HFD group (P>0.0002; P>0.000), however there were no significant change showed in group IIb. There was a significant decrease in ALP in all treated groups compared to control negative (P> 0.0001; P> 0.0001; P> 0.003), were there no significant changes in all treated groups compared with HFD group except group (IId) that showed a significant reduction (P>0.009) compared with HFD. These results are in agreement with Bolanle [2011] who reported that A significant increase ($P<0.05$) was observed in the activity of liver enzymes (AST, ALT, ALP) of hypercholesteremic rats when compared with normal control while a significant decrease ($P<0.05$) was observed when comparing the hypercholesterolemia rats with the group of rats treated with ginger powder at 5% and 10% level and a similar result was reported by Al-Naqeeb [2003] who reported that the administration of aqueous extract of *ginger* to rats, orally and intraperitoneally, at two different levels of doses, significantly decreased the activities of some serum

enzymes such as (AST) and (ALT). Also, these results are in agreement with Albasha and Azab [2014] who reported that aqueous extract of rosemary has significantly decreased the release of AST. This may be due to the hypercholesterolemia diet could have 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 hypercholesterolemia diet. Histological examination supported biochemical alteration and protection by ginger or rosemary oils. Our study showed that consuming a HFD for 6 weeks successfully induced relevant intrahepatic fat deposition, inflammation and simple steatosis in rats. Rats supplemented with ginger or rosemary of combined inhibited intrahepatic fat deposition in these animals. No inflammatory infiltration or fatty lesions. The combined effect is more potent than individual ones.

Conclusion

It was concluded that, rosemary and ginger oils exerts hypocholesterolemia effect in HFD rats. this is promising in protection against CVD.

Acknowledgment

The authors would like to thanks Prof. Soad Shakir, Anatomy Department Faculty of Medicine for her histo pathological examination and King Abdul-Aziz City for Science and Technology for its financial support under grand number (390-35-AT).

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