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Antioxidant activity of the volatile oils of Zingiber officinale (ginger)

Zingiber officinale (zencefil) uçucu yağının antioksidan etkinliği

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SUMMARY

AIM: The present study aimed to investigate the effects of Zingiber officinale Roscoe (ginger) extract on diminish hypercholesterolemic atherosclerosis and it had antioxidant activity.
METHODS: Lipid profile (cholesterol, triglycerides and lipoproteins levels of cholesterol and triglycerides (low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels in the homogenate aorta and in serum of hypercholesterolemic animals were estimated. The development of atherosclerosis in aortic tissues and anti-oxidant effect of ginger extract by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay were evaluated in albino mice.
RESULTS: The present results revealed a significant reduction in the levels of cholesterol, triglycerides, phospholipids and both levels of VLDL and LDL cholesterol and triglycerides in serum and tissue of aorta with high levels dose of ginger extract ($P < 0.01$). Moreover, the antioxidant effect of ginger extract was studied by measuring its radical scavenging activity (RSA), using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay showed strong antioxidative activity with high levels dose of ginger extract ($P < 0.01$).
CONCLUSION: The ginger extract has reduced in serum LDL cholesterol, total cholesterol, triglycerides and phospholipids levels, as well as cellular cholesterol accumulation, reduce DPPH absorption, scavenge free radicals and it has potential to improve the histopathological lesion occurring in different layers of the arterial tissue. In the other word it is effective in attenuating of atherosclerosis development.
Key Words: Ginger extract, LDL-cholesterol, Lipoproteins, Cholesterol, Triglycerides, Phospholipids, Atherosclerosis, Hypolipidimic.

ÖZET

AMAÇ: Bu çalışmada, Zingiber officinale Roscoe (Zencefil) ekstresinin hiperkolesterolemik aterosklerozun azaltılması üzerine etkileri ve sahip olduğu antioksidan etkinliğin araştırılması amaçlanmıştır.
YÖNTEM: Hiperkolesterolemik hayvanların serumlarında ve aort homojenatlarında lipid profili (kolestrol, trigliseritler ile kolestrol ve trigliseritlerin lipoprotein seviyeleri (düşük dansiteli lipoprotein (DDL) ve çok düşük dansiteli lipoprotein (ÇDDL)) seviyeleri hesaplandı. Aort dokusundaki ateroskleroz gelişimi ve zencefil ekstresinin antioksidan etkisi, 1,1-diphenyl-2-picrylhydrazyl (DPPH) tahlili kullanılarak albino farelerde değerlendirildi.
BULGULAR: Bulunan sonuçlar, yüksek doz zencefil ekstresi ile, aort dokusu ve serumdaki kolesterol, trigliseritler, fosfolipitlerin düzeyleri ile hem DDL hem de ÇDDL kolesterol ve trigliseritlerinin düzeylerinde belirgin biz azalma olduğunu ortaya koydu ($P < 0.01$). Ayrıca, yüksek doz zencefil ekstresinin güçlü antioksidan etkinliğini gösteren hızlı 1,1-diphenyl-2-picrylhydrazyl (DPPH) analizi kullanarak, radikal süpürücü etkinliğinin ölçülmesiyle zencefil ekstresinin antiokdidan etkisi çalışıldı ($P < 0.01$).
SONUÇ: Zencefil ekstresi, hücresel kolesterol birikimi yanında serum DDL kolesterolünü, toplam kolesterolü, trigliseritlerin ve fosfolipitlerin düzeyelerini, DPPH absorbsiyonunu azaltmış, serbest radikalleri süpürmüştür ve arter dokusunun farklı katmanlarında gelişen histopatolojik lezyonlarını düzeltme potansiyeline sahiptir.
Anahtar Kelimeler: Zencefil ekstresi, DDL-kolestrolü, Lipoproteinler, Kolesterol, Trigliseritler, Fosfolipitler, Ateroskleroz, Hipolipidemi.

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INTRODUCTION

Ginger rhizomes (*Zingiber officinale Roscoe*, Family *Zingiberaceae*) have a long history for its health benefits and uses as a traditional medicine in the Asia and Africa. The dried extract of ginger contains monoterpenes and sesquiterpenes. Besides, the main active antioxidants in ginger are the gingerols and shogaols as well as some phenolic ketone derivatives [1,2]. The high content of potassium in ginger protects the body against bone fragility, paralysis, sterility, muscle weakness, mental apathy and confusion, kidney damage, and damage to the heart. In addition to potassium's role in blood pressure regulation, it also regulates heartbeat [3].

Coronary artery disease develops as a result of various risk factors, including increased total cholesterol, triglycerides, and phospholipids levels. The presence of phenolic compounds and flavonoids in the diet has been shown to be inversely associated with morbidity and mortality from coronary heart disease [4]. Moreover, the dietary consumption of nutrients rich in polyphenols, such as the crude extract of licorice, derived from the roots of the Asian plant *Glycyrrhiza glabra***,** protects LDL against lipid peroxidation and inhibits the development of aortic atherosclerotic lesions [4]**.**

Ginger extract possesses antioxidative characteristics, since it can scavenge superoxide anion and hydroxyl radicals [5, 6]. AS well as, the high levels of gingerol, the predominant compound of ginger, inhibited ascorbate/ferrous complex induced lipid peroxidation in rat liver microsomes[7]. Gingerol isolated from *Zingibe*r was shown to interfere with inflammation processes [8], and it was also suggested to inhibit platelet function due to inhibition of thromboxane formation[9]. Furthermore, ginger acts as a hypolipidemic agent in cholesterol-fed rabbits[10]. Furthermore, feeding on ginger elevated significantly the activity of hepatic cholesterol-7alpha-hydroxylase, the rate-limiting enzyme in bile acids biosynthesis, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body of rats [11].

In addition, a pure constituent from ginger [E-8 beta, 17 epoxylabd-12-ene-15,16-dial (ZT)], was shown to inhibit cholesterol biosynthesis in homogenates rat liver [12].

The treatment with ginger caused a decrease in serum cholesterol, serum triglyceride and blood pressure in diabetic rats [13]. Ginger is also has highly effective for motion and morning sickness. Therefore, the present study was carried out to investigate the effect of ginger extract on cholesterol and its derivatives. Also, the development of atherosclerosis in albino mice fed on different levels of ginger extract in relation to plasma cholesterol levels was evaluated.

MATERIALS AND METHODS

Dried rhizome of ginger *Zingiber officinale* Roscoe (*Z. Officinal Rosc*.) were purchased from a local retail spice market in Mansoura, City, Egypt. Biomerriee available kits of triglycerides, cholesterol, phospholipids, LDL cholesterol and VLDL-cholesterol enzymatic colorimetric determinations were obtained from Alkan Co., Egypt. Cholesterol was obtained from Sigma Chemical Co.

Plant materials and extraction

Dry rhizome of *Zingiber officinale* Roscoe were purchased from a local market in Cairo Egypt. 400gm of dry powdered of ginger rhizomes was extracted with 720 ml of methanol (95% v/v) as a solvent, after allowing the system to cool down overnight in a Soxhlet apparatus, the methanolic extract was concentrated under vacuum and dried in a desiccators(yield, 11.25 g, 4.5% w/w) and suspended in 5% gum acacia. The dried active methanolic extract was divided in to two portions the first portion was subjected for pharmacological study and the second portion was subjected for chromatographic analysis as follows:

Analysis of the methanolic extract

By analytical Tin layer chromatography (TLC) method, the second portion of the dried methanol extract of Z. officinale was subjected to perform on GC/MS with a Hewlett-Packard HP5890 Series II plus GC-HP 5972 Mass Selective Detector. The operating conditions were as follows: inlet temperature 250ºC, initial temperature 70ºC, detector temperature 280ºC and final temperature 280ºC (hold for 5min). It was performed with column HP-5 (length 30m, film thickness 0.25mm and internal diameter 0.25mm). The carrier gas was ultra high purity helium (UHP He).

The zones were detected under UV at 254nm using CHCl3-MeOH (9:1 and 4:1) and MeOH (1000 ml each) as eluting solvents to obtain 3 fractions, respectively. Fraction 1 (CHCl3-MeOH; 9:1) was separated by preparative pre-coated TLC on silica gel 60F254 silica plates gel (Merck, 0.5mm thick), activated at 105ºC for 30 min with n-hexane-EtOAc (3:1) as a mobile phase to obtain 5 bands. Band 3 was subjected to Sephadex LH-20 column

chromatography (Pharmacia Biotech) eluting with MeOH to afford 16 {fractions (1a-16a)}. Fractions 8a-10a were further separated by Fractions 8a-10a were further separated by preparing TLC on silica gel plates with n-hexane-EtOAc (5:1) as a mobile phase to yield 6-shogaol as a yellow oil (0.042 g). Fraction 13a, upon standing overnight at room temperature, gave 6-dehydrogingerdione or 1-dehydrogingerdione as yellow crystals (0.032 g). Band 4 of fraction 1 was separated by Sephadex LH-20 column eluting with MeOH to obtain 14 fractions (1b-14b). Fractions 7b-8b were separated by preparative TLC on silica gel plates using n-hexane-EtOAc (3:1) as a mobile phase to afford 6-gingerol as yellow oil (0.078 g).

Free radical scavenging activity Assay:

The free radical scavenging activity of methanol extract of the 6-shogaol, 6-dehydrogingerdione or 1-dehydrogingerdione and 6-gingerol isolated compounds from ginger methanolic extract were measured by using 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the method described by Shimada et al. [14]. The 0.1 mmol/L solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of the crud ginger extracts at different levels (50 –250 µg and also from isolated compound from ginger methanolic extract. After 30 min absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The results were expressed as percentage of inhibition, % inhibition = [(Acontrol - Asample)/Acontrol x100]. The EC50 value (effective concentration of sample required to scavenge DPPH radical by 50%) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

Male albino mice weighing 28-35gm supplied by the experimental animal's house of National Cancer Institute, Cairo, Egypt were used through this study.

Hundred mice were kept under normal healthy conditions and fed on mice chow pellets daily and a once weekly, vegetable diet of carrots, celery and cabbage and carrot, then they divided randomly to five groups (n=20) according to the following scheme:

Group 1: Mice fed on the commercial rodent chow and vegetable diet throughout the experiment and given 1.1% alcohol and water (11 ml of alcohol in 1L water) (Negative control).

Group2: Mice fed on high cholesterol diet (2% cholesterol w/w in food pellet) (Positive control).

Group3: Mice fed on high cholesterol diet and orally 25µg of ginger extract in 1.1% alcohol and water administered by gastric tube daily for 6 months.

Group4: Mice fed on high cholesterol diet and orally by gastric tube administered daily with 250 µg of ginger extract as group three for 6 months.

Group5: Mice fed on high cholesterol diet orally by gastric tube administered daily with 2500 µg of ginger extract as group three for 6 months.

At the end of the experimental(6months) animal were fasted and Blood sample was by heart puncture and serum was separated by centrifugation of the blood for determination LDL, cholesterol, triglycerides as well as determination of lipoproteins levels of cholesterol and triglycerides.

The mice were then anesthetized with ethyl ether in a local nasal container. The heart together with the aorta (2-3cm length) was rapidly excised from each animal. The aorta was cut at the origin and removed from the heart processed for histopathological examination.

The aorta was fixed in aqueous solution of10% formal saline solution. The pthomorphological changes were assessed on the basis of paraffin preparations, the aorta sections were soaked in 10% formal saline solution then processed to obtain 5mm section for hematotoxylin & eosin (H&E) staining and examined under a light microscope for observation of structural abnormality**.** The remaining aorta was soaked in deionized water and homogenized for biochemical analysis.

Biochemical analysis

For determinations Triglycerides levels in the serum and aorta using Biomerriee available kits according to the methods described by Fletcher [15], determination the Lipid was first extracted from serum and aorta using the method of Folch *et al.* [16], before the cholesterol and phospholipids levels were determined, The cholesterol [17], Phospholipids [18], LDL cholesterol [19], and HDL-cholesterol was determined according to the previously described methods [20].

Statistical analyses

Statistical Package for Social Sciences (SPSS) version 12 was used. Quantitative variables were summarized using mean and SD values. Qualitative data were summarized using frequencies and percentage. Comparison between different measurement values before and after treatment within each group were analyzed using paired-t test. Differences were considered significant when p was < 0.05 and highly significant when $p < 0.01$ [21].

Table1: Ginger volatile oil constituents.

No	Compound identified	Retention time (min)	Area (%)
1	Dimethylephosphene-D1-2 –chloropropinyl chloride Alpha ethane (methulsulfonyle)	1.31	0.73
2	Ethanone, 1(2-furanyl), 4-4dimethyl-2-cyclopenten-1-oneborneol, bicycle(2.2.1)heptan	9.43	0.43
3	Benzene,1-(1,5-dimethyl-4-hexenyl	16.31	4.62
4	α - zingiberen, Heptane, 6-methyl-2-p-Toyl, Hezingiberen, 1,2 cyclohexadien	16.55	17.6
5	α - amorphene, α - amorp, γ -Cadinene,Naphathalene,1,2,3,4,5,6,8a-oct	16.66	1.14
6	ß- bisabolene, cyclohexene	16.82	5.36
7	ß- sesquiphellandrene	17.15	7.65
8	d-Nerolidol,1,6,10 dodecatrien-5,beta-cholan-24-oic acid 2,6,10- Didecatrienoic acid	17.92	1.13
9	6-Benzofuranone, 3,5-dihydro-5,4-hydroxyphenethylene glycol tripf	18.49	0.58
10	4-(3'-isopropoxy-2',6',6'-trimethy,4,4-bis(methoycarbonyl-1-isoprop cis-1-(2-pyrazyl)-1-octene	10.93	0.66
11	sesquisabinene hydrate zingiberenol,4-dimethyamino-2'-carboxyl -Azoben	19.26	0.82
12	Zingerone (4-(4-hydroxy-3-methoxyp,2-butanone,4-(4-hydroxy-3-methoxyZingerone, Vanillyl Acetone	19.66	2.51
13	1-h-3a-7-methaoazulene,2,3,4,7,8,Alpha,-Cadrene	20.34	0.76
14	8(15)-Cedren-9-ol,1,4-Hexadiene,3,3,5-trimethyl-1,5-Heptadiene, 2,3,6-Trimethyl-	20.47	1.01
15	N(4-Hydroxy-3-Methoybenzyl)-8-MetCapsaicin, Trans-1-Trimethylsilyhex	22.49	0.78
16	2-Cyclochepten-1-one,pregn-5-en-3-ol, 20-amino-	23.4	0.23
17	Methyl Commate D,5,12-epoxy-1,2 Ethyl(bis(Trimeythlesilyl)amino)Iod	23.85	0.47
18	Benzenebutanoic Acid,2,5-Dimethyl Benzene,1-methyle-4-(1,2,2-trimethyl	24.85	0.47
19	Hexadecanoic acid,palmatic acid	25.00	6.32
20	3,5-ditertbutyl-b-4-dihdroxy-NSalin,17.alpha –estra-1,,3,5,1-Cyano-1-p-Nitrophenyl-2-Dimethyl	25.79	2.81
21	9,12-octadecadienoic acid,Linoleic acid	27.75	12.88
22	9-Octadecenoic acid, Docosanolide, Oxacyclotricosan-2	27.84	13.77
23	Docosanolide, Oxacyclotricosan-2, cyclopropaneoctanal, Stearic acid, Octadecanoic acid	28.15	2.89
24	Carpachromene, 6H-benzo1,2 Obovatin methyl Ether	28.79	1.15
25	2',4'- Dhydroxy propiophenone, 2, benzeneacetic acid, 4-hydroxy-3-meSaline, Trimethyl(4-methyl-3-pente	29.34	0.67
26	Cis-6-shogaol (4-Decen-3-one,1-N-2-oxa- hyroxyyethyl-azacrown(18) (spiro[5'-hydroxy-2'methyl-cyclop	30.88	6.69
27	cis-hydroxy-6- Gingero l, Zingerone, Vanillyl Acetone Gingerol, 3-Decanone,	33.52	4.09
28	6-dihydro gingerdione, 3-beta-methoxy-4-cholesten-6, alpha 5,6-beta-cyclo-beta –homo-5-beta	37.45	1.24

RESULTS

Chemical analysis of the volatile oils

Table 1, showed that the GC/MS analysis of the volatile oil of Z. officinale was contained at least 28 compounds, three of them were identified, with geranial (% area 33.0) and neral (% area 26.6) being the main compounds. The main Compounds isolated from the volatile oil are 6-shogaol, 6-dehydrogingerdione or 1-dehydrogingerdioneand 6-gingerol) (Table 1) were isolated from the methanol extract of Z. officinale.

Antioxidant activity evaluation of ginger extract

As shown in Table 2, it can be noticed that the addition of 50 mg of ginger extract/L to DPPH solution induced a 48% decrease in the optical density at 517 nm within 100 Sec. At a higher dose of 250 mg of ginger extract /L, the optical absorbance of DPPH at 517 nm was reduced after 100 Sec. by 89.6%. Addition of vitamin E (50 µmol/L), which served as a positive control, to the DPPH solution induced a rapid decrease in the absorbance at 517 nm up to 96%, which reached a plateau already within 200 Sec.

Table 2: Antioxidant activity of crud Z. officinale methanolic extract

Times (second)	Absorbance at 517nm*		
	Vit. E (50 µmol/L)	Ginger extract (50 mg/L)	Ginger extract (250 µg/ml)
0	1.25	1.25	1.25
6.25	1.10	0.30	0.95
12.5	1.05	0.25	0.80
50	1.00	0.20	0.75
75	0.95	0.18	0.70
100	0.90	0.13	0.65
125	0.85	0.11	0.60
150	0.80	0.096	0.57
200	0.05	0.13	0.92

*Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The results depicted in Table 3 revealed that the butylated hydroxytoluene was used as positive standard for antioxidative Strong radical scavenging activity was exhibited by 6-shogaol and 6-gingerol with EC50 values ranging from 2.5-4.7 µg/ml. This was about 2-4 times more active than butylated hydroxytoluene.

Table 3: EC50 values against DPPH radical of the crud Z. officinale methanolic extract and Compounds isolated from Z. officinale.

No	Compounds	Compounds EC50 against DPPH(µg/ml, mean±SD)	Antioxidant against DPPH (%inhibition± SD) at 100 µg/ml, n=3)
1	6-shogaol	(2.5±0.5 µM)	52.5±0.4 (n=3)
2	6-dehydrogingerdione	(4.2±0.3 µM)	16.7±0.1 (n=3)
3	6-gingerol	(4.7±0.4 µM)	14.4±0.1 (n=3)
4	Volatile oil of Crud ginger	(1.9±0.7, µM)	86.6±0.0 (n=3)
SD	Butylated hydroxytoluene (positive standard)	(37.3±0.9 µM)	8.2±0.2 (n=6)

n = number of samples tested., **SD=**Standard for DPPH radical

Table 4: Effect of crud Z. officinale methanolic extract on the cholesterol, triglycerides and phospholipids levels in serum and aorta of mice.

Groups	Cholesterol		Triglyceride		Phospholipid	
	Serum (mg/ml)	Aorta (mg/g protein)	Serum (mg/ml)	Aorta (mg/g protein)	Serum (mg/ml)	Aorta (mg/g protein)
Group 1	23.5±1.3	24.44±1.23	7.31±2.53	28.00±6.25	0.12±0.01	1.06±0.02
Group 2	28.6±2.1	33.30±4.63*	22.47±5.29*	46.93±1.72*	0.14±0.01	2.48±0.50*
Group 3	23.6±2.1	24.46±1.82**	8.80±0.46**	42.57±5.17**	0.13±0.01	1.80±0.17**
Group 4	*16.7±1.6	17.56±1.82**	7.30±0.46*	33.87±5.17**	0.12±0.01	1.07±0.17**
Group 5	*16.7±1.6	18.98±1.24	6.40±0.4	19.14±5.14	0.10±0.01	1.01±0.09

The data are presented as mean ± SEM of 6 animals. * Significantly different when compared to untreated group (Negative control), $p < 0.05$; **Significantly different when compared to group given high cholesterol diet(Positive control), $p < 0.05$ ginger extract (µg/day).

Table 5: Effect of crud Z. officinale methanolic extract on lipoproteins levels of Cholesterol and triglycerides in the serum of mice.

Groups	VLDL		LDL	
	Cholesterol (mg/ml)	Triglyceride (mg/ml)	Cholesterol (mg/ml)	Triglyceride (mg/ml)
Group 1	10.7 ± 1.06	1.7 ± 0.07	8.6 ± 0.4	0.47 ± 0.006
Group 2	13.5 ± 1.05	2.1± 0.03	9.2± 0.5	0.8± 0.01
Group 3	12.5 ± 1.32	1.8 ± 0.03	7.1 ± 0.2	0.4 ± 0.053
Group 4	*7.2 ± 0.43	*1.1 ± 0.05	*4.0 ± 0.5	*0.19 ± 0.08
Group 5	*6.2 ± 0.52	*0.98 ± 0.05	*3.0 ± 0.2	*0.17 ± 0.0

All results are expressed as means ± SD, n =6, *P < 0.01.

Furthermore the methanol extract of the crud Z. officinale possess strong antioxidative activity against the DPPH radical with % inhibition in the crud methanolic extract of Z. officinale is 86.6-92.5% while the i the DPPH radical with % inhibition in 6-Segoale is 52.5% In general, the methanol extract of crude Z. officinale was more active against the DPPH radical than the isolated compound from Z. officinale but the (6-shogaol) methanolic extract is more active against than other isolated compound from Z. officinale rhizome. The radical scavenging activity of the crude Z. officinale and the three gingerol derivatives 6-shogaol, 6-dehydrogingerdione or 1-dehydrogingerdioneand 6-gingerol could be attributed to the hydroxy group of the 4-hydroxy-3-methoxyphenyl moiety.

Biological evaluation of ginger extract:

Data presented in Table 4 show that feeding on high cholesterol diet (2% cholesterol w/w) had significantly increase the cholesterol, triglycerides and phospholipids levels in the aorta compared with those of the control group. Concerning serum lipid values, the triglycerides level was significantly increased about three folds (22.47 mg/ml), than those observed in the control group (7.31 mg/ml). On the other hand, no significant change was observed in the other two lipid compounds levels in the serum.

Fig. 1: Common carotid artery of untreated (Negative control) mice, stained with H & E (100×).

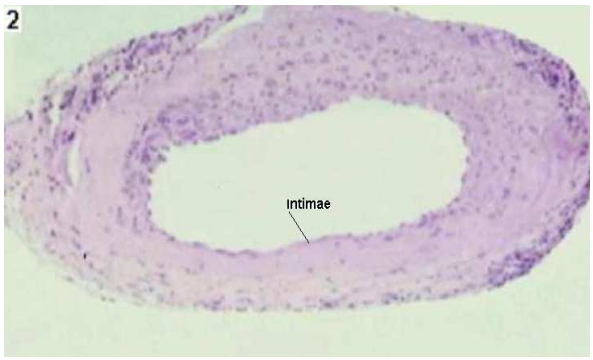

Fig. 2: Hyper cholesterol diet Group Aorta. Atheromatous plaque. Foam cells in the intimae (Positive control)(H&E staining). 100x

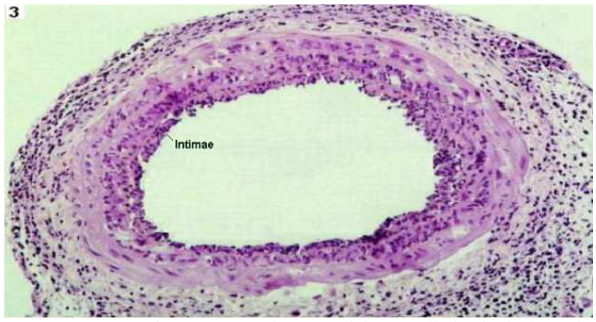

Fig. 3: Aorta. Atheromatous plaque and foam cells in the intimae in mice group feeding on a hyper cholesterol diet plus crud Z. officinale methanolic extract ginger extract. H&E staining. 100 xs.

From the data presented in the same table, it could be clearly observed that addition of ginger extract (25 µg/ml/day) to the high cholesterol diet, inducing marked decrease of the cholesterol, triglycerides and phospholipids levels in the homogenate aorta compared to the levels of the hypercholesterolemic animals ($p < 0.05$) but a slight effect on the phospholipids and cholesterol levels in the serum. However, lowering effect of the serum triglycerides level was observed (Table 4).

Data presented in Table 5 illustrated that feeding on high cholesterol diet (2% cholesterol w/w) had significantly increased levels of the VLDL and LDL cholesterol as well as VLDL and LDL triglycerides in serum compared with those of the control group. Consumption of different levels (25- 2500 µg/ml/day) for 6 months of ginger extract on hyperlipidemic mice showed a significant ($P < 0.01$) reduction in the levels of VLDL and LDL cholesterol in serum. Similarly, significant ($P < 0.01$) reductions were also obtained in the levels of VLDL and LDL triglycerides in serum (Table 5).

Histological evaluation of ginger extract: The effect of ginger extract on aortic atherosclerotic tissues lesion.

The dyslipidemic condition arising from a high-cholesterol diet ($p<0.05$), as shown in Fig. 2 Histological results indicate that ginger extract significantly reduced arterial wall atherosclerotic lesions, when compared to the highcholesterol groups ($p<0.05$), in the high cholesterol diet group caused occur high deposits and spaces in the tissues of aorta. These spaces had originally contained fat droplets which were dissolved during the hemotoxylin and eosin (H & E) staining procedure and also showed the presence of atherosclerotic changes including reduction in the vascular caliber to less than half normal diameter by a greatly thickened intima, The intima nearest the lumen consists of dense fibrous tissue, apart from one area rich in lipid filled macrophages results from atherosclerotic thickness grading, in the ginger extract group were decreased significantly compared to the high-cholesterol diet group Fig 3 and Compared to Untreated healthy Control aorta tissues Fig 1.

On the other hand TC, TG and LDL cholesterol and increase HDL cholesterol atherogenic in serum difference between the ginger extract group and the normal diet group is significant, whereas atherosclerotic thickness grading difference between these two group is not significant. This suggests that decrease in lesions in the group receiving ginger extract has likely been due to antioxidative and anti-inflammatory properties other than the effect of the extract on plasma lipoproteins.

DISCUSSION

The present data revealed that the volatile oil from the rhizome of Z. officinale was characterized by the presence of acyclic oxygenated monoterpenes mainly composed of geranial (Ecitral) and neral (Z-citral).It accordance with the results reported previously by Chen et al. [22] they said that the volatile oil of the rhizome of Z. officinale was contained 6-dehydrogingerdione or 1-dehydrogingerdione Charles et al. [23], and 6-gingerol (Yamada et al. [24] respectively.

The present work suggests that the Z. officinale rhizomes were commonly used as spices and medicinal plants in the world has a potential source of antioxidants (by acting as free radical scavengers) Further study in an animal model is strongly recommended in order to evaluate whether these plants are promising for clinical trials. This evidence

supported that the assumption of gingerols was responsible for antioxidant activity of Z. officinale rhizome (Sekiwa et al., [25]; Chung et al., [26]).

The present data demonstrated the scavenging of free radical capacity of ginger extract which is dose dependent these results are agreed with those reported by You *et al.* [27] and Stoilova *et al.* [28].

Microscopic Assessment of Aorta

The histopathological change observed in both high cholesterol animal diet group and high cholesterol animal diet accompanied with increasing the ginger extract levels (25 -2500 µg/day). There was a focal hyperplasia in the intima, completely covered and fat was also noticed in intercellular spaces with laminas in high cholesterol animal diet group but observed a single, non-complete small atheromatous plaques in high cholesterol animal diet plus ginger extract levels (25 -2500 µg/day) groups, the improvement may be related to the free radical scavenging activity of ginger extract. The present results are in accordance with those reported by Uma et al., [29] they reported that the ethanolic extract of *Zingiber officinale* Roscoe can protect the tissues from lipid peroxidation. The extract also exhibit significant lipid lowering activity in diabetic rats. The present study is the first pilot study to assess the potential of *Zingiber officinale* in lipid lowering activity and diabetic dyslipidaemia these results agree with the study performed by Ali et al., [30] the demonstrates that the ethanol ginger extract causes a decrease in lipid peroxidation, an increase in plasma antioxidant capacity and a reduction in renal nephropathy.The anti-atherogenicity of ginger extract could also be attributed to its direct antioxidative effects on serum LDL which lead to increase in the lipid peroxidation [31].

The effect of consumption of different dose levels of ginger extract on hyperlipidemic mice serum and aorta cholesterol, triglycerides and phospholipids levels. From these results, it could be concluded that there is appositive correlation between these effect on a dose levels (25 -2500 µg/ml/day) used.

These Similar results were reported by Sanjay et *al*. [32], who found that the addition of ginger to the high cholesterol animal diet caused a decrease in serum cholesterol, serum phospholipids, serum triglycerides and blood pressure in diabetic rats. Furthermore the raw ginger may be of great possesses hypoglycaemic, hypocholesterolaemic and hypolipidaemic potential in rats and also the decrease in the serum cholesterol level in this study could be attributed to excretion of more cholesterol in the bile and feces of hypercholesterolemic animals. Also, the decrease in the serum phospholipids level could possibly be due to a higher level of phospholipase that metabolized the blood phospholipids in hypercholesterolemic animals. [33].

Furthermore, consumption of different levels of (25- 2500 µg/day) for 6 months of ginger extract on hyperlipidemic mice showed a significant ($P < 0.01$) reduction in the levels of VLDL and LDL cholesterol in serum (Table1).

These results are in agreement with those reported in [34] and [10], they reported that hyperlipidemia and antiatherosclerotic effects of ginger volatile oil were demonstrated in cholesterol-fed rabbits. Ginger extract consumption can result in accumulation of active ingredients within the cells, as well as in the plasma membrane, thus affecting cellular enzymes, and plasma membrane receptors [35]. From the aforementioned data, it could be clearly concluded that dietary consumption of ginger extract by hyperlipidemia mice significantly reduced the development of aortic atherosclerotic changes, along with an impressive reduction in the levels of serum VLDL and LDL cholesterol.

The hypercholesterolemic effect of ginger could have possibly resulted, at least in part, in the inhibition of cellular cholesterol biosynthesis observed after consumption of ginger extract.

Consumption of ginger extract inhibited the progression of aortic atherosclerosis in atherosclerotic mice. This effect was associated with a significant reduction in the serum and LDL cholesterol levels. Atherosclerosis is a multi-factorial disease associated with different risk factors. Hypercholesterolemia is a major risk factor for atherosclerosis [36].

In conclusion the present study concluded that consumption of the ginger extract were improved beneficial properties in attenuation of atherosclerosis development and a reduced cellular cholesterol accumulation. Also, the hallmark of early atherosclerosis associated with a significant reduction in plasma LDL cholesterol, total cholesterol,triglycerides and phospholipid levels, in adding to that the ginger extract has a great capacity to reduce DPPH absorption and its radical scavenging activity finally the ginger extract has the potential to improve beneficial properties in attenuation of atherosclerosis development. So, it recommended further study to use it as anti atherosclerotic agents.

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