The Role of Natural Products in Coronary Artery Diseases Caused By Hyperlipidemia

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Abstract

Objective: Hyperlipidemia is responsible for the development of coronary artery diseases, which could be treated with cinnamon and ginger.

Methods: Lipid concentrations, Malondialdehyde (MDA), Glutathione Peroxidase (GPx), collagen I, Matrix Metalloproteinase 2 (MMP-2) and Tissue inhibitor of metalloproteinase 2 (TIMP-2) were done in male albino rats weighing 220-250 gm. The rats were divided into five groups, kept on either control diet or high fat diet (HFD). HFD rats treated with cinnamon (one group)(400 mg/kg body weight/day), or with ginger (one group)(500 mg/kg body weight/day), and with cinnamon and ginger combination (another group) via oral route for 40 days.

Results: Hyperlipidemic control (HC) rats which fed on a HFD, developed hypercholesterolemia, hypertriglyceridemia, increased LDL-C, MDA, collagen I, MMP-2 and decreased HDL-C,GPx, TIMP-2 when compared to rats fed on control diet**.** Treatment of the HFD rats with either cinnamon or ginger alone caused a significant decrease in triacylglycerol (TAG), total cholesterol, LDL-C, MDA, collagen I, MMP-2, and a significant increase in HDL-C, GPx, TIMP-2. The combined treatment caused a prominent significant difference between groups.

Conclusion: Hyperlipidemia could induce coronary artery diseases, and that combined treatment had a great protective role against the metabolic abnormalities better than the treatment with either cinnamon or ginger alone.

KEYWORDS: Hyperlipidemia, MMP-2, GPx, Atherosclerosis, Anti-oxidants.

Introduction

Hyperlipidemia is the condition in which the level of plasma lipids, primarily cholesterol and triacylgycerol, are higher than the normal range. Hyperlipidemia is an important risk factor in atherosclerosis, which can lead to coronary artery disease (CAD). The coronary arteries are particularly susceptible to

atherogenesis,atherosclerosis of the coronary arteries may lead to angina pectoris and myocardial infarction ***(Jellinger, 2000)***.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reactions can produce free radicals. Antioxidants are widely used as ingredients in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness***(Baillie et al., 2009)***.

A wide variety of therapeutic agents in modern medicine are available for the treatment of hyperlipidemia. However, most hypolipidemic drugs cause potentially serious side effects and include digestive disturbances, nausea and vomiting. Regular usage of many herbs has been recommended in the management of hyperlipidemia and its complications ***(Ahmida and Abuzogaya, 2009)***.

Cinnamonzeylanicum belongs to genus *Cinnamomum*, family *Lauraceae.* Cinnamon has an antidiarrhoeal, antioxidant and antimicrobial potential ***(Singh et al., 2007)***. Cinnamon was classified as a herbal drug which has cardiovascular effects as it increases the coronary blood flow and provoked piuitrin induced reduction of blood flow. Also it reduced peripheral vascular resistance, suggesting an undeviating vasodilation of peripheral vessels ***(Kamal et al., 2009)***. Cinnamon extract improved lipid profile by extensively decreasing total cholesterol, triglycerides (TAG) and LDL-C levels with increasing serum HDL-C. It also hampers HMG-CoA reductase activity in liver thereby lowering the cholesterol levels ***(Yadav and Bhatnagar, 2007)***. Cinnamon has the ability to inhibit initiation as well as propagation of lipid peroxidation due to their polyphenol content, strong reducing power and superoxide radical scavenging activity ***(Sheng et al., 2008)***.

Ginger (*Zingiberofficinale* Roscoe, Zingiberacae) is a medicinal plant that has been widely used all over the world, since ancient times, for a wide range of unrelated ailments that include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis***(Ali et al., 2008)***. Gingerol is the active ingredient of ginger, that's thought to relax blood vessels, stimulate blood flow and relieve pain. Also, it is an anti-inflammatory agent, which may be useful in fighting heart disease, cancer, Alzheimer's disease, arthritis. Also, it is used as antimicrobial and anti-thrombotic agents ***(Afzal et al., 2001)***.

Consumption of ginger extract inhibits the progression of aortic atherosclerosis. The anti-atherogenicity of ginger extract could also be attributed to its direct anti-oxidative effects on macrophages as well as on plasma LDL. Feeding rats with ginger significantly elevated the activity of hepatic cholesterol-7α-hydroxylase, the rate-limiting enzyme in bile acids biosynthesis, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body ***(Fuhrman et al., 2000)***.

Aim of work:

The aim of this study is to:

1. Clarify the adverse effects of hyperlipidemia on cardiac manifestations as well as to focus on the characteristic features of cardiac remodeling induced by hyperlipidemia particularly coronary artery diseases to address the molecular mechanism underlying them.

2. Evaluate the effect of cinnamon, ginger either monotherapy and in combination on modulating cardiac complications in experimentally hyperlipidemic rats. This is can be provided by measuring of lipids profile, Malondialdehyde (MDA), glutathione peroxidase (GPx), gene expression of collagen III & matrix metalloproteinase 2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2).

Materials and methods

Animals:

One hundred male Albino rats weighing 130- 150 g purchased from Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt) were recruited for the present study. All rats had free access to standard chow diet and tap water. They were housed under control condition at temperature (25°C) with 12 h light/dark cycle.

Diet and Drugs
Induction of hyperlipidemia

One week after acclimatization, ninety rats were switched from standard chow diet to high fat diet for two months to induce hyperlipidemia. The rats weighing220-250g, were selected in this study.

High Fat Diet

Protein (casein) 15 %, carbohydrates (sucrose 10 %, corn starch 31 %), fat (lard) 37 %, cellulose 2 %, vitamin mix 1 % and mineral mix 3.5 % ***(Park et al., 2007).***

Cinnamon Water Extract Preparation

Cinnamon extract was extracted based on the method of ***(Sheng et al., 2008,Soliman et al., 2012)***. Briefly, cinnamon powder (100 g) was dissolved in 1000 mL double distilled water then subjected for revolving evaporator in vacuum state using vacuum pump till the volume of water reduced to 50%. The supernatant was filtered through Whatman paper no. 1 to obtain cinnamon water extract.
Cinnamon Dose: 400 mg/kg body weight/day orally ***(Mahmood et al., 2011).***

Zingiberofficinalis (Ginger) Water Extract Preparation:

The roots ofZingiberofficinale was washed, peeled, cut into small pieces, dried at room temperature and crushed in electrical grinder and powdered. The aqueous extract powder (500 g) was soaked in 2 liters of distilled water for two days. The mixture was filtered, frozen and dried by using lyophilizer for 72 hours, then froze at -

30^{o} C. The powder was taken and weighted for 26 g which dissolved in 520 ml distilled water ***(Lemhadri et al., 2004).***
Ginger Dose: 500 mg/kg body weight/day orally ***(Thomson et al., 2002).***
Experimental design:

Normal and hyperlipidemic rats were assigned into the following experimental groups:

Group I: Normal rats, normal control group (NC) **(n: 10).**

Group II:Hyperlipidemic rats, hyperlipidemic control group (HC) **(n: 10).**

Group III:Hyperlipidemicrats, were treated (1 week after the induction of hyperlipidemia) with cinnamon 400 mg/kg body weight/day orally for 40 days **(n: 10).**

Group IV: Hyperlipidemicrats, were treated (1 week after the induction of hyperlipidemia) with ginger 500 mg/kg body weight/day orally for 40 days **(n: 10).**

Group V: Hyperlipidemicrats, were treated (1 week after the induction of hyperlipidemia) with a combination of cinnamon and ginger for 40 days **(n: 10).**

Sampling

At the end of experiment, animals were fasted overnight, blood was collected via retro-orbital bleeding in dry centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. Serum was collected and divided into aliquots and kept at -20^{o}C for further assay of lipids profile.

Tissue sampling

Following blood collection, rats were killed by decapitation, hearts were removed and washed with buffered 0.9% Nacl (pH 7.4). The atria, extraneous fats and connective tissue were removed. The left ventricles were harvested quickly and rapidly frozen in liquid nitrogen and stored at -80^{o}C for further determination of MDA, GPxspectrophotometrically and collagen-I, MMP-2 and TIMP-2 using real time polymerase chain reactions (RT-PCR).

Methods

Serum TGs were estimated by GPO-POD enzymatic method ***(Nagele et al., 1984)*** using a Biocon kit (India). TC concentration was determined utilizing enzymatic colorimetric CHOD-PAP method ***(Fascse, 1982)*** using Biocon kit (India). HDL−C was determined by the same method after the precipitation of very low density lipoprotein cholesterol (VLDL-C) and LDL-C ***(Warnick et al., 1982)***, and finally, LDL-C was calculated by Using Friedewald's Formula ***(Friedewald et al., 1972)***:

LDL− C (mg/dl) = TC – (HDL− C + TAG/5).

For the detection of MDA, itwas determined colorimetrically according to the method of ***Janero, (1998)***.

GPx was determined by GPx enzymes which catalyze the reduction of hydrogen peroxide (H_2O_2) and a wide variety of organic peroxides (R-OOH) to the corresponding stable alcohols (R-OH) and water using cellular glutathione as the reducing reagent ***(Mannervik, 1985)***.

For the detection of collagen-I, MMP-2 and TIMP-2 gene expression, RNA was extracted, reverse transcribed into cDNA and amplified by PCR. Total RNA was extracted from heart tissue using SV Total RNA Isolation System (Promega, Madison, WI, USA). The extracted RNA was reverse transcribed into cDNA using RT-PCR kit (Stratagene, USA). Then amplification of specific DNA sequences using two primers that hybridize to opposite strands and flank target DNA region.

Sequence of the primers used for real-time PCR.

Gene	Primer sequence
MMP-2	Forward:5'GAGATCTGCAAACAGGACAT-3' Reverse :5'-GGTTCTCCAGCTTCAGGTAA-3'
TIMP-2	Forward:5'-AATGACATCTATGGCAACCCC-3' Reverse :5'-AAGAACCATCACTTCTCTTG -3'
Collagen	Forward:5'- CAAGAATGGCGACCGTGGTGA-3' Reverse :5'- GGTGTGACTCGTGCAGCCATC-3'
β –actin	Forward:5′ - ATCATGTTTGAGACCTTCAACACC-3' Reverse :5'- TAGCTCTTCTCCAGGGAGG 3′

Statistical analysis

The results were expressed as mean ± SEM. To determine the statistical significance of laboratory findings, multiple comparisons were achieved using independent samples T-Test and ANOVA followed by Tukey test as post hoc test. P-value ≤ 0.05 was considered statistically significant.

Results and Discussion

Lipids profile:
Triacylglycerols (TAG):

T**able (1)** and **figure (1)** showed that HC rats recorded a significant elevation in triacylglycerol levels as compared to normal control group by 77.56 % ($P<0.05$).

While in treated groups, there was a significant decrease in serum triacylglycerol levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 17.71 % and 15.36 % respectively. Moreover, there was no significant difference between ginger and cinnamon groups. Furthermore, there was a significant decrement between ginger and cinnamon in combinationeither with cinnamon by 21.59 %, ginger treatment by 23.77 % or hyperlipidemic group by 35.48 % ($P<0.05$).

Total cholesterol (TC):

T**able (1)** and **figure (1)** showed that HC rats recorded significant elevation in total cholesterol levels as compared to normal control group by 76.19 % ($P<0.05$).

While in treated groups, there was a significant decrease in serum total cholesterol levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 20.95 % and 20.05 % respectively. Moreover, there was no significant difference between ginger and cinnamon groups. Furthermore, there was a significant decrement between ginger and cinnamon in combinationeither with cinnamon by 21.03 %, ginger treatment by 21.92 % or hyperlipidemic group by 37.58 % ($P<0.05$).

High Density Lipoprotein Cholesterol (HDL-C):

T**able (1)** and **figure (1)** showed that HC rats recorded significant decrease in HDL-C levels as compared to normal control group by 26.62 % ($P<0.05$).

While in treated groups, there was a significant increase in serum HDL-C levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 18.44 % and 11.39 % respectively. Moreover, there was a significant decrease in ginger group as compared to cinnamon group by 5.96 %. Furthermore, there was a significant increment between ginger and cinnamon in combinationas compared to either ginger treatment group or hyperlipidemic control group by 9.44 % and 21.91 % respectively. But, there was no significant difference between ginger and cinnamon in combinationas compared to cinnamon group ($P<0.05$).

Low Density Lipoprotein Cholesterol (LDL-C):

T**able (1)** and **figure (1)** showed that HC rats recorded significant increase in LDL-C levels as compared to normal control group by 146.64 % ($P<0.05$).

While in treated groups, there was a significant decrease in serum LDL-C levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 29.50 % and 27.17 % respectively. Moreover, there was no significant difference between ginger group and cinnamon group. Furthermore, there was a significant decrement between ginger and cinnamon in combinationas compared to either ginger treatment group by 31.46 %, cinnamon treatment group by 29.20 % or hyperlipidemic control group by 50.09 % ($P<0.05$).

OXIDATIVE STRESS MARKERS:

Malondialdehyde (MDA):

T**able (1)** and **figure (2)** showed that HC rats recorded a significant increase in Malondialdehyde (MDA) levels as compared to normal control group by 433.33 % ($P<0.05$).

While in treated groups, there was a significant decrease in serum MDA levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 34.70 % and 31.58 % respectively. Moreover, there was no significant difference between ginger group and cinnamon group. Furthermore, there was a significant decrement between ginger and cinnamon in combinationas compared to either ginger treatment group by 36.78 %, cinnamon treatment group by 33.75 % or hyperlipidemic control group by 56.74 % ($P<0.05$).

Glutathione peroxidase (GPx):

T**able (1)** and **figure (3)** showed that HC rats recorded a significant decrease in Glutathione peroxidase (GPx)levels as compared to normal control group by 42.77 % (P<0.05).

While in treated groups, there was a significant increase in serum GPx levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 37.62 % and 41.56 % respectively. Moreover, there was no significant difference between ginger group and cinnamon group. Furthermore, there was a significant increment between ginger and cinnamon in combinationas compared to either ginger treatment group by 15.32 %, cinnamon treatment group 18.62 % or hyperlipidemic control group 63.25 % (P<0.05).

CORONARY ARTERY DISEASES MARKERS:

Collagen I:

T**able (1)** and **figure (4)** showed that HC rats recorded a significant increase in Collagen I levels as compared to normal control group by 1100 % (P<0.05).

While in treated groups, there was a significant decrease in serum collagen I levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 34.09 % and 40.15 % respectively. Moreover, there was a significant decrease between ginger group as compared to cinnamon group. Furthermore, there was a significant decrement between ginger and cinnamon in combinationas compared to either ginger treatment group 37.97 %, cinnamon treatment group 43.68 % or hyperlipidemic control group 62.88 % (P<0.05).

Matrix Metalloproteinase-2 (MMP-2):

T**able (1)** and **figure (5)** showed that HC rats recorded a significant increase in Matrix Metalloproteinase-2 (MMP-2) levels as compared to normal control group by 684.21 % (P<0.05).

While in treated groups, there was a significant decrease in serum MMP-2 levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 44.97 % and 45.64 % respectively. Moreover, there was no significant difference between ginger group and cinnamon group. Furthermore, there was a significant decrement between ginger and cinnamon in combinationas compared to either ginger treatment group by 44.44 %, cinnamon treatment group by 45.12 % or hyperlipidemic control group by 69.80 % (P<0.05).

Tissue Inhibitor of Metalloproteinase-2 (TIMP-2):

T**able (1)** and **figure (6)** showed that HC rats recorded a significant decrease in Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) levels as compared to normal control group by 77.94 % (P<0.05).

While in treated groups, there was a significant increase in serum TIMP-2 levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 143.33 % and 123.33 %. Moreover, there was a significant decrease in ginger group as compared to cinnamon group. Furthermore, there was a significant increment between ginger and cinnamon in combinationas compared to either ginger treatment group by 56.72 %, cinnamon treatment group by 43.84 % or hyperlipidemic control group by 250 % (P<0.05).

Discussion

The present study was undertaken to clarify the cardiovascular complications particularly coronary artery disease associated with hyperlipidemia to address the underlying molecular mechanism as well as to explore more effective therapeutic agents that will prevent, retard or reverse coronary artery disease.

This study showed that, there was a significant change in lipids profile in hyperlipidemic rats compared with the normal group. It was found that the levels of triacylglycerol (TAG), total cholesterol (TC), and low density lipoprotein cholesterol (LDL-C) were significantly increased, but HDL-C was significantly reduced.These results are in agreement with***Leaf (2008),*** who reported that, triglyceride-rich lipoproteins come from 2 sources, often described as the endogenous and exogenous pathways. In the exogenous pathway, dietary fats (triglycerides) are hydrolyzed to free fatty acids (FFAs) and monoglycerides and are absorbed, with cholesterol, by intestinal cells. They are then reesterified and combined with apolipoproteins and phospholipids to form a nascent chylomicron, a process requiring microsomal triglyceride transfer protein (MTP). Chylomicrons enter the plasma via the thoracic duct, where they acquire two other soluble apolipoproteins, apo C and apo E, from high-density lipoprotein (HDL).

Our result revealed that there is a significant increase in malondialdehyde (MDA) levels in hyperlipidemic control group when compared to control healthy group.Our study is clarified with the study of***Lovri et al., (2008)*** who stated that, there is an extensive evidence that links hypercholesterolemia with increased lipid peroxidation and increased oxidative stress. Oxidatively modified LDLs, which have been heralded as an initiating factor in atherogenesis, possess numerous unfavorable biological effects, including the induction of endothelial dysfunction, activation of endothelial adhesiveness, monocyte differentiation and adhesion, and smooth muscle cell proliferation. Thus, the assessment of lipid peroxidation is usually performed by analyzing secondary oxidation products such as malondialdehyde.

In our results, there is a significant decrease in glutathione peroxidase (GPx) in hyperlipidemic control group as compared to control healthy group.Our data are in harmony with ***Yang et al., (2008)*** who demonstrated that, increased lipid peroxidation thought to be a consequence of oxidative stress which occurs when the dynamic balance between prooxidant and antioxidant mechanism is impaired. The increase of ROS production and/or deficiency of antioxidant defense system leads to decreased activities of SOD and GSH-Px in higher lipid subjects. Thus, insufficient detoxification of these reactive oxygen species by antioxidant enzymes may lead to an occurrence of imbalance between antioxidant and oxidant systems. Low SOD and GSH-Px activity could also attribute to enzyme inactivation by ROS bringing about damage to proteins.

On the other hand, in our results, there is a significant increase in glutathione peroxidase (GPx) in treated groups as compared to hyperlipidemic control group.

In our results, there is a significant increase in collagen in hyperlipidemic control group as compared to control healthy group.***Pongnimitprasert(2009)*** revealed that, in the atherosclerotic process, macrophage foam cells are formed with the rapid

transformation of phagocytic monocytes penetrated into the sub endothelial space and atherogenic lipoproteins like modified low density lipoprotein (LDL) are uptaken by receptor-mediated endocytosis mechanism. Endothelial dysfunction has been proposed as long-term atherosclerotic lesions which initiates the inflammatory mechanisms and is used as an important diagnostic and prognostic factor, which leads to proliferation of intimal smooth muscle cells, accumulation of extracellular matrix components such as collagen, elastic fibers and proteoglycan, and cholesteryl ester and free cholesterol accumulation within the cells and in the surrounding connective tissues.

On the other hand, in our results, there is a significant decrease in collagen in treated groups as compared to hyperlipidemic control group.Our results are in a line with ***Qin et al., (2009)*** who reported that, in vitro studies have shown that cinnamon can increase the expression of PPAR-γ/α and their target genes such as LPL, CD 36, GLUT 4, and ACO in 3T3-L1 adipocyte. Furthermore, this spice in vivo was found to activate PPARγ and α, resulting in improved insulin resistance and reduced fasting glucose, FFA, LDL-cholesterol, and aspartate aminotransferase levels in high-caloric-diet-induced obesity and db/db mice in its water extract form which in turn decrease the extracellular matrix protein which decrease the collagen content in heart.

In our results, there is a significant increase in matrix metalloproteinase 2 (MMP-2) and decrease in tissue inhibitor of metalloproteinase 2 (TIMP-2) in hyperlipidemic control group as compared to control healthy group.Our study is in harmony with ***Xie et al., (2004)*** who illustrated that, there is a direct effect of hyperlipidemia on the myocardium rather than an indirect effect through coronary sclerosis. That peroxynitrite ($ONOO^-$) is involved in preconditioning and that hyperlipidemia leads to enhanced formation of $ONOO^-$. A significant cellular target of $ONOO^-$ is activation of a large family of zinc-dependent endopeptidases, matrix metalloproteinases (MMPs), via a nonproteolytic oxidative mechanism resulting in fully active proenzymes.Furthermore, signaling pathways involved in the mechanism of preconditioning influence the expression or activation of MMPs, e.g., activation of protein kinase C-ζ and -θ subtypes increases expression of MMP-2 in rat cardiac fibroblast culture and decrease in tissue inhibitor of metalloproteinase 2 (TIMP-2).

On the other hand, in our results, there is a significant decrease in MMP-2 and a significant increase in TIMP-2 in treated groups as compared to hyperlipidemic control group.***Lu et al., (2010)*** revealed that, procyanidins are found in cinnamon, which have been found to inhibit angiogenesis and tumor growth. Cinnamon extract inhibits vascular endothelial growth factor *(*VEGF*)* receptor-2 on endothelial cells and suppresses endothelial cell proliferation, migration, and tube formation in vitro. The cinnamon extract suppressed tumor microvessel density, and the expression of angiogenic factors VEGF, FGF, and TGF-βas well as COX-2 and HIF-1alpha, which promote angiogenesis, which lead to decrease in MMP-2 and increase in TIMP-2.

Our study is in line with ***Szlosarek and Balkwill (2003)*** who reported that, ginger oil can significantly inhibit the expression of TNF-α at concentrations of 0.05% and 0.1%. TNF-α was primarily discovered as a molecule responsible for antitumor activity. Increase in TNF-α level increase the property of tissue invasion and metastasis through the up regulation of NF-κB pathway. TNF-α secreted by tumors induce the secretion of matrix metalloproteases (MMPs) that help in the

process of invasion and metastasis, and also increases the production of VEGF. So, ginger oil can decrease TNF-α, down regulation of NF-κB pathway, which decrease the MMPs and also increase the TIMP-2.

Acknowledgements:
Special thanks to dr. LubnaJamil for her help in the tissue examination.
"This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors".

References:

-Afzal, M., Al Hadidi, D., Menon, M., Pesek, J. and Dhami, M.S. (2001) Ginger: an ethnomedical, chemical and pharmacological review. Drug Metabolism and Drug Interactions. 18, 159-190.

-Ahmida, M. H. and Abuzogaya, M. H. (2009)The effects of oral administration of green tea and ginger extracts on serum and hepatic lipid content in rats fed a hyperlipidemic diet.Der Pharmacia Lettre. 1 (1), 56-64.

-Ali, H.B., Blunden, G., Tanira, O.M. and Nemmar, A. (2008) Some phytochemical, pharmacological and toxicological properties of ginger (Zingiberofficinale Roscoe), a review of recent research. Food Chem. Toxicol.46, 409–442.

-Baillie, J.K., Thompson, A.A.R., Irving, J.B., Bates, M.G.D., Sutherland, A.I., MacNee, W., Maxwell, S.R.J. and Webb, D.J. (2009) Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial. QJM. 102 (5), 341–348.

-Fascse, C.F. (1982) Enzymatic colorimetric method determination of total cholesterol in human serum. Clin Chem. 28, 901-907.

-Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem. 18 (6), 499-502.

-Fuhrman, B., Roseblate, M., Hayek, T., Coleman, R. and Aviram, M. (2000) Ginger Extract Consumption Reduces Plasma Cholesterol, Inhibits LDL Oxidation and Attenuates Development of Atherosclerosis in Atherosclerotic, Apolipoprotein E-Deficient Mice. J Nutr. 130, 1124-1131.

-Janero, D. (1998) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology & Medicine, 9, 515-540.

-Jellinger, P.S. (2000)Medical Guidelines for Clinical Practice for the Diagnosis and Treatment of Dyslipidemia and Prevention of Atherogenesis.AACE Lipid Guidelines, EndocrPract.6 (No. 2).

-Kamal, A., Thanaa, A. and Abd El-Twab, M. (2009) Oxidative markers, nitric oxide and homocysteine alteration in hypercholesterolimic rats, role of atorvastatine and cinnamon. Int J ClinExp Med. 2: 254–265.

-Leaf, D.A. (2008) Chylomicronemia and the chylomicronemia syndrome: a practical approach to management. Am J Med. 121 (1), 10-12.

-Lemhadri, A., Zeggwagh, N. A, Maghrani, M., Jouad, H. and Eddouks, M. (2004) Anti-hyperglycaemic activity of the aqueous extract of Origanumvulgare growing wild in Tafilalet region.Journal of Ethnopharmacology. 92 (2-3), 251-256.

-Lovri, J., Mesi, M., Macan, M., Koprivanac, M., Kelava, M. And Bradamante, V. (2008) Measurement of malondialdehyde (MDA) level in rat plasma after simvastatin treatment using two different analytical methods.PeriodicumBiologorum. 110 (1), 63–67.

-Lu, J., Zhang, K., Nam, S., Anderson, R. A., Jove, R. and Wen, W. (2010) Novel angiogenesis inhibitory activity in cinnamon extract blocks VEGFR2 kinase and downstream signaling. Carcinogenesis. 31(3), 481–488.

-Mahmood, S., Talat, A., Karim, S., Khurshid, R. and Zia, A. (2011) Effect of cinnamon extract on blood glucose level and lipid profile in alloxan induced diabetic rats. Pak J Physiol. 7 (1).

-Mannervik, B. (1985): Glutathione peroxidase. Methods Enzymol. 113, 490-495.

-Nagele, U., Hagele, E.O., Sauer, G., Wiedemann, E., Lehmann, P., Wahlefeld, W.A. and Gruber, W. (1984) Reagent for the enzymatic determination of serum triglycerides with improved lipolytic efficiency. J ClinChemClin Bio. 22, 165-174.

-Park, Y.S., Yoon, Y. andAhn, H.S. (2007) Platycodongrandiflorum extract represses up-regulated adipocyte fatty acid binding protein triggered by a high fat feeding in obese rats. World J Gastroenterol. 13 (25), 3493-3499.

-Pongnimitprasert, N. (2009) Atherosclerosis and NADPH Oxidase. Silpakorn U Sci Tech J. 3 (1), 13–24.

-Qin, B., Dawson, H., Polansky, M.M. and Anderson, R.A. (2009) Cinnamon extract attenuates TNF-alpha-induced intestinal lipoprotein ApoB48 overproduction by regulating inflammatory, insulin, and lipoprotein pathways in enterocytes. HormMetab Res. 41, 516–522.

-Sheng, X., Zhang, Y., Gong, Z., Huang, C. and Zang, Y.Q. (2008) Improved insulin resistance and lipid metabolism by cinnamon extract through Activation of peroxisome proliferator-activated receptors. PPAR Res PMID. 19096709.

-Singh, G., Maurya, S., DeLampasona, M.P. and Catalan, C.N. (2007)A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem. Toxicol.45, 1650–1661.

-Soliman, M.M., Attia, H.F., El-Shazly, S.A. and Saleh, O.M. (2012) Biomedical effects of cinnamon extract on obesity and diabetes relevance in Wistar rats. Am J BiochemMol Biol. 2, 133-145.

-Szlosarek, P.W. and Balkwill, F.R. (2003) Tumour necrosis factor?: a potential target for the therapy of solid tumours. The Lancet Oncology 4 (9), 565-573.

-Thomson, M., Al-Qattan, K.K., Al-Sawan, S.M., Alnaqeeb, M.A., Khan, I.and Ali M. (2002) The use of ginger (Zingiberofficinale Rosc.) as a potential anti-inflammatory and antithrombotic agent.Prostaglandins LeukotEssent Fatty Acids. 67 (6), 475-478.

-Warnick, G.R., Benderson, J.M. and Albers, J.J. (1982) Dextran sulphate-Mg2+ precipitation procedure or quantification of high density lipoprotein cholesterol. Clin Chem. 28 (6), 1379-1388.

-Xie, Z., Singh, M., and Singh, K. (2004) Differential regulation of matrix metalloproteinase-2 and -9 expression and activity in adult rat cardiac fibroblasts in response to interleukin-1β. J Biol Chem. 279**,** 39513–39519.

-Yadav, A.S. and Bhatnagar, D. (2007) Modulatory effect of spice extracts on iron-induced lipid peroxidation in rat liver. Biofactors.29, 147–157.

-Yang, R.L., Shi, Y.H., Hao, G,. Li, W.and Le, G.W. (2008) Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation bet ween Malondialdehyde andAtherogenic Index. J ClinBiochemNutr. 43 (3), 154-158.

Table 1: Effect of treatment of hyperlipidemic rats with either cinnamon (400 mg/kg body weight/day) or ginger (500 mg/kg body weight/day) individually or in a combination for 40 days on fasting serum lipids, tissue MDA, GPx, collagen-I, MMP-2 and TIMP-2. Values were expressed as Mean ± SEM (n=10)

	Normal Control	Hyperlipidemic Control	Cinnamon	Ginger	Cinnamon+Ginger
TAG (mg/dL)	62.22 ± 2.38	110.48 ± 1.75 ^	90.91 ± 2.46 *	93.51 ± 0.66 *	71.28 ± 1.78 *#a
Total Cholesterol (mg/dL)	116.50 ± 0.97	205.26 ± 1.99 ^	162.25 ± 2.56 *	164.11 ± 1.28 *	128.13 ± 0.71 *#a
LDL-C (mg/dL)	42.49 ± 0.58	31.18 ± 0.38 ^	36.93 ± 0.36 *	34.73 ± 0.49 *#	38.01 ± 0.70 *a
HDL-C (mg/dL)	61.62 ± 0.55	151.98 ± 2.34 ^	107.14 ± 2.66 *	110.68 ± 1.70 *	75.86 ± 1.18 *#a
MDA (µmol/mg protein)	1.14 ± 0.02	6.08 ± 0.09 ^	3.97 ± 0.09 *	4.16 ± 0.22 *	2.63 ± 0.10 *#a
GPx (µmol/mg protein)	44.31 ± 0.48	25.36 ± 0.99 ^	34.90 ± 0.84 *	35.90 ± 0.52 *	41.40 ± 0.31 *#a
Collagen I	0.11 ± 0.01	1.32 ± 0.11 ^	0.87 ± 0.01 *	0.79 ± 0.01 *#	0.49 ± 0.03 *#a
MMP-2	0.19 ± 0.01	1.49 ± 0.05 ^	0.82 ± 0.02 *	0.81 ± 0.03 *	0.45 ± 0.04 *#a
TIMP-2	1.36 ± 0.09	0.30 ± 0.03 ^	0.73 ± 0.02 *	0.67 ± 0.01 *#	1.05 ± 0.03 *#a

Values are expressed as mean ± SEM. Number of rats per group n = 10.

TAG, Triacylglycerol, LDL-C, Low density lipoprotein cholesterol, HDL-C, High density lipoprotein cholesterol, MDA, Malondialdehyde,GPx, Glutathione peroxidase, MMP-2, Matrix metalloproteinase 2, TIMP-2, Tissue inhibitor of metalloproteinase 2.

^ Significant difference from normal control at P < 0.05.
*** Significant difference from hyperlipidemic control at P < 0.05.**
 # Significant difference from cinnamon treatment at P < 0.05.
a….., Means of treated groups with different superscript are significantly different from ginger at P < 0.05.

Captions of the figures:

Figure (1): Lipids profile measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

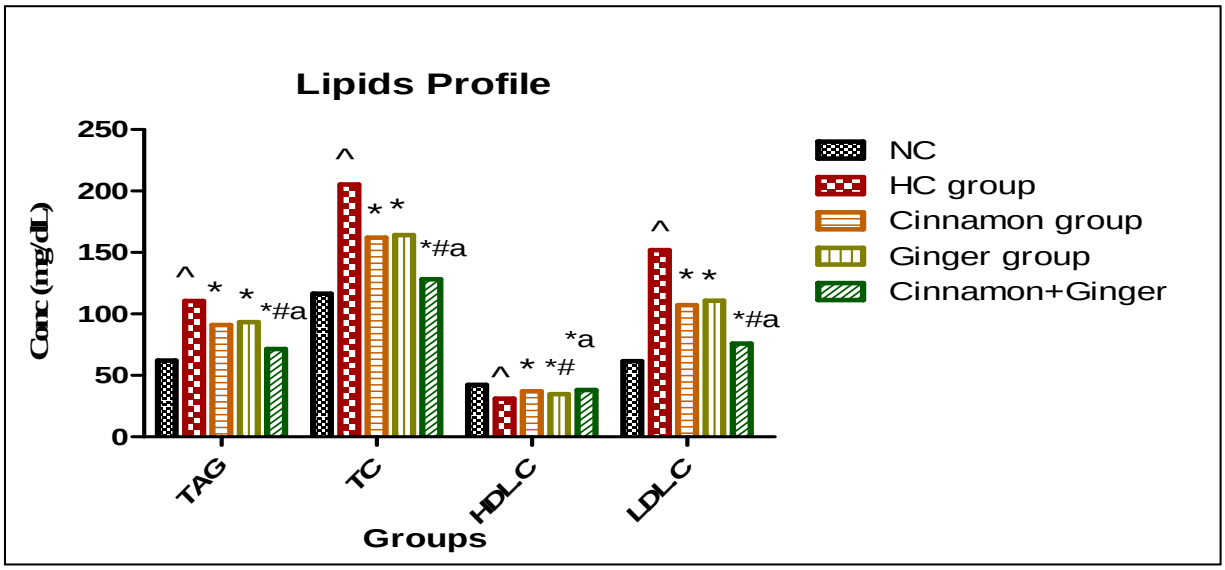
Figure (2): Malondialdehyde (MDA) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

Figure (3): Glutathione peroxidase (GPx) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

Figure (4): Collagen I measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

Figure (5): Matrix metalloproteinase-2 (MMP-2) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

Figure (6): Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.



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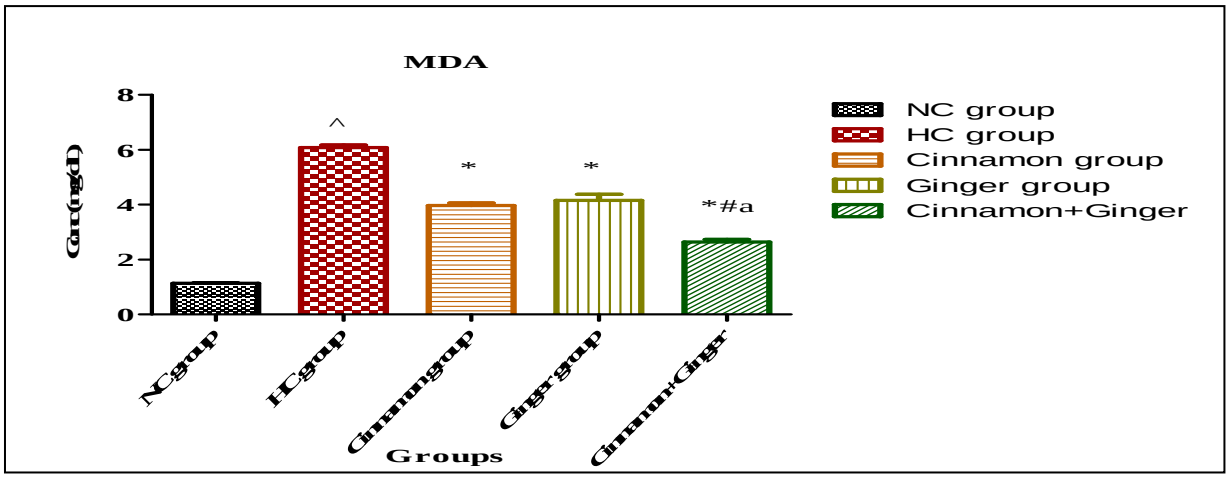

Figure (2): Malondialdehyde (MDA) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

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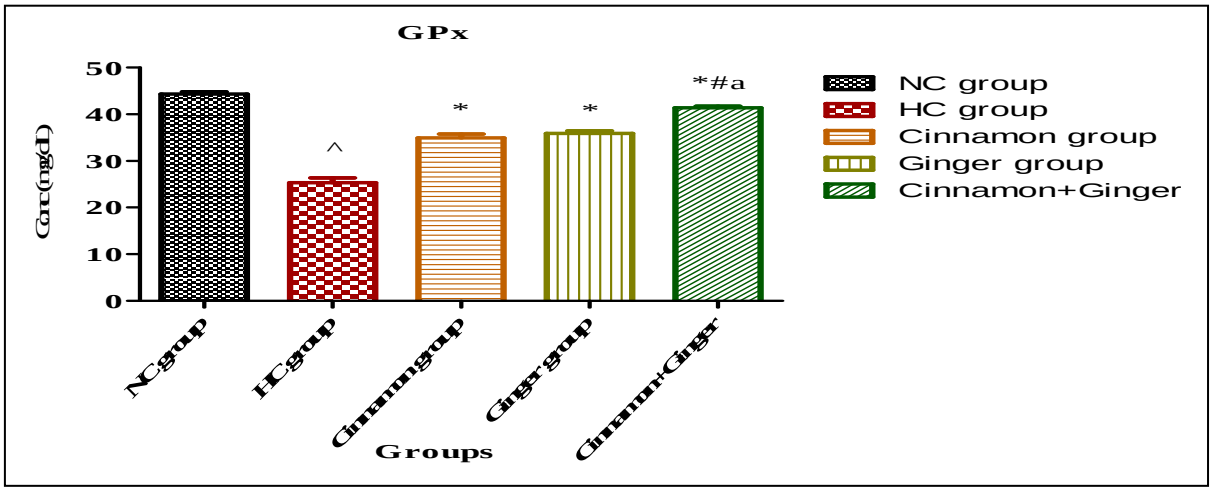

Figure (3): Glutathione peroxidase (GPx) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

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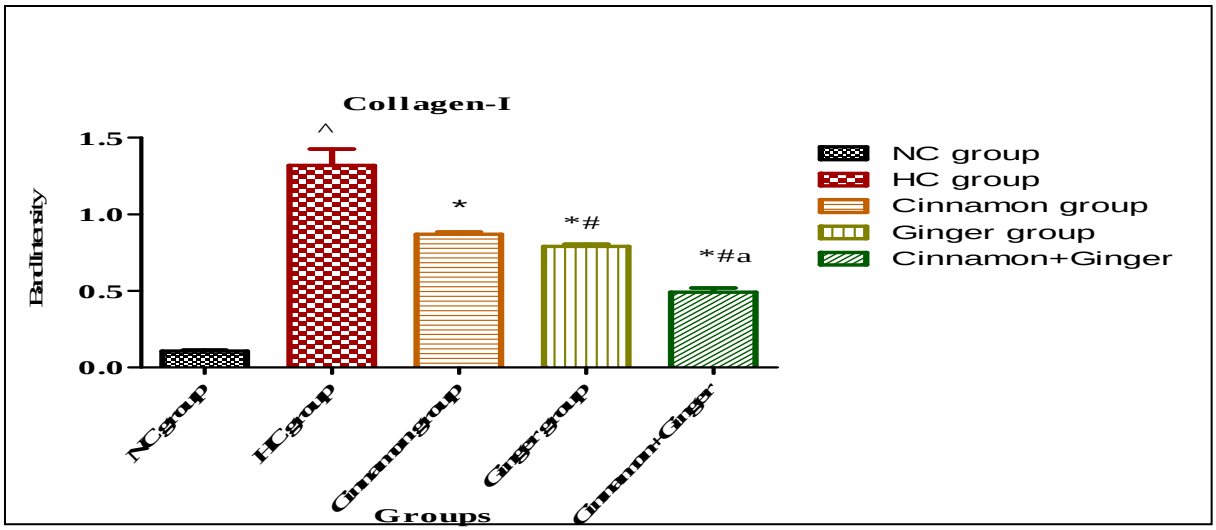

Figure (4): Collagen I measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

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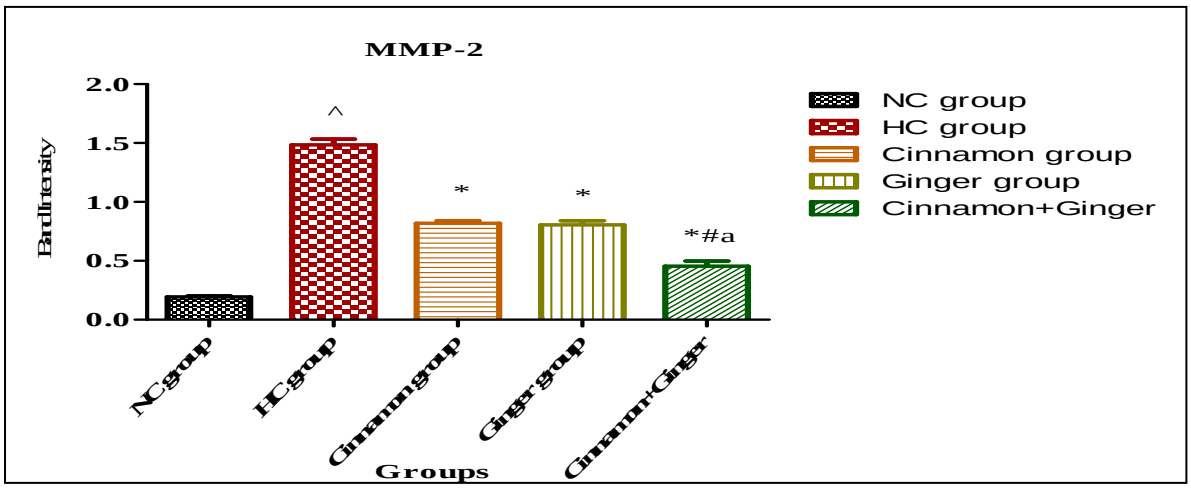

Figure (5): Matrix metalloproteinase-2 (MMP-2) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

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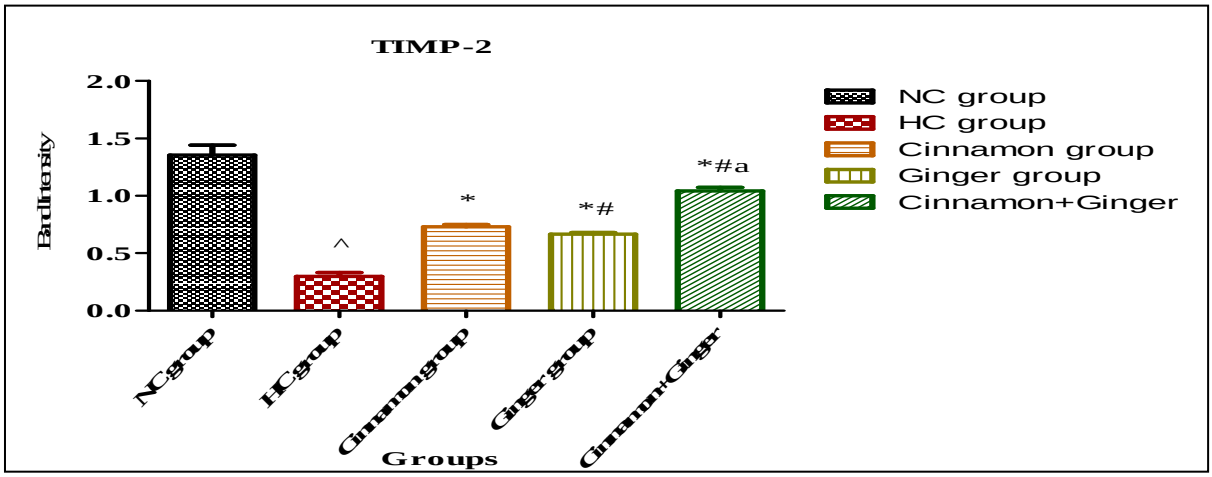

Figure (6): Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) measurementsin control healthy rats, HC rats, HC rats treated with cinnamon or ginger individually and in combination with each other for 40 days.

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