Oxidative Stress Markers and Homocysteine Alteration in Hyperlipidemic Rats: Role of Cinnamon and Gingerin Treatment of Coronary Artery Diseases

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Abstract

Hyperlipidemia is accused of being responsible for the development of coronary artery diseases, which could be treated with cinnamon and ginger.Lipid concentrations, Malondialdehyde (MDA), Nitrite (NO_2), Tumor Necrosis Factor Alpha (TNF-α), Homocysteine and histopathological examination were done in male albino rats weighing 220-250 gm body weight. 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.Hyperlipidemic control (HC) rats which fed on a HFD, developed hypercholesterolemia, hypertriglyceridemia, increased MDA, NO_2, TNF-α and homocysteine 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) levels, total cholesterol, LDL-C, MDA, NO_2, TNF-α. Also, a significant increase in HDL-C.Also, there was a significant difference in homocysteine in ginger group but, no significance in cinnamon group, when compared to HC rats. Moreover, the combined treatment caused a prominent significant difference between groups. Our conclusion is;hyperlipidemia could induce coronary artery diseases, and that combined treatment had a great protective role against the metabolic abnormalities, especiallyhomocysteine.

KEYWORDS: Hyperlipidemia, Cinnamon, Ginger, TNF alpha, Homocysteine.

Introduction:

Hyperlipidemia is the condition in which the level of plasma lipids, primarily cholesterol and triacylglycerol, are higher than the normal range. Hyperlipidemia is an important risk

factor in atherosclerosis, which can lead to coronary artery disease (CAD). Atherosclerosis is the accumulation of lipid, inflammatory cells, and fibrous tissue in the intima, which causes intimal thickening of large and mid-sized arteries. The clinical manifestations differ depending on the circulatory bed affected. 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 is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. Where, these radicals can start chain reactionsthat can cause damage or death to the cell. 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 antidiarrheal, antioxidant and antimicrobial potential ***(Singh et al., 2007).*** Cinnamon was classified as an 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. Increased cardiac contractile force and beating rate was also exerted by cinnamaldehyde, which is present in cinnamon. Dietary cinnamon increases biliary secretion of cholesterol and phospholipids without affecting the bile content***(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 activates peroxisome proliferator activated receptor gamma (PPAR-γ) resulting in improved insulin resistance and reduced fasted LDL-C, thereby managing obesity related hyperlipidemia and also increases NO levels, which is a potent vasodilator. 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. Furthermore, gingerol isolated from Zingiber inhibits platelet function by inhibiting thromboxane formation. 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).***

The objective and 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), NO or NO_2, tumor necrosis factor alpha (TNF-α) and homocysteine as well as investigating of the histological changes in cardiac tissues of hyperlipidemic rats.

Materials and Methods

Animals:

One hundred male Albino rats weighing 160- 180 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 and 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 of Zingiberofficinale 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, TNF-α and homocysteine.

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 cut into two equal halves by a scalpel. One half of it was rapidly placed in 10% neutral buffered formalin for histopathological staining and the other half was rapidly frozen in liquid nitrogen and stored at -80^{o}C for further determination of MDA and NO_2spectrophotometrically.

Histological studies

The selected paraffin blocks for histological staining were sectioned (2-µm thickness) and stained with Hematoxylin and Eosin (H&E) stain***(Drury and Wallington, 1980).***

Methods

Serum TGs were estimated by GPO-POD enzymatic method ***(Siedel et al., 1983)*** using a Biocon kit (India). TC concentration was determined utilizing enzymatic colorimetric CHOD-PAP method ***(Richmond, 1973)*** using diagnostic kit (Egypt). HDL−C was determined by the same method after the precipitation of very low density lipoprotein cholesterol (VLDL-C) and LDL-C ***(Burstein et al., 1970)***, 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 was determined colorimetrically according to the method of **(Janero, 1998).**

Total tissue nitrite (initial nitrite plus the nitrite produced from sodium reduction) was estimated colorimetrically at 540 nm according to Griess reaction, from a standard curve of sodium nitrite ***(Schmidt et al., 1989).***

Serum TNF-α was determined according to ***(Chen et al., 1998)***by solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) using rat TNF-α kits (RayBiotech, USA) and a microtiter plate reader capable of reading at 450 nm.

Serum homocysteine was determined according to ***(Vijayanet al., 2013)***by a sandwich enzyme immunoassay (ELISA) using rat homocysteine kits (RUO, USA)and a microtiter plate reader capable of reading at 450 nm.

Statistical analysis:

Statistical analysis and correlations were performed using SPSS program version 16.

Data are presented as Mean ± standard error mean (SEM). Student "t" test and analysis of variance (ANOVA) followed by Bonferroni's post hoc analysis were used for comparisons between groups. Moreover, correlations between different parameters were evaluated by Pearson's correlation (*r*). The level of statistical significance was set at probability $P < 0.05$.

Results and Discussion:

Lipids profile:

Triacylglycerols (TAG):

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):

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):

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):

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):

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).

Nitrite (NO_2):

Figure (3) showed that HC rats recorded a significant increase in nitrite (NO_2) levels as compared to normal control group by 451.43 % (P<0.05).

While in treated groups, there was a significant decrease in serum NO_2 levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 54.40 % and 55.44 % 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 32.56 %, cinnamon treatment group by 34.09 % or hyperlipidemic control group by 69.95 % (P<0.05).

Coronary Artery Diseases Markers:

Tumor Necrosis Factor Alpha (TNF-α):

Figure (4) showed that HC rats recorded a significant increase in Tumor Necrosis Factor Alpha (TNF-α)levels as compared to normal control group by 262.38 % (P<0.05).

While in treated groups, there was a significant decrease in serum TNF-α levels in cinnamon group and ginger group as compared to hyperlipidemic control group by 23.02 % and 29.26 %. Moreover, there was a significant decrease in 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 24.03 %, cinnamon treatment group by 30.19 % or hyperlipidemic control group by 46.26 % (P<0.05).

Homocysteine:

Figure (5) showed that HC rats recorded a significant increase in Homocysteinelevels as compared to normal control group by 111.35 % (P<0.05).

While in treated groups, there was a significant decrease in serum Homocysteine levels in ginger group as compared to hyperlipidemic control group by 19.09 %. On the other hand, there was no significant difference in cinnamon group as compared to hyperlipidemic control group. Moreover, there was a significant decrease in ginger group as compared to cinnamon group by 11.34 %. Furthermore, there was a significant decrement between ginger and cinnamon in combinationas compared to either ginger treatment group by 25.31 %, cinnamon treatment group by 33.78 % or hyperlipidemic control group by 39.57 % ($P<0.05$).

Correlations:

This study showed that there was a positive correlation between serum total cholesterol and LDL-C **Figure (6).**

Furthermore, TAG was negatively correlated with serum TNF-α level **Figure (7).**

Histological Studies:

Light microscope examination of hematoxylin and eosin (H&E) stained sections of the cardiac muscle of the normal control (NC) group rats revealed that the cardiac muscle fibers are connected end to end by intercalated discs appeared with central and oval vascular nuclei **(Figure 8).**

Examination of hematoxylin and eosin stained sections of the cardiac muscle of the hyperlipidemic control (HC) group rats showed section in the cardiac muscle fibers showing indistinct and distorted striation fibers (thin arrows) and fat cells (thick arrows) **(Figure 9).**

The cardiac muscle fibers of rats receiving cinnamon treatment alone showed hypertrophied myofibrils (thick arrows), indistinct and distorted striation fibers (thin arrows), fat cells (crossed arrows) and blood vessels inside myofibrils (arrow heads) in hematoxylin and eosin stained sections **(Figure 10).**

The cardiac muscle fibers of rats receiving ginger treatment alone showed normal striations and normal contour fibers (thin arrow), hypertrophied and indistinct striations (thick arrows), many intraepithelial lymphocyte (crossed arrows), distorted fibers (medium size arrows) and fat cells (arrow heads) in hematoxylin and eosin stained sections **(Figure11).**

Histological examination of hematoxylin and eosin stained sections of the cardiac muscle fibers of rats receiving ginger and cinnamon in combination revealed regular striations and myofibrils (normal arrows), central vesicular nucleus (crossed arrows) and normal fibroblasts (arrow heads) **(Figure 12).**

Discussion:

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.

On the other hand, in our study revealed that there is a significant decrease in TAG, TC and LDL-C either in cinnamon or ginger groups or combined treatment when compared to hyperlipidemic control group.

Amin and Abd El-Twab, (2009)confirmed that cinnamon treatment might have a direct role in lipid metabolism, where *cinnamon* bark prevents hypercholesterolemia (HC) and hypertriglyceridemia and lowers the free fatty acids and triglycerides (TG) levels of type 2 diabetic subjects by its strong lipolytic activity. Cinnamate, a phenolic compound found in cinnamon, lowers cholesterol levels in high fat-fed rats by inhibiting hepatic HMG-CoA reductase activity.

Furthermore,***Mathew et al., (2003)*** demonstrated that ginger inhibits the hydroxymethylglutaryl Co A (HMG-Co A) reductase which is a rate limiting enzyme for cholesterol biosynthesis (like that of statins). Also, it promotes excretion and impairs absorption of cholesterol.

Our result revealed that there is a significant increase in malondialdehyde (MDA) levels in hyperlipidemic control group when compared to control healthy group.

On the other hand, our results revealed that, there is a significant decrease in MDA levels in either cinnamon or ginger or combined group when compared to hyperlipidemic control group.

Su et al., (2007)stated that cinnamon had strong antioxidant activity, that cinnamon contains high level of phenolic groups. Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation. Cinnamon essential oil was able to reduce lipid peroxidation in the β-carotene-linoleic acid system. Therefore, MDA concentration is decreased.

Afshari et al., (2007)study is in line with our study that, administering ginger powder caused significant decreases in thiobarbituric acid reactive substances (TBARS) levels. Thus, an augmentation of plasma antioxidant capacity decreases plasma-free radical and lipid peroxidation (MAD), when consuming herbal extracts containing antioxidants, which lead to a decrease in the malondialdehyde level accompanied by significant increases in the activities of GSH-Px and SOD.

Our results demonstrated that, there is a significant increase in nitrite levels (NO2) in hyperlipidemic control group when compared to control healthy group.

On the other side, our results elucidated that, there is a significant decrease in nitrite levels (NO2) in either cinnamon or ginger or combined treatment when compared to hyperlipidemic control group.

Our study is in agreement with***Brahmachari et al., (2009)***who illustrated that, cinnamon contains three major compounds (cinnamaldehyde, cinnamyl acetate and cinnamyl alcohol), which are converted into cinnamic acid by oxidation and hydrolysis, respectively. In the liver, this cinnamic acid is β-oxidized to benzoate that exists as sodium salt (sodium benzoate; NaB) or benzoyl-CoA. NaB attenuates the expression of inducible NO synthase (iNOS) and proinflammatory cytokines in microgalia, astrocytes, and macrophages.

Furthermore***, Li et al., (2011)***revealed that ginger which has [6]-dehydroshogaol, [6]-shogaol and 1-dehydro-[6]-gingerdione as active constituents were shown to be potent inhibitors of nitric oxide (NO) synthesis in activated macrophages. As ginger's main compound [6], gingerol may, have an antioxidant activity against linoleic acid autoxidation and phospholipids peroxidation.

In our results, there is a significant increase in tumor necrosis factor alpha (TNF-α) in hyperlipidemic control group as compared to control healthy group.

On the other hand, in our results, there is a significant decrease in TNF-α in either cinnamon or ginger or combined treatment as compared to hyperlipidemic control group.

This result is supported by***(Gordon, 2007***) who demonstrated that, cinnamon water extract (CWE) inhibits expression of TNF-α. LPS-induced nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) degradation and mitogen-activated protein kinases (MAP) kinase phosphorylation in macrophages was strongly inhibited by the polyphenol-rich CWE fraction. Oral administration of CWE decreased serum levels of LPS-induced TNF-α and IL-6.

Our data are in harmony with***Tripathi et al., (2008)***who illustrated that, the anti-inflammatory effects of ginger might be related to its ability to inhibit prostaglandin and leukotriene biosynthesis. Gingerols actively inhibit arachidonate 5-lipoxygenase, an enzyme of leukotriene biosynthesis. [8]-gingerol, but not [6]-gingerol, was shown to inhibit cyclooxygenase-2 (COX-2) expression, Also it was reported that ginger extract suppresses the activation of tumor necrosis factor α (TNF-α) and expression of COX-2 in human synoviocytes.

In our results, there is a significant increase in homocysteine in hyperlipidemic control group as compared to control healthy group.

On the other hand, in our results, there is a significant increase in homocysteine in ginger or in combined treatment as compared to hyperlipidemic control group. But, there is no significant difference in cinnamon treatment as compared to hyperlipidemic control group.

Our results are consistent with***Tyagi et al (2009)***who revealed that, cinnamon control homocysteine (Hcy) level, may be through influencing activity of key enzymes in Hcy metabolism or cystathio-nine b-synthase (CBS) where cinnamon have insulin-like action and exert a blood glucose suppressing effect by improving insulin sensitivity, signaling and synthesis, where insulin inhibits hepatic CBS activity or slowing absorption of carbohydrates in the small intestine.

Vasanthiand Parameswari, (2010)was in harmony with our study who confirmed that, consumption of ginger extract inhibited the progression of aortic atherosclerosis in atherosclerotic, apolipoprotein-E deficient mice. As hyperhomocysteinemia is a risk factor for cardiovascular disease. Plasma cysteine predicts progression of atherosclerosis. So, cysteine levels are decreased due to the gingerol effect.

Conclusions:

Our study revealed that HC rats developed atherosclerosis and caroronary artery diseases. They also resulted in increased MDA, NO_2, TNF-α and homocysteine. Our results demonstrated that treatment with either cinnamon or ginger alone is able to improve the hyperlipidemia, and to decrease MDA, NO_2, TNF-α and a minimal effect in homocysteine level but within a minimal effect. Moreover, the combined treatment with cinnamon and ginger was able to improve the hyperlipidemia and had a great effect on attenuating the coronary artery diseases by improving thehyperlipidemia, and decreasing theMDA, NO_2, TNF-α and homocysteine but within a greater effect than treatment with either cinnamon or ginger alone. Furthermore, the histopathological examination revealed the marked improvement of cardiac tissues when treated with combined treatment with cinnamon and ginger.

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Conflict of interest statement
The authors declare that they have no competing interests.

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Figure (2): Malondialdehyde (MDA) measurementsin rats
Figure (3): Nitrite (NO2) measurementsin rats
Figure (4): Tumor necrosis factor alpha (TNF-α) measurementsin rats
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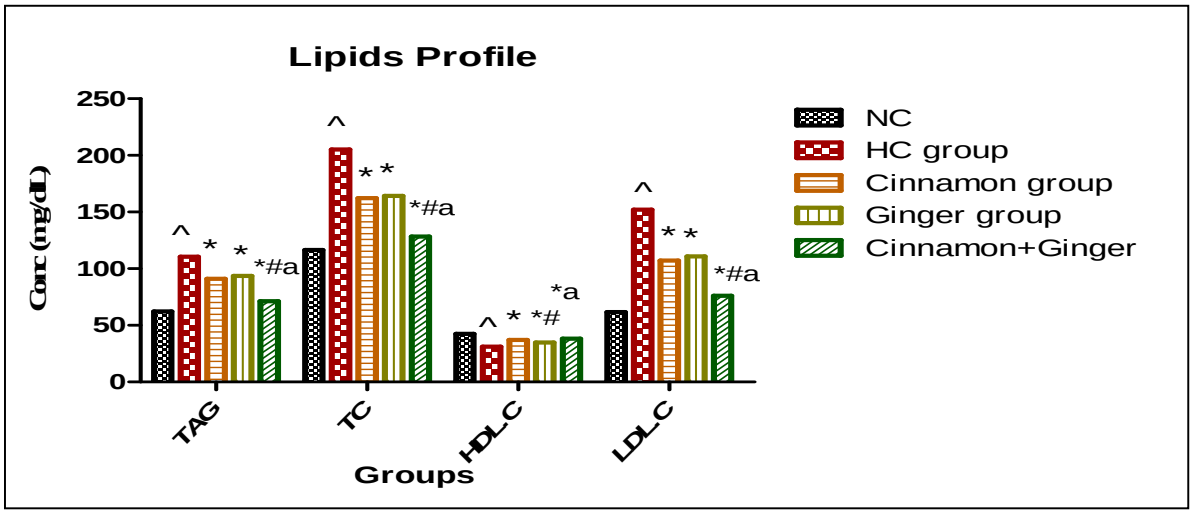


Figure (1): Lipids profile measurementsin rats

^ 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.

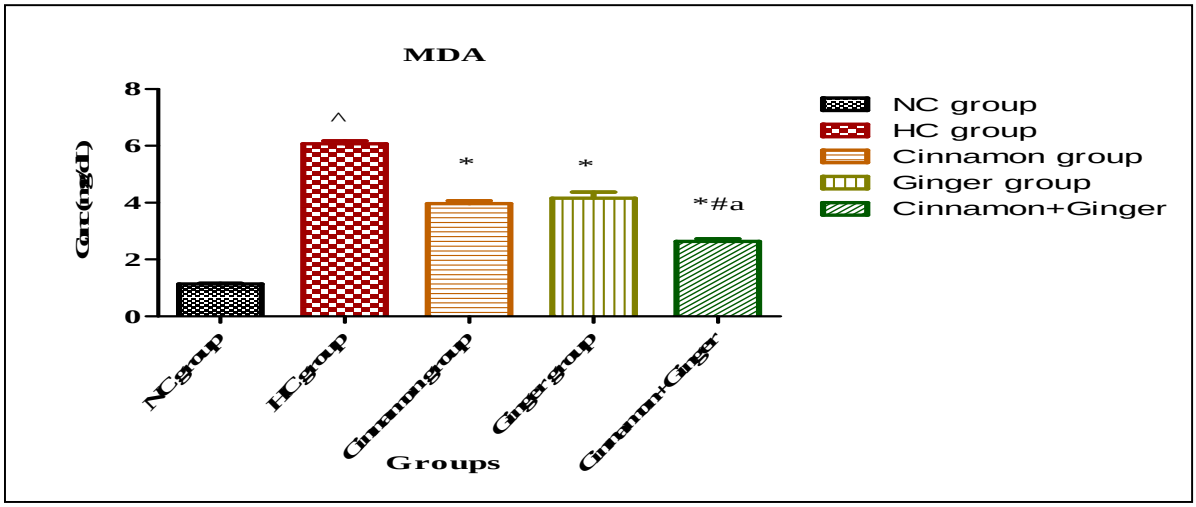


Figure (2): Malondialdehyde (MDA) measurementsin rats

^ 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.

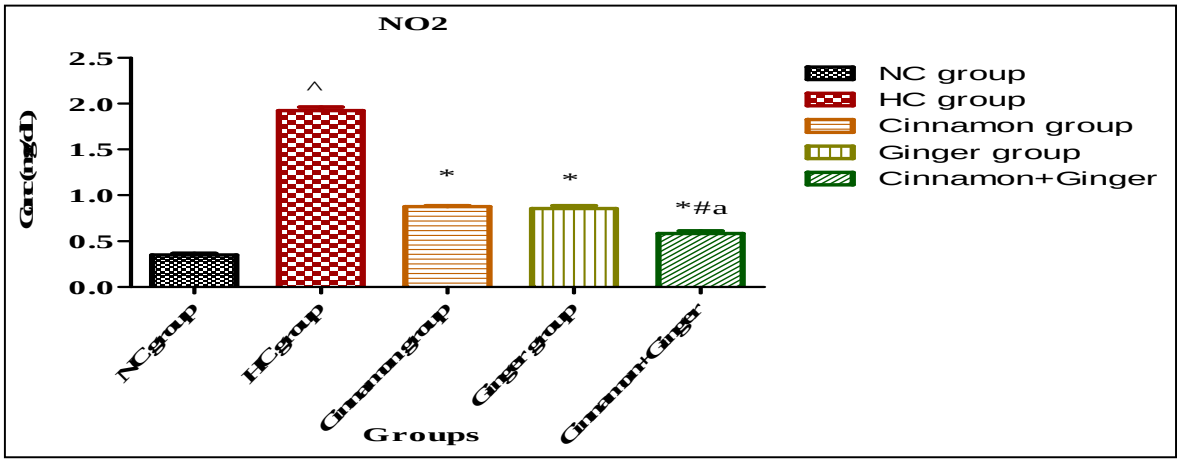


Figure (3): Nitrite (NO2) measurementsin rats

^ 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.

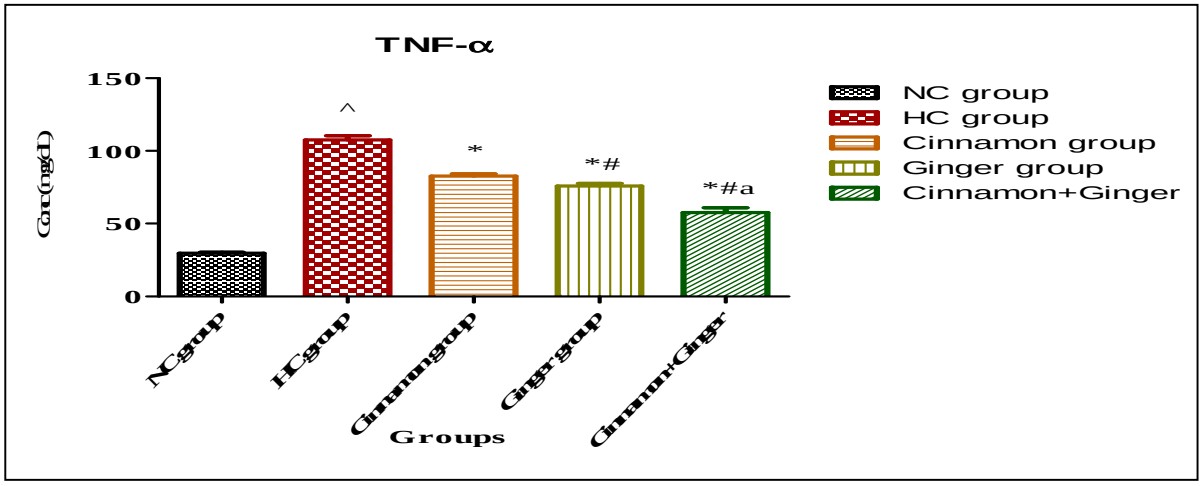


Figure (4): Tumor necrosis factor alpha (TNF-α) measurementsin rats

^ 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.

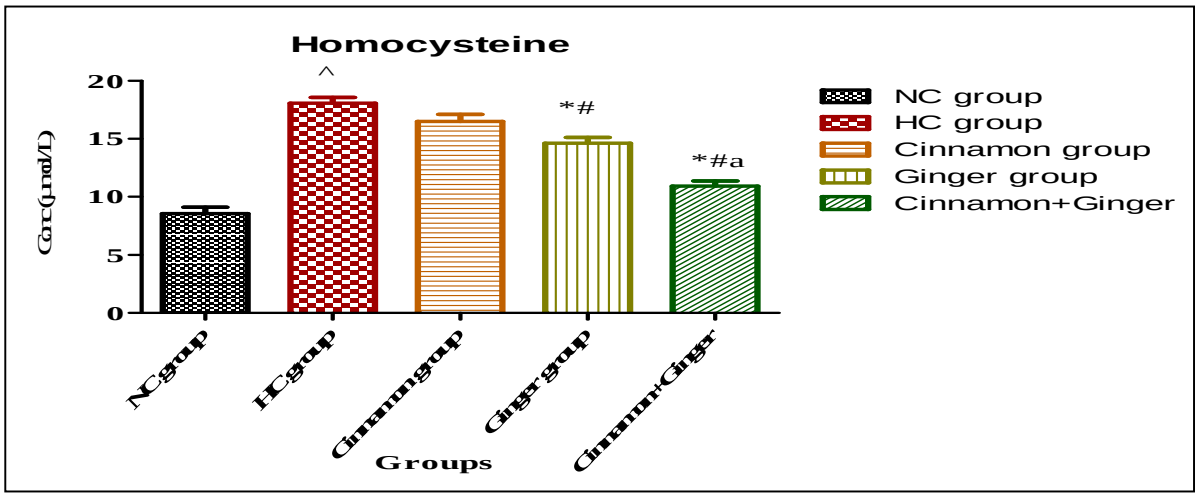


Figure (5): Homocysteine measurementsin rats

^ 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.

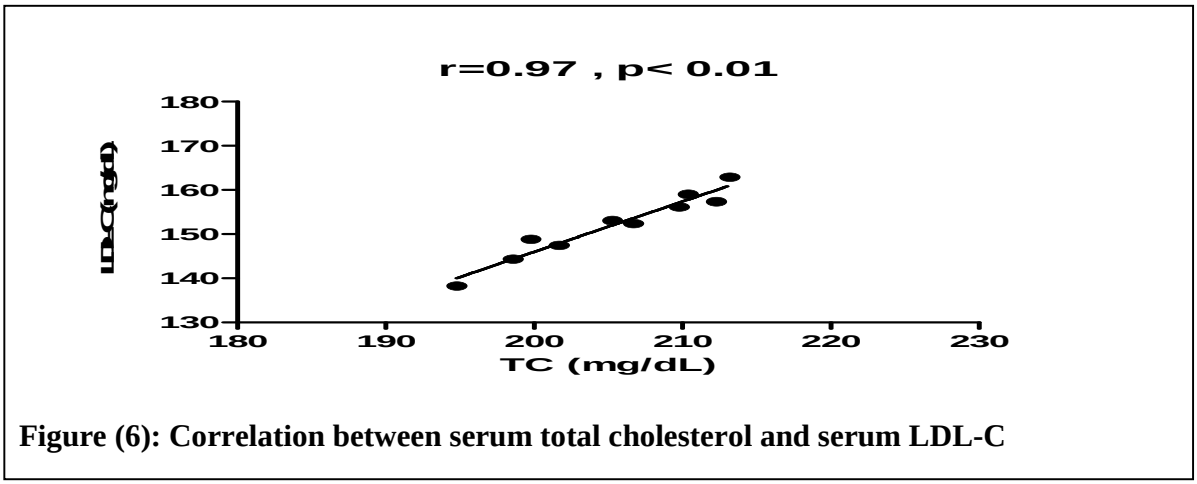


Figure (6): Correlation between serum total cholesterol and serum LDL-C

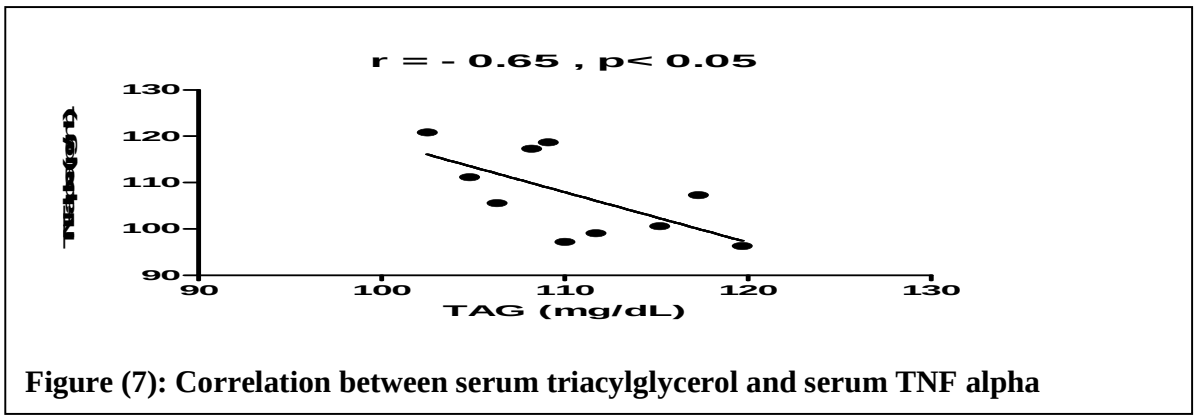
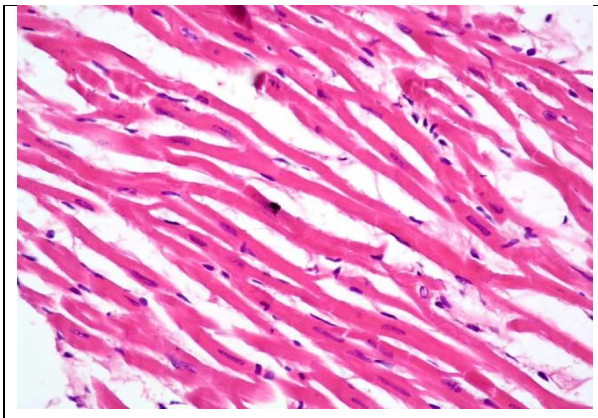
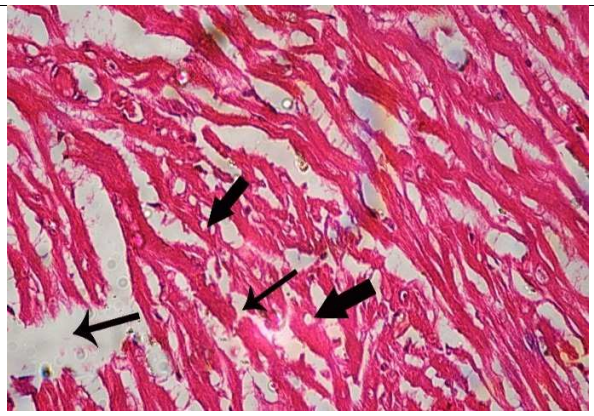


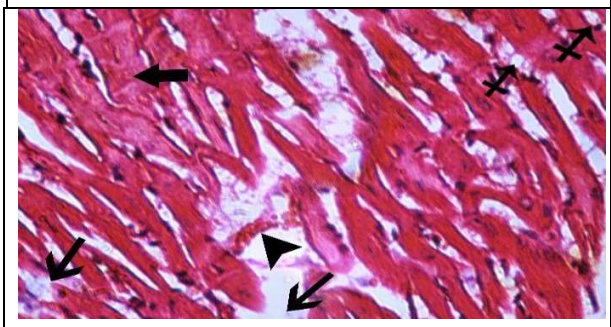
Figure (7): Correlation between serum triacylglycerol and serum TNF alpha



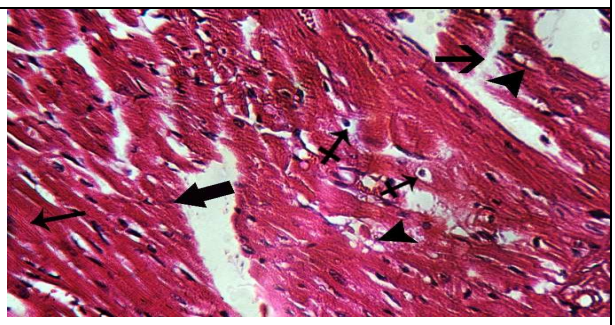
Figure(8): A photomicrograph of a section in cardiac muscle of normal control (NC) rats showing branching anastomosing cardiac muscle fibers connected end to end by intercalated discs containing central and oval vascular nuclei (H &E ×400).



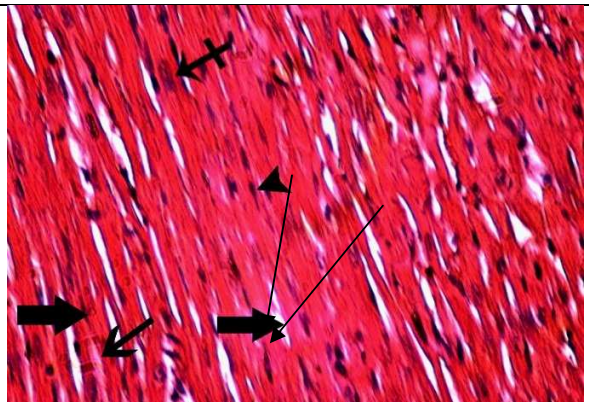
Figure(9): A photomicrograph of a section in cardiac muscle of hyperlipidemic control (HC) rats revealing indistinct and distorted striation fibers (thin arrows) and fat cells (thick arrows) (H & E ×400).



Figure(10): A photomicrograph of cardiac muscle of rats receiving cinnamon treatment showing hypertrophied myofibrils (thick arrows), indistinct and distorted striation fibers (thin arrows), fat cells (crossed arrows) and blood vessels inside myofibrils (arrowheads) in hematoxylin and eosin stained sections(H & E × 400).



Figure(11): A photomicrograph of a section in cardiac muscle of rats receiving ginger treatment showing normal striations and normal contour fibers (thin arrow), hypertrophied and indistinct striations (thick arrows), many intraepithelial lymphocyte (crossed arrows), distorted fibers (medium size arrows) and fat cells (arrow heads) (H & E × 400).



Figure(12): A photomicrograph of a section in cardiac muscle of rats receiving ginger and cinnamon in combination revealed regular striations and myofibrils (normal arrows), central vesicular nucleus (crossed arrows) and normal fibroblasts (arrow heads) (H & E × 400).