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Original Article

Oxidative markers, nitric oxide and homocysteine alteration in hypercholesterolimic rats: role of atorvastatine and *cinnamon*

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Abstract: To investigate the effects of atorvastatin and *cinnamon* on serum lipid profile, oxidative stress, antioxidant capacity, hepatic enzymes activities, nitric oxide (NO) as well as homocysteine (Hcy) in hypercholesterolemic rats, 48 male albino rats, weighing 130-190 gm were divided into 2 groups, normal group fed on basal rat chow diet (n=12) and high cholesterol group (HCD) were fed on 1% cholesterol-enriched diet for 15 day (n=36). Hypercholesterolemic rats were divided into 3 subgroups (n=12 for each) fed the same diet and treated with atorvastatine (HCD+Atorvastatin) or *cinnamon* extract (HCD+cinnamon) or none treated (HCD) for 3&6 weeks. Serum triglycerides (TG), Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), ALT, AST, NO, Hcy, hepatic reduced glutathione (GSH), Malondialdehyde (MDA) and antioxidant enzymes, Superoxide dismutase (SOD) and catalase activity were measured. Results showed that HCD increased significantly TG, TC, LDL-C, ALT, AST, Hcy and hepatic MDA, while lowered significantly antioxidant enzyme activities and NO levels. Atorvastatin therapy significantly increased HDL-C, NO and antioxidant activity while decreased LDL-C, MDA and Hcy concentrations. Serum TG, TC, LDL-C, ALT, AST and hepatic MDA levels were significantly lowered meanwhile, serum HDL, NO values and hepatic antioxidant activities were significantly, higher in *cinnamon*-treated than untreated group. These results indicate that lipid abnormalities, oxidative injury and hyperhomocystienemia were induced by HCD and this study recommend that administration of atorvastatine or *cinnamon* provided protection against the lipemic-oxidative disorder and act as hypocholesterolemic, hepatoprotective agent and improve cardiovascular function through modulation of oxidative stress, NO and Hcy.

Key words: Hypercholesterolemia, homocystein, nitric oxide, oxidative stress atorvastatin, *cinnamon*

Introduction

The liver plays a central role in balancing cholesterol from all sources and regulating plasma LDL levels [1]. The hepatic pool of cholesterol is derived from local biosynthesis and from chylomicron remnants and lipoproteins. Cholesterol biosynthesis is regulated by the rate-limiting enzyme HMG CoA reductase, which catalyzes production of mevalonic acid from HMG CoA and represents the therapeutic target for statins [2].

Hypercholesterolemia (HC) is characterized by coronary endothelial dysfunction. It may also promote ischemic tissue damage by enhancing the vulnerability of the microcirculation to the deleterious effects of ischemia and other inflammatory stimuli [3]. Also there was intracellular lipid accumulation in cardiomyocytes and several alterations in the structure and properties of the myocardium in experimental rats.

One mechanism that may underlie the abnormal coronary vascular function in HC is an alteration in the oxidative status accompanied by increased production of several oxidants such as peroxynitrites, and oxidative end-products such as PGF2-a isoprostanes and increased oxidation of LDL cholesterol [4]. Furthermore, a shift in oxidative status with a

decrease in NO and an increase in oxygen radicals may have a deleterious effect on vascular permeability. Thus, these mechanisms may play a pivotal role in inducing abnormalities of both myocardial perfusion and vascular permeability associated with HC.

In the hypercholesterolemic state, the net result of combined oxidative and nitrosative stress is a pro-inflammatory phenotype that is manifested as, enhanced leucocyte trafficking, and increased vascular permeability [5].

The biological effects of free radicals are controlled in vivo by a wide range of antioxidants such as vitamins E, C, glutathione and antioxidative enzymes as; SOD and GSH-Px both detoxify hydrogen peroxides and converts lipid hydroperoxides to non-toxic alcohols [6].

HMG-CoA reductase inhibitors, known as statins, are widely used to lower LDL-C. The beneficial effects of statins on coronary artery disease are not only related to plasma cholesterol levels, but also pleiotropic effects in addition to lipid-lowering properties [7] that include reduction of plaque thrombogenicity, inhibition of cellular proliferation and improvement of endothelial function. Statin therapy inhibits the production of cytokines in the endothelium and reduces free-radical production in the vascular wall.

NO is a potent vasodilator in the vasculature. It inhibits platelet adherence and regulates endothelial permeability to lipoproteins. NO is produced from the amino acid L-arginine by nitric oxide synthase (NOS), Statins increased endothelial NO production and decreased endothelin-1 expression, factors favorable to improve endothelial function. Also statins upregulate endothelial NOS expression. Pravastatin increases bioavailability of NO in atherosclerotic arterial walls and activates cNOS independently of its cholesterol-lowering effect [8].

Herbs have been the basis for many medicinal therapies, among these herbes *cinnamon* belongs to genus *Cinnamomum*, family *Lauraceae* which are distributed in India, Egypt, China, Srilanka and Australia. *Cinnamon* leaves and bark are used extensively as spices in food or to produce essential oils. In ancient Egypt *cinnamon* was used as medicinally and as flavoring for beverages, it was also used in embalming, where body cavities were filled with spiced preservatives. The plant has a hot taste and emits a spicy odor when crushed [9]. Previous studies on biochemical activities from *Cinnamon* were mainly focused on its essential oils [10] which included antioxidant, antimicrobial activity [11] and antidiarrhoeal activity, where irradiation of *cinnamon* did not affect the antioxidant potential of the *cinnamon* compounds.

In India and Europe *cinnamon* was traditionally taken as a warming herb for cold conditions. The herb stimulates circulation, especially in the fingers and toes. Moreover, *cinnamon* extract has a regulatory role in blood glucose level and lipids [12]. Thus the aim of this study was to determine the effect of *cinnamon* and atorvastatine on markers of oxidative stress, antioxidant capacity and endothelial function indicators, NO and Hcy in hypercholesterolemic rat.

Materials and methods

Diets

In this experiment we used normal rat chow diet and the special diet high in cholesterol where cholesterol was purchased from Sigma Company (United Kingdom) for induction of HC in rats. HC was induced by addition of cholesterol powder (1%) 1gm cholesterol /100 gm diet, bile salts 0.25% and beef tallow 4% [13] to the standard normal diet for 15 days before the period of the start of treatment.

Experimental animals

White male albino rats (*Rattus norvegicus*) weighing about 130-190g were kept under observation for about 2 W. before the onset of the experiment to exclude any intercurrent infection. The chosen animals were separately housed in stainless steel cages at normal atmospheric temperature 25±5°C as well as under good ventilation and received water and standard balanced diet. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt.

Our study was carried out in accordance with the guidelines of Beni Suef University for animal use and these animals were used for experimental study.

Experimental treatments and drugs

Table 1. Effect of atorvastatin and *Cinnamon* for 3 and 6 weeks from treatments on serum lipid contents and liver enzyme activities in HCD fed rats

Parameters		Normal	HCD	HCD + Atorvastatin	HCD + *cinnamon*
TC (mg/dl)	3W	70.68±3.79^{f}	86.16±4.78^{ab}	81.73±3.24^{bc}	77.26±5.24^{cde}
	6 W	71.81±3.11^{ef}	91.50±9.26^{a}	78.75±5.21^{cd}	76.75±5.46^{cdef}
TG (mg/dl)	3W	54.20±3.85^{e}	70.47±10.12^{ab}	66.316±4.96^{bc}	64.72±5.55^{bcd}
	6 W	57.77±5.64^{de}	74.75±13.91^{a}	62.70±5.35^{bcd}	58.77±4.21^{cde}
LDL (mg/dl)	3W	28.83±6.76^{d}	50.69±5.54^{a}	41.28±5.45^{b}	38.35±6.43^{bc}
	6 W	32.02±6.25^{cd}	57.57±9.42^{a}	36.20±8.78^{bcd}	34.96±7.21^{bcd}
HDL (mg/dl)	3W	31.03±3.84^{a}	21.37±2.60^{d}	27.19±3.17^{bc}	26.30±2.08^{c}
	6 W	28.17±4.35^{abc}	19.54±3.01^{d}	30.35±4.69^{ab}	30.05±2.56^{ab}
ALT (U/L)	3W	10.17±1.57^{c}	14.17±4.17^{ab}	11.75±3.57^{bc}	12.08±2.15^{abc}
	6 W	10.25±2.68^{c}	15.29±2.40^{a}	12.33±3.28^{abc}	11.67±3.63^{bc}
AST (U/L)	3W	33.00±7.87^{c}	46.17±7.88^{ab}	39.17±4.62^{bc}	39.67±6.055^{bc}
	6 W	33.67±4.72^{c}	50.66±8.92^{a}	39.83±5.81^{bc}	39.33±4.97^{bc}

Means have different letters indicate significant variation at ($P< 0.05$), while the same letters indicate non significant variation.

Table 2. Effect of atorvastatin and *Cinnamon* for 3 and 6 weeks from treatments on NO, Hcy and lipid peroxidation in HCD fed rats

		Normal	HCD	HCD + Atorvastatin	HCD + *cinnamon*
S NO (μ mol/ml)	3W	0.81±0.14^{ef}	0.72±0.11^{g}	0.89±0.08^{cde}	0.94±0.03^{abcd}
	6 W	0.87±0.04^{de}	0.70±0.06^{g}	0.95±0.04^{abcd}	1.027±0.11^{a}
S Hcy (Mmol/L)	3W	9.77±0.95^{e}	17.51±2.65^{a}	12.92±1.13^{c}	12.05±0.80^{bc}
	6 W	9.67±1.04^{e}	17.81±2.67^{a}	14.86±1.73^{b}	13.55±1.53^{bc}
MDA (n mole/g)	3W	14.18 ±2.51^{c}	20.33±4.72^{b}	17.50±2.59^{bc}	15.35 ±2.91^{c}
	6 W	15.01±0.90^{c}	24.33±3.79^{a}	16.13±3.83^{c}	14.38±3.82^{c}

Means have different letters indicate significant variation at ($P< 0.05$), while the same letters indicate non significant variation.

Aorvastatin was purchased from Delta pharma S.A.E) and *cinnamon* barks were purchased from local market.

Preparation of cinnamon extract

Cinnamon bark (*Cinnamomum zeylanicum*) was purchased from the local market at Egypt. The bark was dried and finely powdered in a mechanical mixer. 10 g of finely-powdered cinnamon was weighed and mixed with 100 ml of water and kept in a water bath at 60°C for two hours and filtered [14] and [15]. This extract was diluted with water (1:10) and was administered orally to rats at dosage of 20mg/day/rat.

Experimental design and animal grouping

The experiment continued for 5-8 W. and divided into 2 periods, one was induction of HC and the other was treatment period.

Table 3. Effect of atorvastatin and *Cinnamon* for 3 and 6 weeks from treatments on hepatic antioxidant status in HCD fed rats

Antioxidant activity		Normal	HCD	HCD+ Atorvastatin	HCD+ *cinnamon*
Catalase ($K\times10^{-2}$)	3W	53.34±2.69^{a}	48.48±3.10^{b}	52.77±3.39^{a}	53.61±1.75^{a}
	6 W	53.61±2.34^{a}	45.24±3.38^{b}	54.68±5.57^{a}	54.70±5.16^{a}
Hepatic peroxidase	3W	2.00±0.48^{abc}	1.62±0.33^{cd}	1.98±0.32^{abc}	1.84±0.18^{abc}
	6 W	2.24±0.50^{a}	1.42±0.29^{d}	1.78±0.24^{bcd}	2.20±0.23^{ab}
Hepatic SOD (u/g)	3W	88.37±8.49^{ab}	84.58±8.09^{bc}	88.29±6.79^{ab}	90.75±4.08^{ab}
	6 W	89.00±6.74^{ab}	77.17±7.98^{c}	90.71±4.89^{ab}	95.21±9.37^{a}
GSH nmol/100mg	3W	37.64±2.54^{abc}	32.50±1.68^{de}	38.63±1.74^{ab}	35.07±2.64^{cd}
	6 W	37.78±1.60^{ab}	30.70±1.36^{e}	36.34±2.79^{bc}	39.32±3.16^{a}

Means have different letters indicate significant variation at ($P< 0.05$), while the same letters indicate non significant variation

Table 4. Correlation coeffecient between NO, Hcy, MDA, Catalase, peroxidase and SOD parameters in HCD fed rats

	No	Hcy	MDA	Catalase	Peroxidas	SOD
Hcy	-0.44	-	-	-	-	-
MDA	-0.77	0.83	-	-	-	-
Catalase	0.88	-0.72	-0.96	-	-	-
peroxidase	0.68	-0.82	-0.86	0.81	-	-
SOD	0.92	-0.57	-0.91	0.94	0.79	-
GSH	0.77	-0.74	-0.86	0.88	0.92	0.84

-ve indicate negative relationship while +ve indicate positive relationship

Induction period continues for 15 day and was period of nutritionally experimental induction of HC by feeding basal normal diet with 1% cholesterol powder, 0.25% bile salts and beef tallow in percentage of 4% [13] for 15 days. So during this period rats were divided into 2 groups, normal and Hypercholesterolemic group. Normal group, rats were maintained on basal rat chow diet during the entire experiment and include 12 rats. Hypercholesterolemic group, rats were maintained on HCD and include 36 rats.

Treatment period starts from the beginning of 3rd W. and continue for 6 W. more. During this period, hypercholesterolemic rats were divided into 3 subgroups (12 rats for each) according to the type of treatment which administered to each group. The three subgroups were (HCD), HCD+Atorvastatin and HCD+ *cinnamon* group.

Hypercholesterolemic (HC) group, rats were continued to maintain on HCD for 6 W. HCD+ Atorvastatin group, where rats maintained on HCD with administration of atorvastatin at a dosage of 0.2 mg/kg b. wt. and administered orally by gastric intubation daily for 6 W. [16]. HCD+*cinnamon*, rats maintained on HCD with administration of *cinnamon* extract at a dosage of 20mg/day/rat [15] in saline and

administered orally by gastric intubation daily for 6 W.

Blood and tissue sampling

At the 3rd and 6th W. from the treatments, six rats from each group were fasted overnight and sacrificed under diethyl ether anesthesia. Blood samples were collected and allowed to coagulate at room temperature then centrifuged at 3000 rpm for 30 minutes. The clear, non haemolysed supernatant sera were quickly removed and stored at -20C° for subsequent biochemical analysis of serum lipid profile (TG, Cholesterol, LDL and HDL). Also ALT and AS T activities, serum NO and Hcy concentrations were measured.

After sacrification by decapitation and dissection, liver tissue was immediately removed and weighted. 0.5 gm of liver tissue was homogenized in 5ml of 0.9% NaCl. The homogenate was centrifuged and the clear supernatant was kept in deep freezer at -20C° for biochemical analysis. The rest of liver tissue used for measurement of oxidative stress markers as, liver GSH, catalase, SOD and peroxidase.

Analytical methods

The serum lipid contents was assayed using enzyme-based kits for cholesterol [17], triacylglycerols [18], Serum LDL concentration [19] and HDL [20], using reagent kits obtained from Spinreact Company (Spain). ALT and AST activities were determined using of commercial kits according to the manufacturers' instructions. Oxidative antioxidant markers, MDA [21], GSH [22], Catalase activity [23], SOD, activity [24], Peroxidase activity [25] were measured. Serum nitric oxide and homocysteine concentration were determined according to the method described by Miranda et al. [26] and Araki and Sako [27] respectively.

Statistical analysis

The data were analyzed using 2 ways ANOVA followed by LSD analysis to compare various groups with each other and the effect of time, treatment and their interaction. Results were expressed as mean±standard deviation and values of P<0.05 and P<0.01 were statistically significant and highly significant different, respectively.

Results

Serum TG in the two tested periods, cholesterol and LDL-cholesterol concentrations in both experimental periods were significantly increased while the concentration of HDL-C in both tested periods was decreased in hypercholesterolemic in comparison with normal rats.

Treatment of HCD with either atorvastatin for 6 W. or *cinnamon* for both tested periods (**Table 1**) led to significant ameliorative effects on the disturbed serum cholesterol. When the treatment period was extended to 6 W, the decrement in TG became highly significant (P<0.01) in either atorvastatin or *cinnamon*. Moreover both atorvastatin and *cinnamon* significantly decreases LDL while significantly increase HDL in both tested periods (**Table 1**).

Serum ALT and AST activity showed significant increases (P< 0.05) at the 3rd &6th W. in the HCD when compared to the normal group and the treatment with atorvastatin for 3&6 W. showed a non significant change in ALT while *cinnamon* treatment at 6th W., there was a significant (P<0.05) decrease in serum ALT and AST when compared to the HCD group.

MDA was significantly increased in hepatic tissue of HCD group at the 3rd and 6th W. and decreased by administration atorvastatin and *cinnamon* at the 6th W. from treatment (**Table 2**). However *cinnamon* had more beneficial effect at the end of the 6th W.

Hepatic SOD at the 6th W., catalase at the 3rd &6th W. and peroxidase at the 6th W. were significantly decreased in HCD in comparison with normal group (**Table 3**) and atorvastatin administration for 3 and 6 W. increased catalase activities while non significant for peroxidase. Moreover, SOD significantly increased after 6 W. of treatments.

Serum NO was decreased significantly in HCD at 3rd and 6th W. compared to normal group, while improved by treatment with atorvastatin and *cinnamon* at the 3rd and 6th W. (**Table 2**). On the other hand, serum homocysteine increased markedly in serum of hypercholesterolemic rats at 3rd and 6th W. and lowered with treatments (**Table 2**).

There were positive correlation coefficient between NO and catalase, (r=0.88), Peroxi-

dase, (r =0.68), SOD (r =0.92) and GSH (r=0.77) while, there were negative correlation coefficient between NO and Hcy (r =0.44) and MDA (r =0.77) (**Table 4**).

Discussion

Effect of HCD, atrovastatine and cinnamon on lipid profile in rat

The present study revealed that administration of diet supplemented with 1% cholesterol and 0.25% cholic acid to male albino rats was suitable to induce HC and there was a significant ($P<0.05$) increased in serum TC, TG, and LDL-C concentrations at 3&6 W. Whereas, HDL-C in both tested periods was decreased in of HCD as compared with normal group (**Table 1**).

These changes may be attributed to the better cholesterol absorption favored by cholic acid supplementation. Feeding rats with cholic acid and cholesterol down-regulates the transcription of CYP7A1 [28]. CYP7A1 is the rate-determining enzyme in the biosynthetic pathway of bile acids from cholesterol in the liver for its excretion into the bile which accounts for about 50% of the daily cholesterol excretion [29].

LDL-C elevation in hypercholesterolemic rats might be attributed to the reduction in the number of LDL receptor or reduced LDL binding to its receptor in these subjects. Changes in hepatic LDL-receptor contribute to the elevation in blood cholesterol levels induced by HCD as well as to the reduction that follows hepatic cholesterol depletion.

Another risk factor for developing atherosclerosis is the reduction in HDL-C level which attributed to its central function in the reverse of cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism [30]. Moreover, increased TG and decreased HDL-C levels in hypercholesterolemic rats may be attributed to decreased activity of lipoprotein lipase.

Treatment of with either atorvastatin or *cinnamon* improve lipid profile since they significantly decrease TC, TG, and LDL-C levels and a significant increase of serum HDL-C which became more pronounced after 6 W. of treatment (**Table 1**).

These findings are in accordance with the result of Shepherd [31] who reported that statins as fluvastatin, pravastatin, atorvastatin, and rosuvastatin administration to hypercholesterolemic rats induce an inhibition of cholesterol production in rat liver by blocking HMG-CoA reductase, but do not impact intestinal cholesterol absorption. As a result, hepatocytes become depleted of cholesterol and respond by increasing LDL-C clearance from the blood via up regulation of hepatic LDL-C receptors and decreasing entry of LDL-C into the circulation [32]. These actions, in turn, give rise to lower LDL-C levels. Moreover, atorvastatin lowered the LDL-C in dietary induced HC in normal rabbits through the direct inhibition of LDL production rather than enhanced clearance. There was a significant reduction in TG in guinea pigs treated with atorvastatin when compared to controls.

Cinnamon treatment might have a direct role in lipid metabolism, where *cinnamon* bark at different doses (1, 3 and 6 g/day) prevents HC and hypertriglyceridemia and lowers the free fatty acids and TG levels of type 2 diabetic subjects by its strong lipolytic activity [33]. Cinnamate, a phenolic compound found in *cinnamon*, lowers cholesterol levels in high fat-fed rats by inhibiting hepatic HMG-CoA reductase activity compared with lovastatin [34].

Cinnamon, a widely used spice in food preparation is found to activate PPARgamma and alpha, resulting in improved insulin resistance, reduced fasted LDL-c, and AST levels in high-caloric diet-induced obesity mice in its water extract form so managing obesity-related diabetes and hyperlipidemia [35].

Effect of treatments on liver enzyme activities in Hypercholesterolemic rats

Liver transaminases (ALT & AST) in the hypercholesterolemic rats at the 3rd &6th were significantly higher than normal animals (**Table 1**), which may be attributed to hyperlipidemia resulted in injury of liver tissue so when cell membrane is damaged, these enzymes which are normally located in the cytosol, leak into the blood streams [16].

High serum cholesterol level can cause liver damages [36] and treating for 6 W. with *cinnamon* caused amelioration in the activity

of these enzymes (**Table 1**). Elevated serum ALT and AST usually indicate hepatocyte damage and the most common presentation is fatty liver. In mice, AST and ALT were increased notably, and 3 W. of *cinnamon* treatment significantly decreased their levels suggesting that *cinnamon* may play an important role in improving liver function [35].

Effect of treatments on oxidant/antioxidants markers in HC rats

Lipid peroxidation products MDA increased significantly in hypercholesterolemic rats, these data are in accordance with those of Martinet et al. [37]. In addition Davi, et al. [4] demonstrated that HC is associated with increased production of oxygen radicals and increased LDL-C oxidation.

Increased oxidative stress in HCD, contribute to HC induces ROS overproduction which could in turn initiate lipid, protein and DNA oxidative modifications that could be involved in HC-induced vasculopathies [37]. Moreover HC stimulates the release of platelet-activating factor, which in turn increases the synthesis and release of inflammatory cytokines known to stimulate polymorph nuclear leukocytes to produce ROS.

Imbalance between free radical production and antioxidant led to oxidative stress which is obvious from depressed enzymatic and non-enzymatic antioxidant defense system in HCD fed group in our study. Thus diminished antioxidant defense in HCD group resulted in free radical induced damage of lipid so called lipid peroxidation. Lipid peroxidation serves as a marker of cellular oxidative stress and recognized as a major causative factor of oxidative damage in diseases as atherosclerosis and cancer.

Cardiovascular disease patients have manifested a significant increase in lipid peroxidetion which is correlated to the severity of HC. Therefore, in respect of HC, our recent interest has been focused on strategies that enhance the removal of ROS, either by using antioxidant or drugs that enhance endogenous antioxidative system.

The present results indicated that treatments of hypercholesterolemic rats with either atorvastatin or *cinnamon Zeylanicum* produced significant decrease in lipid peroxidation products. These obtained data are concomitant with the results of Sakabe et al. [38] who found that atorvastatin significantly reduced the MDA and RLP-cholesterol concentrations. Bolayirli et al. [39] reported that atorvastatin therapy to hypercholesterolemic rabbits for 4 W. resulted in significantly decreased MDA concentration and attributed this decrease to the significantly decreased "aged LDL" by atorvastatin is more prone to oxidation. However their findings reveal non-significant difference in homocysteine levels as a result of atorvastatin therapy that was in disagreement with our results.

Short-term atorvastatin therapy can induce rapid alterations in lipid profiles, antioxidant effects, improvement in endothelial function [40] and NO availability almost completely after 3 days in HC probably by decreasing oxidative stress. This improvement seems to be more rapid than the accompanying decline in LDL-C and not related to these lipid changes. This finding can support the concept of lipid-independent effects of statins [41].

The significant decrease in MDA level in hypercholesterolemic rats treated with *cinnamon* (**Table 3**) attributed to its antioxidant activity due to the presence of cinnamate supplementation in the *cinnamon* bark [34].

The antioxidants are believed to intercept the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl group, thereby forming a stable end-product which does not initiate or propagate further oxidation of lipid [9]. Another possible mechanism is the ability to prevent insulin resistance possibly through activating insulin signaling via the nitric oxide pathway [42]. As insulin resistance is associated with increased lipid oxidation and decreased carbohydrate oxidation leading to an oxidative stress followed by accumulation of fat in the hepatocytes [43].

Regarding hepatic antioxidant system there was a significant inhibition of the antioxidant defense system during experimental HC, specifically a decrease of catalase, peroxidase and SOD activities and parallel fall in GSH content (**Table 3**). These data correspond to reports of Mahfouz et al., [44] with diminution of the respective enzyme mRNA expressions after cholesterol feeding stress. It could be concluded that HC diminishes the antioxidant defense system and decreases the activities of

SOD, CAT and elevating the lipid peroxide content.

The current results revealed that either atorvastatin or *cinnamon* resulted in signifycant increase in the antioxidant enzymes activities and GSH contents in liver homogenate (**Table 3**). Atorvastatin inhibits angiotensin-induced superoxide formation by NAD(P)H oxidase in isolated rat vascular smooth muscle cells and in rats *in vivo* by down regulating mRNA expression of NADPH oxidase and inhibition of GTPase translocation from cytosolic compartment to the cell membrane which is required for its activation [45]. Atorvastatin also prevented hyperglycemia-enhanced superoxide formation in coronary artery segments.

The overall mechanisms by which atorvastatin in animal models act as antioxidants are decreasing MDA level, LDL oxidation while increasing antioxidant enzymes activities and GSH contents. Statins also modulate oxidation of lipoprotein, superoxide generation, scavenger receptor expression and endothelial NO synthase [46].

The antioxidant enzyme activities were found to be significantly enhanced, whereas GSH content was markedly restored in rats treated with *cinnamon*. This spice partially counteracted increase in lipid conjugated dienes and hydroperoxides, the primary products of lipid peroxidation. Thus, these spices exert antioxidant protection through their ability to activate the antioxidant enzymes because of cinnamate a phenolic compound in cinnamon bark [34]. The antioxidant effect of phenolic compounds in *cinnamon* extracts is mainly due to their redox properties and could result in various mechanisms including: free-radical scavenging activity, transition-metal-chelating and singlet-oxygen-quenching capacity.

Antioxidant effect of atorvastatin and *cinnamon* can be used to treat cardiovascular and hepatic diseases and may provide health-promoting effects.

The link of the antioxidant capacity of either atorvastatin or cinnamon and liver injury is to protect the liver tissue by removing and scavenging the free radical leading to normalizing the liver injury indicators, ALT, AST and MDA.

Effect of treatments on serum nitric oxide and homocysteine in HC rats

The present study demonstrated that MDA and homocystiene increased significantly in hypercholesterolemic rats at the 3rd and 6th W. (**Table 2**). A significantly increased susceptibility to free radical-induced lipid peroxidation measured as MDA and high level of homocystiene in hypercholesterolemic rabbits [39]. On the other hand, nitric oxide concentration decreased significantly in serum of hypercholesterolemic rats (**Table 2**). This finding parallels with that of Szilvassy et al. [47] who reported that cholesterol-enriched diet caused a significant decrease in vascular NO production.

Production of oxygen radicals may decrease the bioavailability of NO, thus leading to inadequate endothelium-dependent responses. The bioavailability of NO is impaired either through decreased synthesis of NO or through increased breakdown of NO. Moreover impaired NO activity in proatherosclerotic states caused mainly by increased degradation of NO via oxygen radicals, where oxidative stress plays a central role in smoking-mediated dysfunction of NO biosynthesis in endothelial cells [48].

The most interesting finding is that elevated homocysteine levels seem to be of crucial importance for deterioration of endothelial function, through nitric oxide mediated vasodilatation especially if other cardiovascular risk factors such as HC preexist.

HC reduces endothelial function either by decreasing the synthesis and release of endothelium-derived relaxing factors or by inactivating NO through its reaction with superoxide radicals [49]. Moreover, decreased biological activity and bioavailability of NO may be due, in part, to the action of circulating endogenous NO synthase inhibitor asymmetric dimethyl arginine (ADMA) and may be involved in homocystiene associated endothelial dysfunction [10].

The elevated level of homocystiene in our study may be ascribed to the fact that oxidative stress is involved in the effects of homocystiene related to its tendency to form disulphide bonds and to generate oxygen derived free radicals promoting LDL lipid peroxidation [50].

Hyperhomocysteinema (HHCY) is an independent putative risk factor for cardiovascular disease and the redox activity of Hcy may contribute to enhanced oxidative inactivation of NO which is an endogenous anti-atherosclerotic molecule [49]. Hcy act through the formation of disulfides and generation of H_2O_2, O^{-}_2 and increases oxidative degradation of NO [51]. Total Hcy increase endothelial generation of ADMA by inhibiting the activity dimethyl-arginine dimethyl-aminohydrolase (DDAH) that is responsible for the metabolism of ADMA [52].

An association between hyperlipidemia and HHCY has been suggested. Higher plasma Hcy was associated with lower HDL level. Moreover, HHCY was associated with disturbed plasma lipids or fatty liver. It seems that hypomethylation associated with HHCY is responsible for lipid accumulation in tissues. Decreased methyl group resulted in decrease the synthesis of phosphatidylcholine, major phospholipids required for vLDL assembly and homeostasis. The effect of Hcy on HDL is probably related to inhibiting enzymes or molecules participating in HDL-particle assembly [53].

Homocysteine stimulates MDA elevation and there were +ve correlation r=0.83 (**Table 4**). Homocysteine induces formation of the peroxynitrite biomarker nitrotyrosine. Taken together these results suggest that the Hcy-mediated responses leading to nitric oxide impairment are mainly coupled to MDA elevation. Thus Hcy decreases nitric oxide bioavailability [54] and led to lipid peroxidation, endothelial dysfunction and is an independent risk factor for cardiovascular disease.

Treatments of HCD with either atorvastatin or *cinnamon* produced significant decrease in serum Hcy level at the 6th W., while NO concentration was increased in comparison with hypercholesterolemic non- treated ones at the 3rd &6th W. (**Table 2**).

Treatment of hypercholesterolemic rats with atorvastatin influence Hcy metabolism, possibly through effects on glomerular filtration, or by influencing activity of key enzymes in Hcy metabolism or cystathionine b-synthase (CBS) [55]. Moreover atorvastatin administration resulted in decline in serum cholesterol level, which could reduce the levels of superoxides, possibly affects NO production and increased serum nitrate concentration in hypercholesterolemic rabbits [56]. Also atorvastsatine increase activity of endothelial nitric oxide synthase.

This is the first report indicating that *cinnamon* produce elevation in NO while lowered homocysteine level and oxidative stress markers which improve hepatic and cardio-vascular risk indicators. *Cinnamon* control 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 [57] and exert a blood glucose-suppressing effect by improving insulin sensitivity, signaling and synthesis [12], where insulin inhibits hepatic CBS activity or slowing absorption of carbohydrates in the small intestine. Further investigation needed to clarify its mechanism of action.

Actually, Hcy can be harmful to cells because it evokes oxidative stress through the production of ROS, binds to nitric oxide and produces homocysteinylated proteins, or led to the accumulation of its precursor, S-adenosyl homocysteine, a potent inhibitor of biological transmethylations. Nutritional or biochemical interventions have been proposed in the treatment of hyperhomocysteinemia, while the results of large clinical trials designed to assess the mechanism of lowering homocysteine levels in reducing cardiovascular risk, are pending.

Hcy induced expression of iNOS and decreased eNOS expression, which led to a decreased NO bioavailability. Hcy competes with GABA-A receptors, inducing the oxidative stress transduction pathway [58]. Future studies need to explore the exact pathomechanisms of HHCY in CHF. Moreover, larger intervention trials are needed to clarify whether modification of plasma HCY by B-vitamin supplementation improves the clinical outcome in CHF patients [75].

There were positive correlation coefficient between NO and catalase, (r=0.88), Peroxidase, (r= 0.68), SOD (r=0.92) and GSH (r=0.77) while, there were negative correlation coefficient between NO and Hcy (r=-0.44) and MDA (r=-0.77) (**Table 4**) indicating that oxidetive stress and high Hcy associated with decrease NO level and availability for endothelial dysfunction and risk factor for cardiovascular disease.

The data showed that the tested agent significantly decreased serum level of Hcy (Table 2) which is considered a risk factor for cardiovascular disease. HCD induces ROS overproduction and administration of the tested agent resulted in decline in serum cholesterol and LDL level, which could reduce the levels of superoxides and the cardiovascular disease correlated to the severity of HC and LDL. Moreover indirect recovered hepatic antioxidant capacity led to improve cardiovascular function. Bioavailability of NO a potent vasodilator decreased by Hcy and the tested agent produced significant decrease in serum Hcy level consequently improve cardiovascular function and provide protection from the risk factors.

Conclusion

Lipid abnormalities, oxidative injury and hyperhomocystienemia were induced by a HCD while administration of atorvastatine or *cinnamon* afforded protection against the lipemic-oxidative injury and was time dependant. Atrovastatine improved lipid parameters and decreased hepatic lipid peroxidation whereas increase GSH and antioxidant markers. *Cinnamon* extract has the capability to reduce hypercholestrolemia and the modulation of the oxidative stress, Hcy and enhances nitric oxide level. So, act as a hypocholesterolemic agent, hepatoprotective and improve cardiovascular function apart from using chemical drug with possible side effects.

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