Research Square

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Renin Angiotensin System Blockage by Losartan Neutralize Hypercholesterolemia-Induced Inflammatory and Oxidative Injuries

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Abstract

Background: Hypercholesterolemia induces several metabolic diseases via oxidative and pro-inflammatory pathways. Renin angiotensin system (RAS) contributes to the pathogenesis of hypercholesterolemia-associated metabolic changes. Therefore, this study aims to explore the protective role of losartan (LT) against oxidative and inflammatory damages in different physiological systems including heart, liver and kidney tissues in hypercholesterolemic rats.

Methods: After induction of hypercholesterolemia by high cholesterol diet for 6 weeks, LT was administered for 4 weeks. In serum samples, the levels of lipoproteins, aminotransferases, creatine kinases, urea, apoptosis and inflammatory markers were measured. In cardiac, hepatic and renal tissues, lipid peroxidation product and glutathione as well as antioxidant enzymatic activities were assayed. Finally, histopathological assessment evaluated the structural damage in in cardiac, hepatic and renal tissues.

Results: Serum markers of cardiac, hepatic and renal toxicities including creatine kinases, aminotransferases and urea were attenuated by LT in hypercholesterolemic animals. Moreover, LT markedly corrected the elevated levels of lipoproteins, apoptosis and inflammatory biomarkers. Hypercholesterolemia-induced lipid peroxidation, low glutathione levels and diminished activities of antioxidant enzymes were prominently improved in LT treated animals. Histopathological alterations by hypercholesterolemia in heart, liver and kidney tissues were ameliorated by LT.

Conclusion: This study confirmed the pathological enrolment of renin-angiotensin system in hypercholesterolemia-associated metabolic alterations. LT had a significant cardiac, hepatic and renal protective role against these impairments through down-regulation of oxidative damage, inflammation and necrosis.

Background:

High cholesterol is a common metabolic disorder, which is closely related to diabetes and obesity [1–3]. Hypercholesterolemia may deposit fats and triglycerides into the liver, which usually leads to cirrhosis of the liver or even cellular liver cancer [4, 5]. Experimental hypercholesterolemia can impair fat metabolism leading to high blood lipids and tissues [6]. Furthermore, studies showed that high cholesterol diet (HCD) could induce hypercholesterolemia even after short term exposure, which is markedly linked to oxidative stress [7]. Furthermore, hypercholesterolemia has multiple serious consequences for different physiological systems. It is considered among the major risk factors for many health problems including ischemic heart disease, fatty liver and kidney disease. Changing systolic and diastolic cardiac function as well as contractile-induced dysfunction in rodents that are fed to HCD [8] have been reported. In addition, cardiac phagocytosis has recently been shown to prevent high cholesterol in mice [9]. Increased cholesterol intake was found to impair the renal functions and to provoke kidney damages in rodents [10].

Several molecular pathways have been investigated to explore the mechanisms underlying metabolic disorders associated with high cholesterol. Among these contributing mechanisms, oxidative stress and overproduction of reactive oxygen species (ROS) are commonly documented pathways. Several experimental observations have reported that significantly excess cholesterol load leads to imbalances in the state of oxidation and reduction within tissues and ROS accumulation. Fat peroxide in cellular membranes is also involved as a caustic mechanism [11]. Furthermore, studies have revealed the relationship between oxidative stress and inflammation, which was closely related to tissue necrosis and apoptosis during high cholesterol. Biomarkers of inflammation and elevated programmed DNA damage by HCD were found in rodents [12]. The activation of transcription factors such as nuclear factor kappaB (NF-κB) and the generation of oxidized low-density lipoprotein may explain this association [13].

The renin-angiotensin system (RAS) has one precursor called the angiotensinogen. It is cracked by kidney derived renin to angiotensin (Ang) I, which is subsequently trimmed to Ang II by angiotensin conversion enzyme (ACE). Ang II is a biologically active peptide in RAS. The main receptor of the pathophysiological and physiological effects of Ang II is the Ang II receptors type 1 (AT1R). Several studies have shown that ACE and AT1R blockers prevent vascular injury caused by high cholesterol in various types [14–17]. Losartan (LT) is an angiotensin II receptor antagonist of the first type used mainly for the treatment of hypertension and diabetic nephropathy. LT intervention to lower the end-point in hypertension showed that LT was therapeutically superior to atenolol in reducing cardiovascular morbidity and mortality, with a similar decrease in blood pressure [18]. An additional study found that the therapeutic benefits of LT in terms of cardiovascular events were due in part to low levels of uric acid in the blood [19]. Thus, the present study aims to explore the potential protective role of LT on the metabolic and redox status in animals fed on HCD.

Methods:

Animals and food preparation:

Male albino Wistar rats, approximately 70 to 80 grams, were obtained from the Animal Care Center at King Saud University. After 10 days of acclimatization in standard conditions of temperature, humidity and day/night cycles, six rats were fed with normal cholesterol rat chow (NCRC), while eighteen animals were fed HCD for 6 weeks. Water and food were allowed to free access in this whole experiential duration. HCD in pellet form was prepared by adding 1% cholesterol + 0.5% cholic acid with NCRC powder content: protein 20%, fat 4%, fiber 3.5%, ash 6%, total energy 2850 Kcal/kg. The experimental procedures in the present study followed the National Institute of Health guide care policies (NIH 1996). In addition, this study received ethical approval from the Ethical Committee of Pharmacy College, Animal Care Center, King Saud University.

Experimental design:

After six weeks, the HCD fed animals were randomly divided in to three groups (n=6) as follows: Group-1, Control group of rats fed with rat chow was treated with vehicle. Group-2, HCD fed rats were

treated with vehicle. Group-3, HCD fed rats were treated with LT (10 mg/kg/day, orally) for four weeks and Group-4, HCD fed rats were treated with LT (20 mg/kg/day, orally) for four weeks. During the LT supplementation, HCD feeding was continued until the end of experiment. Weekly animals' body weight and general health conditions were carefully monitored during the whole period. Under light anesthesia, blood samples were collected through cardiac puncture and centrifuged at 4,000 rpm for 10 minutes. Serum samples were stored after suppuration at -20 ºC till analysis. Then, animals were decapitated and dissected to collect heart, liver and kidney. Tissues were immediately dipped into liquid nitrogen for 1 min, and then stored at -80 º C until analysis. A cross section of heart, liver and kidney were preserved in 10% formaldehyde for histopathological evaluations.

Serum analysis:

Total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL) and high density lipoprotein-cholesterol (HD), creatine kinase-B (CK-B), lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea (BUN) levels were estimated by using commercially available diagnostic kits (Human, Wiesbaden, Germany). Inflammatory biomarkers including tumor necrosis factor-alpha (TNF-α), interlukin-1beta (IL-1β), interlukin-6 (IL-6), prostaglandin E-2 (PGE-2), caspase 3 and nitric oxide (NO) levels were estimated by using ELISA kits for rats (R&D systems Inc., USA).

Tissue analysis:

In homogenate of heart, liver and kidney tissues, thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels were measured by using ELISA kits (Cayman Chemical Co., USA). In Post-mitochondria supernatants of heart, liver and kidney, enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were assayed by using ELISA kits (R&D systems Inc., USA).

Histopathological procedures:

Across sectional portions of heart, liver and kidney tissues from each group were preserved in 10% buffered formalin. After embedding in paraffin blocks, samples were sectioned by rotary microtome to 5 µm sections. These sections were H&E stained and examined for histopathological changes in a blinded manner. The extent of cardiomyocytes damage and hemorrhage, hepatic inflammation and ballooning degeneration and glomerular damage were histologically assessed and scored according to Ma et al [20].

Statistical Analysis:

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple comparisons test (n = 6). Differences between groups were considered statistically significant when $P \leq 0.05$. All statistics tests were conducted using Graph Pad Prism (v. 5) software.

Results:

In HCD fed rats, levels of TC, TG and LDL were increased significantly (P<0.001) compared to control animals. Treatment with LT (10 and 20 mg/kg/day) resulted in a significant reduction in the serum levels of TC (P<0.001), TG (P<0.01) and LDL (P<0.05) compared to the HCD group. However, HDL levels did not markedly alter in HCD group when compared to controls. In HCD rats, serum activities of CK and CK-MB were increased (P<0.001), while LT (10 and 20 mg/kg/day) treatment revealed significant (P<0.01) inhibition of CK and CK-MB compared to HCD. AST and ALT were increased significantly (P<0.01) in rats fed with HCD, which was corrected by LT (P<0.01) as compared to the HCD group. Creatinine and urea levels were significantly (P<0.001) increased in HCD fed animals, which were markedly reduced (P<0.05) in LT treated animals **(Table 1)**.

Serum levels of IL-6, IL-1β and TNF-α were significantly (P<0.001) increased in HCD fed animals. LT treatment to hypercholesteremic rats markedly reduced the levels of IL-6 (P<0.05 and P<0.001; respectively), IL-1β (P<0.01) and TNF-α (P<0.01) compared to HCD group. NO and caspase-3 activities were significantly (P<0.001) increased in HCD rats and these levels were markedly (P<0.05 and P<0.01) reduced in LT (10 and 20 mg/kg/day) groups. Serum PGE2 levels were significantly (P<0.001) increased in HCD fed rats. LT (20 mg/kg/day) treatment markedly (P<0.05) reduced PGE2 levels **(Figure 1)**.

In cardiac, hepatic and renal cells, TBARS levels were significantly (P<0.001) increased in HCD fed rats. LT treatment (10 and 20 mg/kg/day) markedly reduced the TBARS levels in cardiac (P<0.05 and P<0.001; respectively), hepatic (P<0.01 and P<0.001; respectively) and renal (P<0.05 and P<0.01; respectively) tissues as compared to HCD supplemented rats. HCD fed rats showed significantly decreased GSH levels in heart, liver and kidney tissues (P<0.001, P<0.01 and P<0.001; respectively). The GSH levels were increased significantly by LT treatment in cardiac (P<0.01), hepatic (P<0.05 and P<0.01; respectively) and renal (P<0.05 and P<0.01; respectively) tissues as compared to HCD supplemented rats **(Figure 2)**.

The cardiac, hepatic and renal SOD enzymatic activities were found significantly reduced (P<0.001, P<0.001 and P<0.01; respectively) in HCD fed rats. Both the doses of LT treatment markedly enhanced SOD activities in cardiac (P<0.05 and P<0.001; respectively), hepatic (P<0.05 and P<0.01; respectively) and renal (P<0.05) tissues compared to HCD group**.** CAT enzymatic activities in cardiac, hepatic and renal cells were significantly reduced (P<0.001) in HCD fed rats. LT treatment markedly enhanced CAT activities in cardiac (P<0.05 and P<0.01; respectively), hepatic (P<0.05 and P<0.01; respectively) and renal (P<0.05 and P<0.01; respectively) tissues as compared to HCD group**.** In heart, liver and kidney tissues, GPx activities were significantly (P<0.001) decreased in HCD fed rats. Hypercholesteremic rats treated with LT (10 and 20 mg/kg/day) had markedly enhanced GPx enzymatic activities in cardiac (P<0.05), hepatic (P<0.01 and P<0.001; respectively) and renal (P<0.01) tissues as

compared to HCD supplemented rats. GST enzymatic activities in cardiac, hepatic and renal tissues were significantly diminished ($P<0.01$, $P<0.001$ and $P<0.001$; respectively) in HCD group. Treatment of the HCD supplemented animals with LT (10 and 20 mg/kg/day) markedly enhanced GST activities in cardiac ($P<0.05$), hepatic ($P<0.05$ and $P<0.01$; respectively) and renal ($P<0.05$ and $P<0.01$; respectively) tissues as compared to HCD group **(Figure 3)**.

The quantitative histological assessment of cardiac, hepatic and renal sections was showed in **table 2**. Histological changes in cross sections of heart tissues from the control group showed normal appearance of myocardial cells with oval elongated nuclei and homogenous cytoplasm. Rats fed high cholesterol diet showed marked damage with hemorrhage between muscle fibers. Rats treated with LT (10 mg/kg/day) had less injury and normal morphology of the myocardial cells along with homogeneous cytoplasm and oval-elongate nucleus. The higher dose of LT (20 mg/kg/day) showed significant recovery in cardiomyocytes **(Figure 4)**. Liver sections from a control rats revealed normal architecture of hepatocytes, while liver sections from HCD group showed steatosis and inflammatory infiltration. In LT treated group (10 mg/kg/day), there was incomplete regenerating hepatocytes around the central vein. Liver sections from LT (20 mg/kg/day) treated rats showed complete recovery in hepatocytes with binuclear cells **(Figure 4)**. Representative histology images of renal cortex of control rats revealed normal appearance of the proximal and distal convoluted tubules as well as Bowman's capsule. Rats fed on HCD showed dilated glomerular capillaries, and expanded glomerular tuft and Bowman's space. Treatment of HCD animals with LT in two doses (10 and 20 mg/kg/day) showed improvement in glomeruli and renal tubules **(Figure 4)**.

Discussion:

Hypercholesterolemia due to chronic consumption of HCD has detrimental effects on multiple physiological systems, which may lead to altered redox and metabolic status. In the present study, experimentally-induced hypercholesterolemia following 6 weeks of HCD feeding in rats resulted in impaired cardia, hepatic and renal structural and functions. These effects were associated with aggravated oxidative stress and inflammatory status. The pathological role of RAS during hypercholesterolemia has been documented. In the current study, blockage of terminal step of RAS by LT markedly improved the altered cardia, hepatic and renal architecture and physiology *via* reducing local and systemic oxidative stress and inflammatory cytokines.

In the present study, we used TBARS as a marker for lipid peroxidation. TBARS react with malondialdehyde (MDA), which is a by-product of lipid hydroperoxides during cell membrane hydrolysis. In addition, GSH levels were estimated as it is a well-documented endogenous antioxidant molecule. Glutathione is a tripeptide that has two forms; the reduced GSH form, which scavenges free radicals, and oxidized GSSG form. The ration between the two forms determines the redox status in biological tissues.

Antioxidant enzymes also support the cellular functions and prevent the oxidative destructions of ROS and free radicals. SOD catalyzes the transformation of oxygen radicals (O_2^-) to less harmful species like H_2O_2. CAT hydrolyses H_2O_2 in to water and oxygen. GPx and GST enzymes help in the process of glutathione antioxidant defense against free radicals. They catalyze the transformation two GSH molecules to GSSG, which is associated with reduction of one H_2O_2 molecule to two water molecules.

Hypercholesterolemia is known to trigger the formation of systemic inflammatory cytokines. These inflammatory mediators play critical function in the pathogenesis of multiple metabolic syndromes, which made them a promising panel for assessment of cardiovascular and metabolic disorders. In the present study, HCD feeding led to significant augmentation of IL-6, IL-1β, TNF-α and PGE2 serum levels, which indicate an advanced degree of systemic inflammation. These results are in agreement with Chan et al, where experimental hypercholesterolemia was associated with reduced antioxidant capacity and systemic inflammation [21]. Furthermore, HCD fed animals showed a marked increase in the serum level of caspase-3 and NO. These biological molecules are markers for cellular damage and apoptosis. Studies have showed that hypercholesterolemia could noticeably trigger apoptosis [22] and formation of NO [23]. The elevated systemic inflammation and apoptosis in hypercholesterolemic rats might be attributed to combined oxidative and nitrosative cellular injuries, which heightened vascular permeability as well as leucocyte trafficking [24]. RAS was also linked to the pathophysiology hypercholesterolemia-induced systemic inflammation. Ang II was found to enhance monocytes migration pro-inflammatory cytokine levels throughout hypercholesterolemia [25]. In addition, Ang II is known to promote and regulate the formation of ROS and NO. Correspondingly, inhibition of the eventual step in Ang II cascade through blocking AT1R might show a considerable reduction in systemic inflammatory mediators and caspase-3, which was reported in LT treated animals in the current study.

Elevated levels of cholesterol in the blood exert direct harmful effects on the myocardium. Numerous clinical and preclinical studies explored the deleterious effects of hypercholesterolemia on cardiac performance and contractile dysfunctions [26]. In the present study, serum levels of TC, TG and LDL were markedly increased in HCD fed animals, while HDL levels were reduced, which indicates systemic hypercholesterolemia. Moreover, high cholesterol levels evoked cardiomyocytes necrosis and destruction, which explains the elevation of serum levels of CK-B and CK-MB. In addition, histological analysis revealed hypercholesterolemia-induced alterations among the architecture of cardiac muscle fibers. This comes in agreement with other studies, where cholesterol lowering therapy ameliorated cardiac failure in hypercholesterolemic rodents [27]. Hypercholesterolemia-induced cardiac damage in the current study might be attributed to down-regulation of antioxidant enzymes and glutathione and provoked lipid peroxidation. Other studies showed that free radicals scavenging by antioxidant compounds demonstrated enhanced myocardial necroptosis, properties and redox status in HCD rats [12]. Interestingly, modulation of AT1R was found to reduce hypercholesterolemia associated impairments myocardial ischemic-reperfusion [28]. Furthermore, RAS inhibition resulted in improved serum lipids and energy consumption cardiac tissue in HCD rabbits [29]. Similarly, we found that LT can preserve cardiac muscle from oxidative injuries *via* improving glutathione levels and the antioxidant

enzymes activates and reducing lipid peroxidation. The cardio-protective effects of LT were further confirmed by the histological analysis.

Liver functions and hepatic cellular structure are known to be sensitive to changes in serum cholesterol levels. Hypercholesterolemia was found in multiple studies to impair liver function testes and to alter the histological structure of the liver [30, 31]. In the current study, serum levels of ALT and AST were elevated in HCD rats, while histological features of hepatic slides showed steatosis and inflammation. Triggered oxidative stress and lipid peroxidation explain the reported damage and necrosis. In one study, hypercholesterolemic animals demonstrated significant hepatic lipid peroxidation and reduced SOD and CAT functions [32]. Modulation of AT1R by LT markedly protected against hypercholesterolemia-induced alterations in liver functions and histology structure. LT is known to possess hepato-protective effects in several conditions, where oxidative stress is deemed to play a pathological role. For instance, El-Demerdash et al found that LT attenuates carbon tetrachloride-induced liver fibrosis and oxidative stress [33]. Moreover, LT markedly lowered diabetic associated oxidative hepatic damages in rats in another study [34]. Likewise, we reported that LT treatment corrects the altered redox status and oxidative injuries in liver tissues of hypercholesteremic animals.

Kidney tissues and renal functions were also altered by hypercholesterolemia in the present study. Feeding of the animals with HCD markedly elevated the levels of creatinine and urea, which are markers for renal dysfunction. In addition, hypercholesterolemia provoked alterations in glomerular size and structure. Similar results were reported in other studies, where experimentally-induced hypercholesterolemia by HCD impaired renal functions and structure [35, 36]. Likewise in cardiac and hepatic tissues, hypercholesterolemic rats showed triggered oxidative stress and lipid peroxidation, which is evidenced by diminished glutathione and antioxidant enzymes activities as well as elevated TBARS levels. Hypercholesterolemia is known to promote renal formation of free radicals leading to cellular injury and inflammation [37, 38]. Ang II was found to endorse these effects in previous studies [39]. Furthermore, reversal of RAS activity by ACE inhibition or AT1R blocking showed attenuation of these renal impairments and oxidative stress as reported in the current and other studies [40, 41].

As a limitation to this study, the metabolic and oxidative alterations were measured only in male hypercholesterolemic rats, which may alter the assumption that animal gender and the associated hormonal differences may interfere with the therapeutic effects of RAS inhibitors. Another drawback of the current study is that food and water consumptions were not determined. Calculations of the food and water intake could have explained lipid profile variations between experimental groups.

Conclusion:

Taken together, this study confirmed the involvement of RAS in the metabolic impairments countered following hypercholesterolemia caused by cholesterol rich diet. Inhibition of the eventual step in RAS through blocking of AT1R by LT considerably protects against cardiac, hepatic and renal metabolic abnormalities, oxidative injury and inflammation in hypercholesterolemic animals. Therefore, the

aforementioned beneficial effects of LT may promote it as a useful therapeutic tool to improve lipid profile and hypercholesterolemia associated metabolic and oxidative alterations.

Abbreviations:

RAS: Renin angiotensin system; HCD: High cholesterol diet; ROS: Reactive oxygen species; NF-κB: Nuclear factor kappaB; Ang: Angiotensin; ACE: Angiotensin conversion enzyme; AT1R: Angiotensin II receptors type 1; NCRC: Normal cholesterol rat chow; TC: Total cholesterol; TG: Triglycerides; LDL: Low density lipoprotein-cholesterol; HDL: High density lipoprotein-cholesterol; CK-B: Creatine kinase-B; LDH: Lactate dehydrogenase; CK-MB: Creatine kinase-MB; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interlukin-1beta; IL-6: Interlukin-6; PGE-2: Prostaglandin E-2; NO: Nitric oxide; TBARS: Thiobarbituric acid reactive substances; GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione-S-transferase; SEM: Standard error of the mean; ANOVA: analysis of variance; MDA: Malondialdehyde; O_2^-: Oxygen radicals; LT: Losartan.

Declarations:

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Authors' contributions:

AMSA and FA: made substantial contributions in treatment, analysis and interpretation of the data. HMA and MMA: contributed to the conception and design of the study, interpretation the data and drafting the manuscript. MM and SSA: performed the histological studies, helped in drafting and revision of the manuscript and revised the manuscript for important intellectual content.

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Availability of data and materials:

The datasets generated and/or analyzed in the present study are included in the manuscript.

Ethics approval and consent to participate:

The experimental procedures in the present study followed the National Institute of Health guide care policies (NIH 1996). In addition, this study received ethical approval from the Ethical Committee of Pharmacy College, Animal Care Center, King Saud University.

Consent for publication:

Not applicable.

Competing interests:

The authors declare no competing interests regarding the publication of this paper.

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Tables

Table 1: Effects of LT on hypercholesterolemia-induced changes in serum biochemistry:

Parameters	Control	HCD	LT(10)	LT(20)
TC (mg/dl)	47.95±7.42	112.94±28.51^{***a}	69.84±22.45^{***b}	60±10^{***b}
CK-B (U/L)	10.26±165	22.08±3.74^{***a}	14.21±4.45^{***b}	12.32±2.89^{***b}
CK-MB (U/L)	20.54±3.3	44.19±11.48^{***a}	26.25±6.99^{***b}	23.33±8.3^{***b}
HDL (mg/dl)	37.8±6.23	32.56±6.85	33.33±2.54	34.37±2.82
TG (mg/dl)	21.03±9.24	59.05±12.24^{***a}	43.11±6.67^{**b}	38.477±6.79^{**b}
LDL (mg/dl)	37.79±12.43	55.24±10.59^{*a}	37.94±13.24^{**b}	37.95±6.2^{***b}
AST (U/L)	36.67±6.94	54.17±4.67^{**a}	41.24±7.50^{**b}	37.40±8.75^{b}
ALT (U/L)	17.65±2.16^{***a}	36.93±5.96	28.61±5.10^{**b}	22.12±4.90^{***b}
Creatinine (mg/dl)	2.06±0.67	6.18±2.00^{***a}	4.12±0.92^{*b}	3.95±1.23^{*b}
Urea (mg/dl)	19.87±3.93	59.60±11.79^{***a}	39.19±10.10^{**b}	32.44±10.59^{***b}

Data were expressed as Mean± SEM (n=6) and analyzed using one-way ANOVA followed by Student Newman-Keuls as post hoc test. [a] Control *vs* HCD group; [b] HCD *vs* LT(10) or LT(20). P values consider significant when $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

Table 2: Quantitative assessment of LT effects on hypercholesterolemia-induced changes in histological features of heart, liver and kidney tissues:

Tissue	Control	HCD	LT(10)	LT(20)
Heart	0.12± 0.070	1.05± 0.213^{**a}	0.60± 0.086	0.41± 0.070^{*b}
Liver	0.20± 0.030	2.00± 0.200^{**a}	1.80± 0.170	1.20± 0.150^{*b}
Kidney	0.53± 0.020	1.40± 0.180^{*a}	1.00± 0.160	0.57± 0.210^{*b}

Data were expressed as Mean± SEM (n=6) and analyzed using one-way ANOVA followed by Student Newman-Keuls as post hoc test. [a] Control *vs* HCD group; [b] HCD *vs* LT(10) or LT(20). P values consider significant when $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

Figures

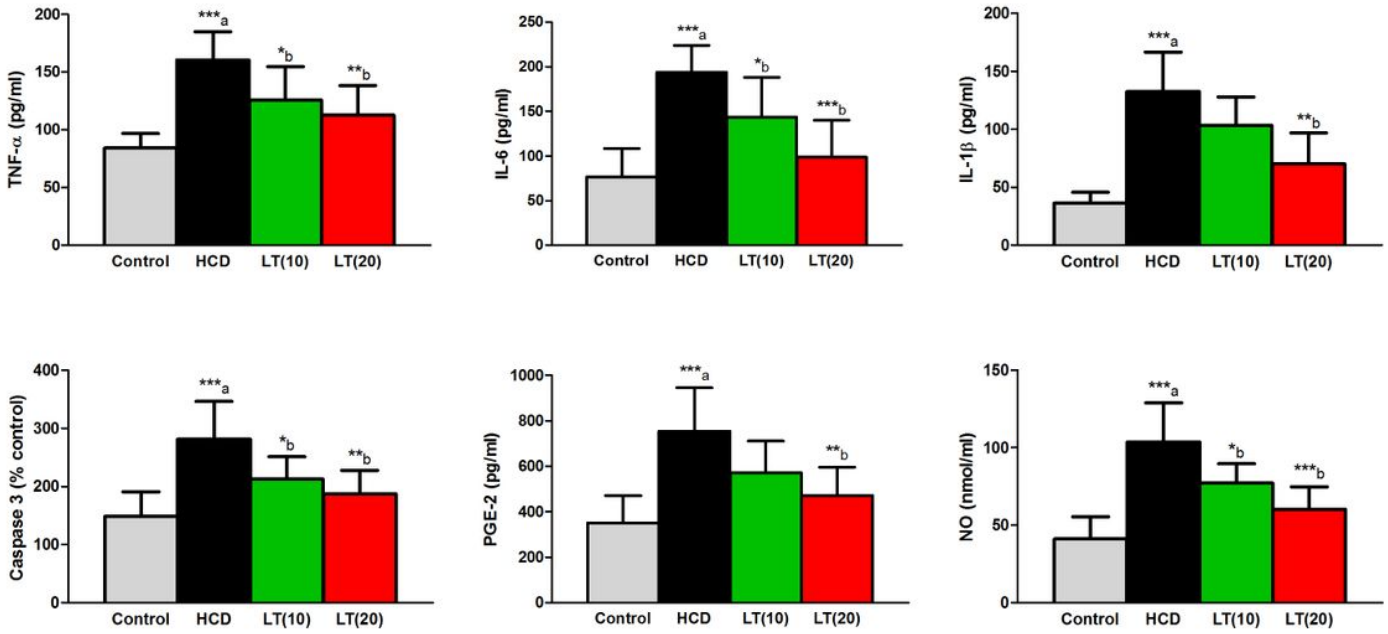


Figure 2

Effect of LT on hypercholesterolemia-induced changes in serum inflammatory biomarkers including TNF-α, IL-6 and IL-1β levels along with serum PGE-2, Caspase 3 and NO levels. Data were expressed as Mean± SEM (n=6) and analyzed using one-way ANOVA followed by Student Newman-Keuls as post hoc test. a Control vs HCD group; b HCD vs LT(10) or LT(20). P values consider significant when *P<0.05, **P<0.01 and ***P<0.001.

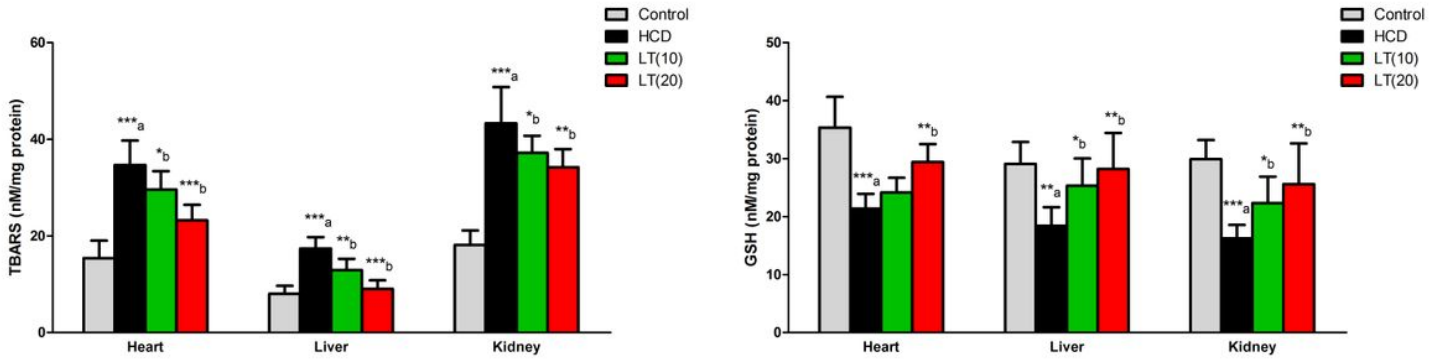


Figure 3

Effect of LT on hypercholesterolemia-induced changes in TBARS and GSH levels in cardiac, hepatic and renal cells. Data were expressed as Mean± SEM (n=6) and analyzed using one-way ANOVA followed by Student Newman-Keuls as post hoc test. a Control vs HCD group; b HCD vs LT(10) or LT(20). P values consider significant when *P<0.05, **P<0.01 and ***P<0.001.

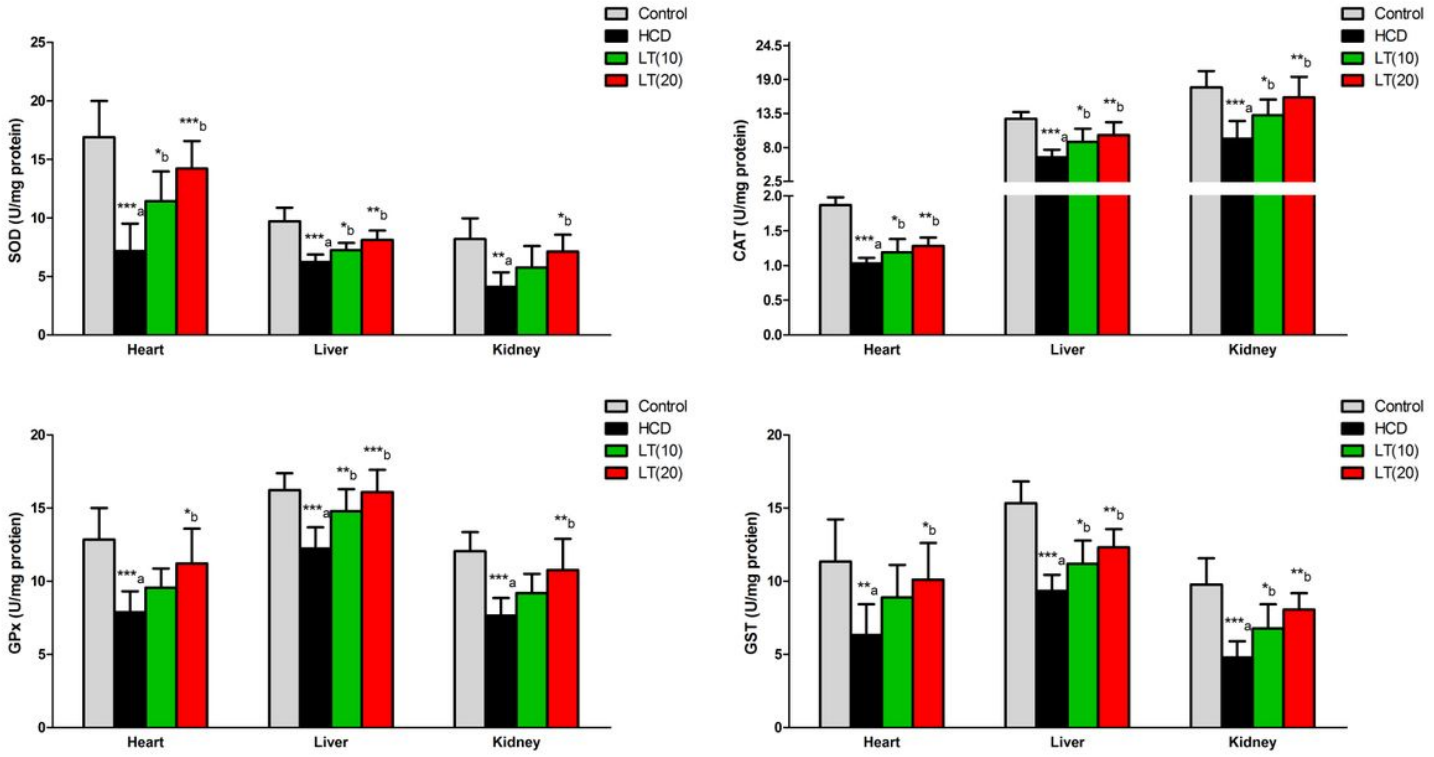


Figure 5

Effect of LT on hypercholesterolemia-induced changes in antioxidant enzymes activities including SOD, CAT, GPx and GST in cardiac, hepatic and renal cells. Data were expressed as Mean± SEM (n=6) and analyzed using one-way ANOVA followed by Student Newman-Keuls as post hoc test. a Control vs HCD group; b HCD vs LT(10) or LT(20). P values consider significant when *P<0.05, **P<0.01 and ***P<0.001.

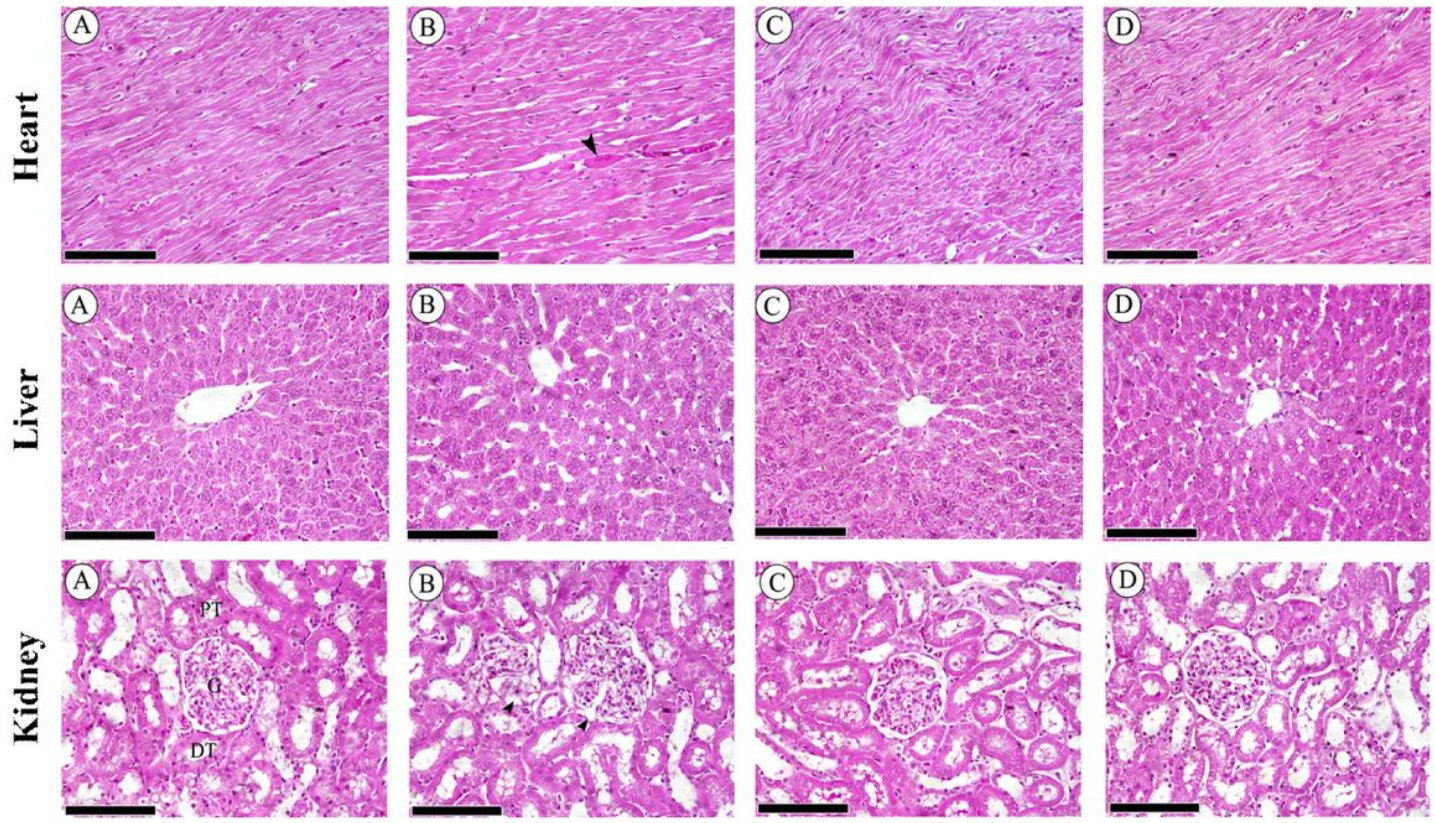


Figure 8

Sections of heart tissues (A to D) showing (A) the control group with normal appearance of myocardial cells, (B) severe damage with hemorrhage between muscle fibers (arrow) in HCD group, (C) less injury and normal myocardial cell morphology with oval-elongate nucleus centrally and homogeneous cytoplasm in LT(10) group, (D) almost normal cardiomyocytes looking in LT(20) group. Sections of hepatic tissues (A to D) showing (A) normal architecture of hepatocytes and central vein in control group, (B) steatosis and infiltration of inflammatory cells in HCD group, (C) incomplete regenerating hepatocytes around the central vein in LT(10) group, (D) complete recovery in hepatocytes with binuclear cells in LT(20) group. Sections of renal cortex (A to D) showing (A) normal appearance of the proximal convoluted tubules [PT], distal convoluted tubules [DT], Bowman's capsule and glomerulus [G] in control group, (B) dilatation in glomerular capillaries (head arrow), increase in glomerular tuft size and expansion in Bowman's space in HCD group, (C) partial improvement in glomeruli and renal tubules in LT(10) group, (D) complete retrieval of glomeruli and renal tubules in LT(20) group. Scale bar = 50 μm.