Baicalein neutralizes hypercholesterolemia-induced aggravation of oxidative injury in rats

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Abstract

Background: Hypercholesterolemia is a major risk factor for several cardiovascular and metabolic diseases through triggering oxidative and pro-inflammatory cascades. Baicalein (BL) is a natural flavone with multiple therapeutic properties. Thus, the present study aims to highlight the protective value associated with BL supplementation in hypercholesterolemic rats. Keywords : hypercholesterolemia, baicalein, inflammation, oxidative stress Methods: In this context, animals were fed for sex weeks on high cholesterol diet (HCD) then BL oral treatments in two doses (25 and 50 mg/kg/day) was started and continued for four weeks. In serum, lipid profile, liver enzymes, cardiac enzymes, renal markers, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL10), caspase-3, nitric oxide (NO) and prostaglandin-2 (PG-2) levels were estimated. In renal, hepatic and cardiac cells, thiobarbituric acid-reactive substance (TBARS), glutathione (GSH), Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were measured. Results: In hypercholesterolemic animals, the altered levels of lipoproteins, aminotransferases, creatine kinases and urea were significantly corrected by BL. Inflammatory and apoptosis biomarkers were also markedly attenuated in HCD groups following BL treatment. Hypercholesterolemia considerably evoked the lipid peroxidation product, TBARS, and oxidative radicals in cardiac, hepatic and renal tissues, which were attenuated by BL treatment, particularly the 50 mg/kg/day dose. BL improved the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) following their suppression in HCD groups. In addition, the histological alterations induced by cholesterol overload in the cardiac, hepatic and renal tissues were ameliorated by BL supplementation. Conclusions: As a conclusion, the up-regulated oxidative damage, inflammation and necrosis observed within the hypercholesterolemic animals could be enhanced by co-administration BL. Activation of the cellular antioxidant enzymes alone with suppression of inflammatory cytokines may be involved to mediate these prominent effects.

Background

Hypercholesterolemia is a major global health problem. Epidemiological reports showed that the incidence of hypercholesterolemia is mainly associated with poor diet habits such as excessive saturated fats and cholesterol containing foods consumption as well as lack of exercise. It is also more reported in females than in males (1). World Health Organization (WHO) has reported around 2.6 million mortalities due to hypercholesterolemia in 2004 (2). Hypercholesterolemia has multiple significant consequences on different physiological systems. It is considered among the major risk factors for several health problems including ischemic heart diseases, fatty liver, and kidney diseases. Altered cardiac systolic and diastolic functions as well as contractile dysfunction were previously reported in rodents fed on HCD (3). In addition, basal cardiac autophagy was recently demonstrated to be suppresses by hypercholesterolemia in rats (4). Hypercholesterolemia was also reported in other studies to trigger lipids accumulation in liver, which negatively influence the hepatic functions (5, 6). Increased cholesterol intake was found to impair the renal functions and to provoke kidney damages in rodents (7).

Several molecular pathways were investigated to explore the mechanisms behind hypercholesterolemia associated metabolic disturbances. Among these contributing mechanisms, oxidative stress and over production of ROS are commonly documented pathways. Numerous experimental observations reported that cholesterol overload markedly induces imbalance in the redox status within the tissues and ROS accumulation. Lipid peroxidation of the cellular membranes is also involved as a caustic mechanism (8). Moreover, studies have revealed the linkage between oxidative stress and inflammation, which were closely correlated with tissue necrosis and cellular apoptosis during hypercholesterolemia. Biomarkers of inflammation and programed DNA damage were found elevated by HCD in rodents (9). Activation of nuclear factor-kappa B (NF-κB) and other similar transcription factors as well as generation of oxidized low-density lipoprotein may explain this correlation (10).

The assumption that several promising natural products, isolated phytochemicals such as flavonoids, may have valuable therapeutic effects against metabolic alterations associated with hypercholesterolemia has been discussed in different investigations. BL is a 5,6,7-trihydroxyflavone. It is considered a primary product of *Scutellaria* species. BL is known for its multiple pharmacological properties such as antioxidant and anti-inflammatory against several disorders cancer, cardiac, neurological, hepatic and renal diseases (11, 12). In addition, other studies have showed the ability of BL to ameliorate diabetic associated metabolic complications via suppression of hyperglycemia, inflammation free radicals production and NF-κB-related pathways (13). One recent study found that BL might demonstrate an effective protection against oxidized low-density lipoprotein-induced oxidative and inflammatory damage (14).

Earlier studies demonstrated that, even short exposure to HCD is capable of inducing hypercholesterolemia and is significantly associated with oxidative stress (15). Furthermore, in our previous studies, HCD was used induce hypercholesterolemia in male Wistar rats (16, 17). Thus, the present investigation aims to explore the potential protective role of BL on the metabolic and redox status in animals fed on HCD.

Methods

Animals

Twenty four male Wistar rats of approximately 70 to 80 gram body weights were attained from Pharmacy College Animal Care Center at King Saud University. All received animals were acclimatized for 10 days prior to start the experiments. The rats were sustained in standard conditions such as 22 ± 1 ºC temperature, 50-55% humidity, and equal 12 h day/night cycles. All the experimental protocol such as euthanasia procedure, blood sampling and final sacrifice were followed by National Institute of Health guide care policy (NIH, 1996) and this animal study was approved by the Ethical Committee of Pharmacy College, Animal Care Center, King Saud University, Riyadh, Saudi Arabia (date 01-01-2018 and No. 663-EACC-2018).

Food composition for normal food and HCD

HCD in pellet form was prepared by adding 1% cholesterol + 0.5% cholic acid with normal cholesterol rat chow (NCRC) powder. Six rats were fed on NCRC (content: protein 20%, fat 4%, fiber 3.5%, ash 6%, total energy 2850 Kcal/kg) and eighteen rats were fed HCF for 6 weeks. Water and food were allowed to free access in this whole experiential duration.

Experimental design

After six weeks the HCD fed animals were randomly divided in to three groups by taking six rats in each group: Group-1; Control group of rats fed with rat chow were treated with vehicle. Group-2; HCD fed rats were treated with vehicle. Group-3; HCD fed rats were treated with BL (25 mg/kg/day, orally) for four weeks. Group-4; HCD fed rats were treated with BL (50 mg/kg/day, orally) for four weeks. During the BL 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. Blood samples were collected through cardiac puncture under anesthesia induced by intraperitoneal injection of ketamine (92 mg/kg, Hikma Pharmaceuticals, Amman – Jordan) and xylazine (10 mg/kg, Bayer, Turk) mixture. Serum samples were collected after blood centrifugation (4,000 rpm) for 10 min and stored at -20 ºC until analysis. Finally, animals were decapitated; heart, liver and kidneys were dissected, weighed, immediately small portion was 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

In serum samples, TC, TG, LDL-cholesterol, LHD-cholesterol, CK-B, LDH, CK-MB, ALT, AST, creatinine and urea levels were estimated by using commercially available diagnostic kits (Human, Wiesbaden, Germany). Inflammatory biomarkers including TNF-α, IL-1β, IL-6, IL-10, PGE-2, caspase 3 and NO levels were estimated by using ELISA kits for rats (R&D systems Inc., USA).

Tissue analysis

Organ's (heart, liver and kidney) small portions were homogenized in physiological buffer (1:10, w/v) and TBARS and GSH levels were measured by using ELISA kits (Cayman Chemical Co., USA). In Post-mitochondria supernatants of heart, liver and kidney, enzymatic activities of SOD, CAT and GPx were measured by using ELISA kits (R&D systems Inc., USA).

Histopathological procedures

Across sectional portion of a heart, liver and kidney tissues from each group of treatment were preserved in 10% buffered formalin. The samples were embedded in paraffin blocks and sections of thickness 5 mm were cut using a Leica CM3050 S Research Cryostat (Leica Bio-systems, USA). The sections were stained with H&E. Finally, they were examined under the microscope for histopathological changes by an observer who was blind with respect to the treatment groups.

Statistical Analysis

Data were expressed as mean ± SEM and analysed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple comparisons test (n = 6). [a] Control *vs* HCD group; [b] HCD *vs* BL(25) or BL(50). P values consider significant when *P<0.05, **P<0.01 and ***P<0.001. All statistics tests were conducted using Graph Pad Prism (v. 5) software.

Results

Serum lipid profile is presented in table 1. In HCD fed rats, TC, TG and LDL levels were significantly (P<0.001) increased compared to control animals. BL treatment to hypercholesteremic rats markedly reduced the TG and TC levels were significantly P<0.05 and P<0.01 inhibited in BL (25 and 50 mg/kg/day) treated groups as compared to HCD group of rats respectively. The high dose of BL (50 mg/kg/day) only inhibited the TC levels significantly (P<0.05) compared to HCD group. However, HDL levels did not markedly alter in HCD group when compared to controls (Table 1). The enzymes of CK, CK-MB and LDH are considered the cardiac markers and these were estimated and shown in Table 1. In HCF administered rats, the serum enzymes of CK, CK-MB and LDH were shown to increases (P<0.001) compared to control group. BL (50 mg/kg/day) treatment showed significant (P<0.05) inhibition in enzymatic activity of LDH compared to HCD. The CK and CK-MB levels were markedly reduced by both the doses of BL (Table 1).

Serum levels of TNF-α, IL-6 and IL-1β were significantly (P<0.001) elevated, while IL-10 levels reduced (P<0.001) in HCD fed animals compared to control rats. BL treatment to hypercholesteremic rats for four weeks markedly reduced the pro-inflammatory cytokines in dose dependent manner. The anti-inflammatory cytokine IL-10 markedly (P<0.01) elevated in BL (50 mg/kg/day) treated group **(**Figure 1**)**. Similarly, the levels of NO as well as caspase 3 activity were significantly (P<0.001) increased, while PGE-2 levels were significantly (P<0.001) reduced in HCD group. BL treatment markedly corrected (P<0.01) the altered levels and activity of NO, caspase 3 and PGE-2 as compared with HCD group **(**Figure 1**)**.

TBARS level was in high significantly (P<0.001) while GSH level was reduced (P<0.001) in cardiac cells of HCD fed rats compared to control animals. BL treatment (25 and 50 mg/kg/day) for 4 weeks to HCF fed rats, the TBARS was reduced markedly (P<0.05 and P<0.001, respectively) and the GSH was increased (P<0.01) in BL (50 mg/kg/day) treated group when compared to HCF supplemented control rats. Enzymatic cardiac antioxidants of SOD, CAT and GPx were found to reduces (P<0.001) in HCF fed rats compared to control group. Both the doses of BL markedly (P<0.05 and P<0.01, respectively) enhanced the enzymatic activities of SOD and CAT compared to HCD group. While the enzymatic activity of GPx was markedly elevated in BL (50 mg/kg/day) treated group **(**Figure 2**).**

TBARS levels were significantly (P<0.001) increased in hepatic cells of HCD fed rats while GSH levels found inhibited markedly (P<0.001) by the HCD supplementation compared to normal healthy control rats. Treatment with BL (25 and 50 mg/kg/day) produced inhibition in TBARS levels (P<0.05 and P<0.0, respectively) compared to HCD group of rats. While, GSH levels were significantly (P<0.05) enhanced by the BL (50 mg/kg/day) treatment. Enzymatic activities of SOD, CAT and GPx were significantly (P<0.001)

inhibited in hepatic cells of HCD fed rats compared to control animals. Treatment with BL (25 and 50 mg/kg/day) markedly ($P<0.05$ and $P<0.01$, respectively) enhanced the SOD and GPx activities in hepatic cells compared to untreated hypercholesteremic rats. However, the CAT activity was significantly ($P<0.05$) increased by the BL (50 mg/kg/day) treatment compared to HCD fed rats **(**Figure 3**)**.

In kidney, TBARS levels were significantly ($P<0.001$) increased in hypercholesteremic rats while GSH levels reduced markedly ($P<0.001$) by the HCD supplementation when compared to normal healthy control rats. Treatment with BL (50 mg/kg/day) produced inhibition ($P<0.01$) in kidney TBARS levels compared to HCD group. The kidney GSH levels markedly ($P<0.01$) inhibited by BL treatment (50 mg/kg/day) to HCD fed rats compared to HCD fed untreated animals. Enzymatic activities of SOD ($P<0.01$), CAT ($P<0.01$) and GPx ($P<0.001$) were significantly inhibited in renal cells of HCD fed rats compared to control animals. BL (50 mg/kg/day) treatment, significantly ($P<0.05$) enhanced the enzymatic activities of SOD and CAT while GPx activity increased more significantly ($P<0.01$) in renal cells compared to untreated hypercholesteremic rats **(**Figure 4**)**.

Histological changes were seen in cross sections of heart tissues from rats fed HCD and treated with two doses of BL (25 and 50 mg/kg): A) The control group showing the normal appearance of myocardial cells with oval elongated nuclei and homogenous cytoplasm. B) Section of heart tissue from rats feeding HCD showed multi focal vacuolar degeneration (heads- arrow) and congestion of blood capillaries (arrow). C) Moderate myocardial cell morphology with oval-elongate nucleus centrally and homogeneous cytoplasm were shown in myocardiocytes of HCD rats treated with (25 mg/kg). D) Normal myocardial cell morphology with oval-elongate nucleus centrally and homogeneous cytoplasm were shown in myocardiocytes of HCD rats treated with (50 mg/kg) **(**Figure 5**)**.

Histological changes were seen in cross sections of liver tissues from rats fed HCD and treated with two doses of BL (25 and 50 mg/kg): A) The liver from a control rat shows normal hepatocytes and CV. B) Liver of rats fed high cholesterol showed marked fat deposition (arrow), dilated sinusoids and pyknotic nuclei (head arrow). C) Liver of HCD treated with (25 mg/kg) BL showed moderate injury in hepatocytes and less fat deposition. D) Liver of HCD treated with (50 mg/ kg) BL showed moderate injury in hepatocytes and less fat deposition. **(**Figure 6**).**

Light micrographs of renal cortex of rats fed high cholesterol diet and administered orally with two doses of Baicalein (25 and 50 mg/kg). Section from the renal cortex of the control group reveals the normal appearance of the PT, DT, Bowman's capsule and glomerulus (G) (A). Renal cortex of rats fed high cholesterol showed dilatation in glomerular capillaries (head arrow), thickening in basal membrane of glomerulus (arrow) and mononuclear cell infiltration was seen (curved arrow) (B). Renal cortex of high cholesterol diet treated with (25 mg/kg) and (50 mg/kg) of Baicalein showed reduced injury in glomeruli and renal tubules. H&E, scale bar = 50 µm. (Figure 6).

Discussion

Dietary cholesterol overload is a major contributing factor for the development of cardiovascular and metabolic disorders. Hypercholesterolemia alters the physiological antioxidant abilities, resulting in ROS generation, and chronic inflammatory responses. Thus, the use of pharmacologically active natural products including BL might alleviate such complications. In this context we observed that BL can restore the cardiac, hepatic and renal antioxidant capacity in hypercholesterolemic rodents. Furthermore, BL showed marked anti-inflammatory and anti-necrosis properties in the present study. The BL associated improvement of hypercholesterolemia-caused significant alterations in the histological architecture of cardiac, hepatic and renal tissues confirmed its protective value.

Multiple lines of evidence support the notion that there is a linkage between cellular oxidative events and inflammation in various disorders induced by lipid discrepancies, particularly cardiovascular diseases (18). Under regular physiological status, the production of free radicals is limited and scavenged by the endogenous antioxidants. However, pathological conditions disrupt this balance in favor of ROS generation, resulting in oxidative stress. In the current study, the experimental observation documented that prolonged cholesterol overload triggers cardiac, hepatic and renal dysfunctions and over-production of ROS, which includes superoxide free radicals, hydrogen peroxide, and singlet oxygen. Markers of depleted antioxidant capacity such as low GSH levels as well as inhibited SOD, CAT and GPx activities were reported in the HCD group compared to normal animals. Free radical generation during HCD exposure was combined with cellular membranes lipid peroxidation with may harm functional cellular components. Our results come consistent with other studies that demonstrated augmented oxidative damage after HCD exposure (9, 19). The provoked lipid peroxidation indicates excessive ROS production that may exceed the detoxification capacity. Growing evidences suggest correlation between HCD and chronic inflammatory state. This assumption plays a crucial role in different diseases pathologies including diabetes and atherosclerosis. Studies have found that elevated cholesterol and fats values cannot initiate the pathological progression of pro-inflammatory cytokines (20). Moreover, the programmed cellular necrosis and its associated markers such as caspase 3 were found to be regulated by inflammatory mediators such as TNF-α (9). These cellular events alone with lipid peroxidation lead to defects in plasma membrane integrity, leakage of essential intracellular components, and damages of nucleic acids (21). Presently, HCD group exhibited profound high levels of TNF-α, IL-1β, IL-6, NO and caspase 3 alone with low IL-10 and PGE-2 levels, which indicates HCD-induced inflammatory response and DNA injury.

Nowadays, phytochemical polyphenolic products are reported for use in multiple therapies. These natural products may protect against cardiovascular, ischemic, diabetes, hepatic and renal pathological conditions (22). BL is commonly promising polyphenolic compound with multiple therapeutic benefits. Several experimental studies reported the antioxidant and anti-inflammatory effects of BL in different biological systems. BL was found to protect against hypoxia re-oxygenation injury through recruitment of its oxidative and inflammatory cytokines suppressive effects (23). Another study reported that BL exhibited prominent ameliorative effects against oxidative and inflammatory injury of myocardial tissues in diabetic animals, which was mediated by PI3K/Akt signaling cascade (24). In addition, the hepatoprotective efficacy of BL was demonstrated in rodents with diabetic live injury (13). Interestingly,

Tsai et al found that BL attenuate the oxidized LDL-induced accumulation of cholesterol and foam cells formation in the subendothelial space, which suggest the potential role of BL against hypercholesterolemia (14). Our present findings are in agreement with these previous studies. BL corrected the elevated levels of TC, TG, and LDL-C, while enhanced HDL-C, which indicates the anti-hypercholesterolemic effects. BL therapy showed cardio-protective effects confirmed by the alleviated CK-B, LDH, and CK-MB activities. Markers of liver toxicity including ALT and AST as well as nephrotoxicity markers such as creatinine and BUN were also restored by BL treatment. These cardiac, hepatic and renal protective effects were associated with repaired histological features in BL groups. Furthermore, BL treatment markedly re-activated the suppressed antioxidant enzymes SOD, CAT and GPx and suppressed the provoked lipid peroxidation in cardiac, hepatic and renal tissues. The unique chemical structure BL elucidates its pharmacological properties. BL has tri-hydroxyl chemical groups at carbon number 5, 6 and 7. It also involves three saturated rings. These structural components are essential tool for free radical scavenger ability of most of flavones.

Limitations encountered in the current study include the unisexual testing of BL effects in hypercholesterolemic male rats. This may interfere with assumption that gender metabolic and physiological differences may influence the protective effects of natural products against hypercholesterolemia and the associated molecular mechanisms. Moreover, the food consumption during the experimental period was not followed, which could have added to the explanation of the body weigh variations between different experimental groups.

Conclusions

Present findings suggest the therapeutic value of BL co-administration in HCD animals. The protective efficacy of BL was considerable in ameliorating cardiac, hepatic and renal oxidative injury *via* restoration of tissues regular histological features and antioxidant status. Regulation of pro-inflammatory and tissue apoptosis cellular mechanisms could contribute to BL protective mechanism against hypercholesterolemia and promotes its ability to attenuate ROS formation and antioxidant enzymes dysfunction.

Abbreviations

Baicalein (BL), high cholesterol diet (HCD), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL10), nitric oxide (NO) and prostaglandin-2 (PG-2), thiobarbituric acid-reactive substance (TBARS), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), World Health Organization (WHO), reactive oxygen species (ROS), nuclear factor-kappa B (NF-κB), normal cholesterol rat chow (NCRC), total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL), high density lipoprotein-cholesterol (HD), creatine kinase-B (CK-B), lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), standard error of the mean (SEM), central vein (CV), proximal convoluted tubules (PT), distal convoluted tubules (DT), glomerulus (G).

Declarations

Ethics approval and consent to participate

All the experimental protocol such as euthanasia procedure, blood sampling and final sacrifice were followed by National Institute of Health guide care policy (NIH, 1996) and this animal study was approved on dated 01/01/2018 No. 663-EACC-2018 by the Ethical Committee of Pharmacy College, Animal Care Center, King Saud University, Riyadh, Saudi Arabia.

Consent of publication

Authors consent form (BioMed Central) is filled uploaded

Availability of data and material

The analyzed raw data and materials as reference available with the corresponding author

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AMSA: Have made substantial contributions to the conception and design of the study, analysis and interpretation of the data and drafted the manuscript. MSA: Have made substantial contributions to the conception and design of the study, diet preparation, biochemical analysis and interpretation of the data. IA: Have made substantial contributions to the conception and design of the study, diet preparation, biochemical analysis and interpretation of the data. AAA: Have made substantial contributions to the conception and design of the study, diet preparation, biochemical analysis and interpretation of the data. AA: Have made substantial contributions to the conception and design of the study, diet preparation, biochemical analysis and interpretation of the data. SSA: Have performed the histological studies, interpreted the data, helped in drafting the manuscript and revised the manuscript for important intellectual content.

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Tables

Table 1:

Parameters	Control	HCD	BL(25)	BL(50)
TC (mg/dl)	47.95±7.42	112.94±28.51^{***a}	92.87±11.77^{*b}	76.64±5.57^{**b}
TG (mg/dl)	21.03±9.24	59±12.65^{***a}	44.55±12.33^{*b}	37.61±5.96^{**b}
HDL (mg/dl)	37.8±6.23	32.58±6.87	25.51±6.89	28.8±3.98
LDL (mg/dl)	10.38±3.05	47.16±10.59^{***a}	42.44±4.37	36.56±5.72^{*b}
LDH (U/L)	136.56±8.24	241.56±11.32^{***a}	237.78±7.65	225.26±8.95^{*b}
CK-B (U/L)	10.26±165	22.08±3.74^{***a}	17.33±4.03^{*b}	13.71±3.85^{**b}
CK-MB (U/L)	20.54±3.31	44.19±11.48^{***a}	28.68±8.06^{**b}	23.44±7.70^{***b}
Urea (mg/dl)	19.87±3.93	59.60±11.79^{***a}	47.68±9.43^{*b}	37.75±7.47^{***b}
Creatinine (mg/dl)	2.06±0.67	6.18±2.00^{***a}	4.94±1.60	3.91±1.26^{*b}
AST (U/L)	36.67±6.94	54.17±4.67^{***a}	45.56±8.34^{*b}	38.89±5.24^{**b}
ALT (U/L)	17.65±2.16	36.93±5.96^{***a}	29.39±4.42^{*b}	24.75±8.09^{**b}

Figures

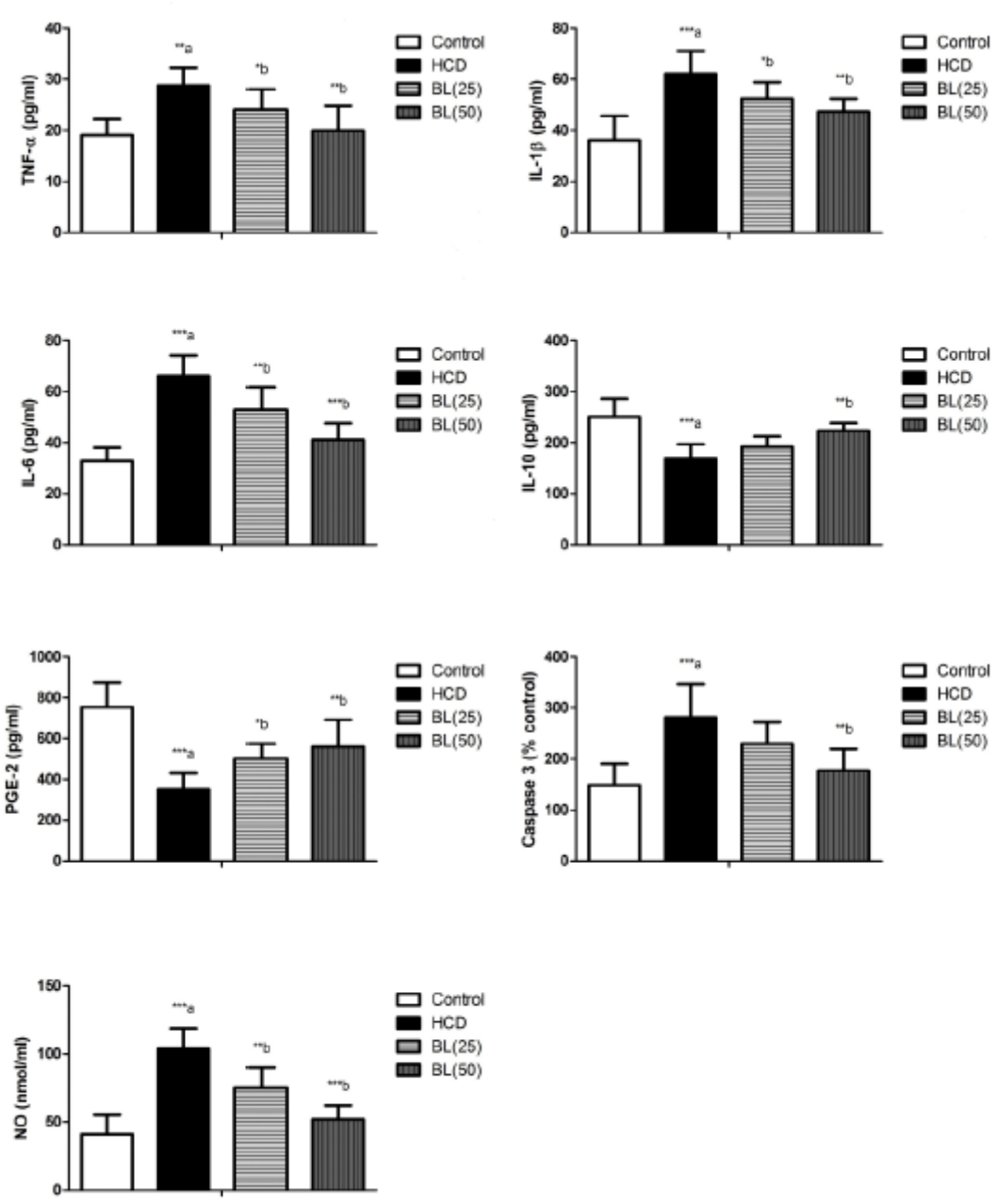

Figure 1

Effect of BL on hypercholesterolemia-induced changes in serum inflammatory biomarkers including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1beta (IL-1β) and interleukin-10 (IL-10) levels along with serum prostaglandin E-2 (PGE-2), Caspase 3 and nitric oxide (NO) levels.

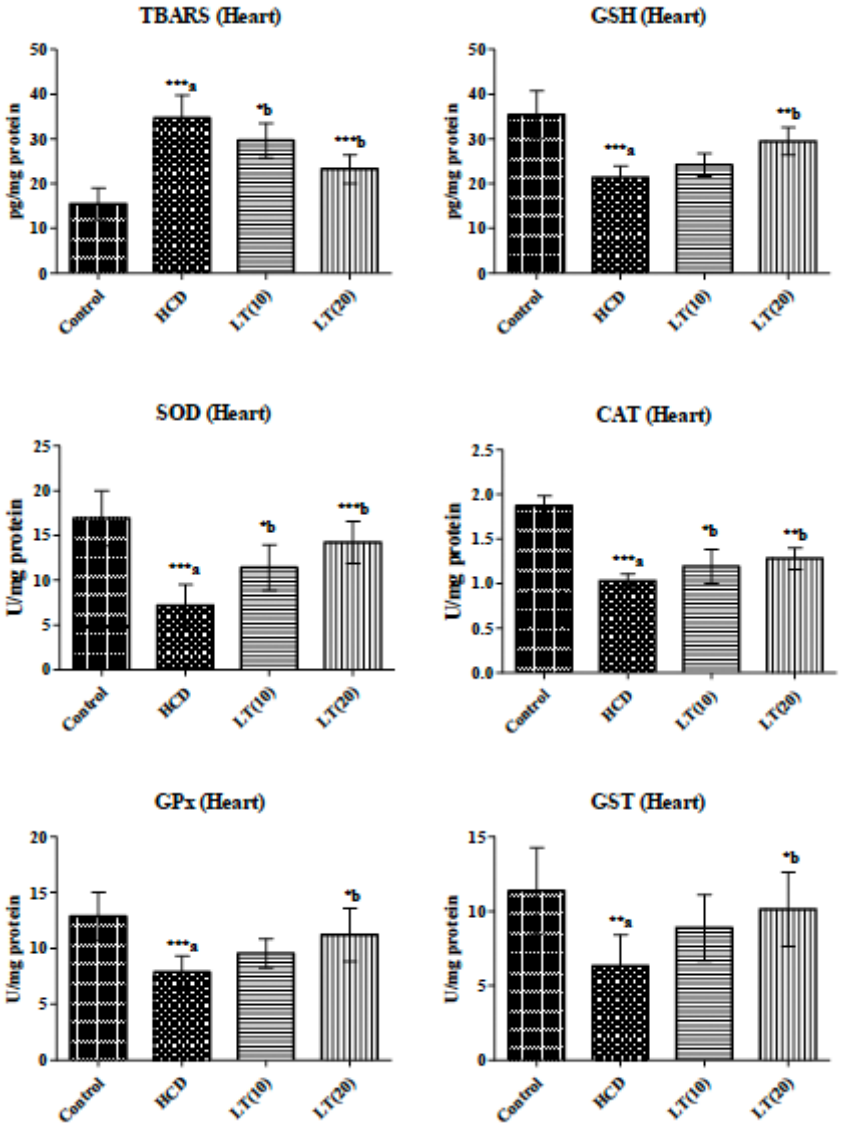

Figure 2

Effect of BL on hypercholesterolemia-induced thiobarbituric reactive substances (TBARS) and glutathione (GSH) levels, and enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione oxidase (GPx) in cardiac cells.

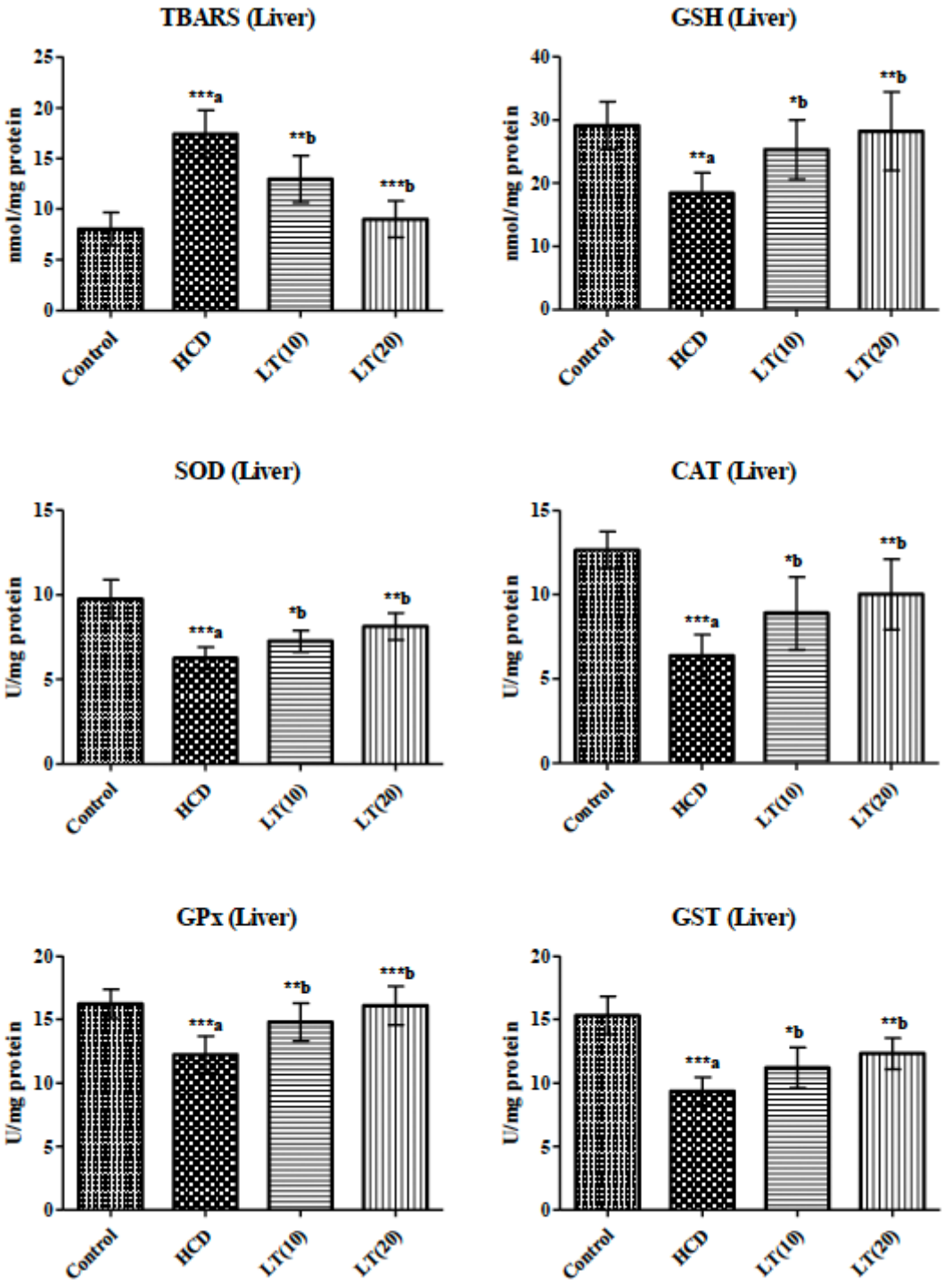

Figure 3

Effect of BL on hypercholesterolemia-induced thiobarbituric reactive substances (TBARS) and glutathione (GSH) levels, and enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione oxidase (GPx) in hepatic cells.

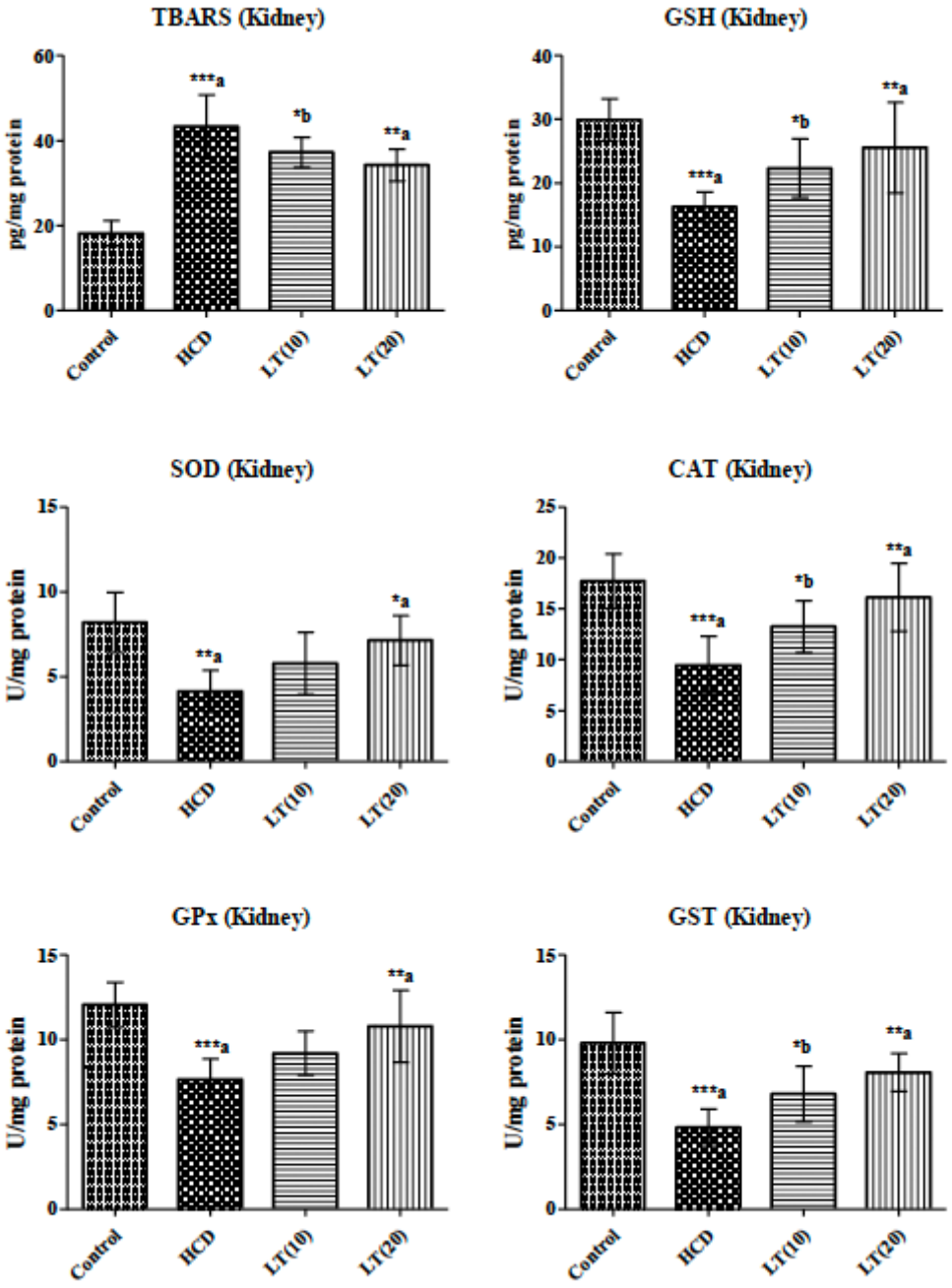

Figure 4

Effect of BL on hypercholesterolemia-induced thiobarbituric reactive substances (TBARS) and glutathione (GSH) levels, and enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione oxidase (GPx) in renal tissue.

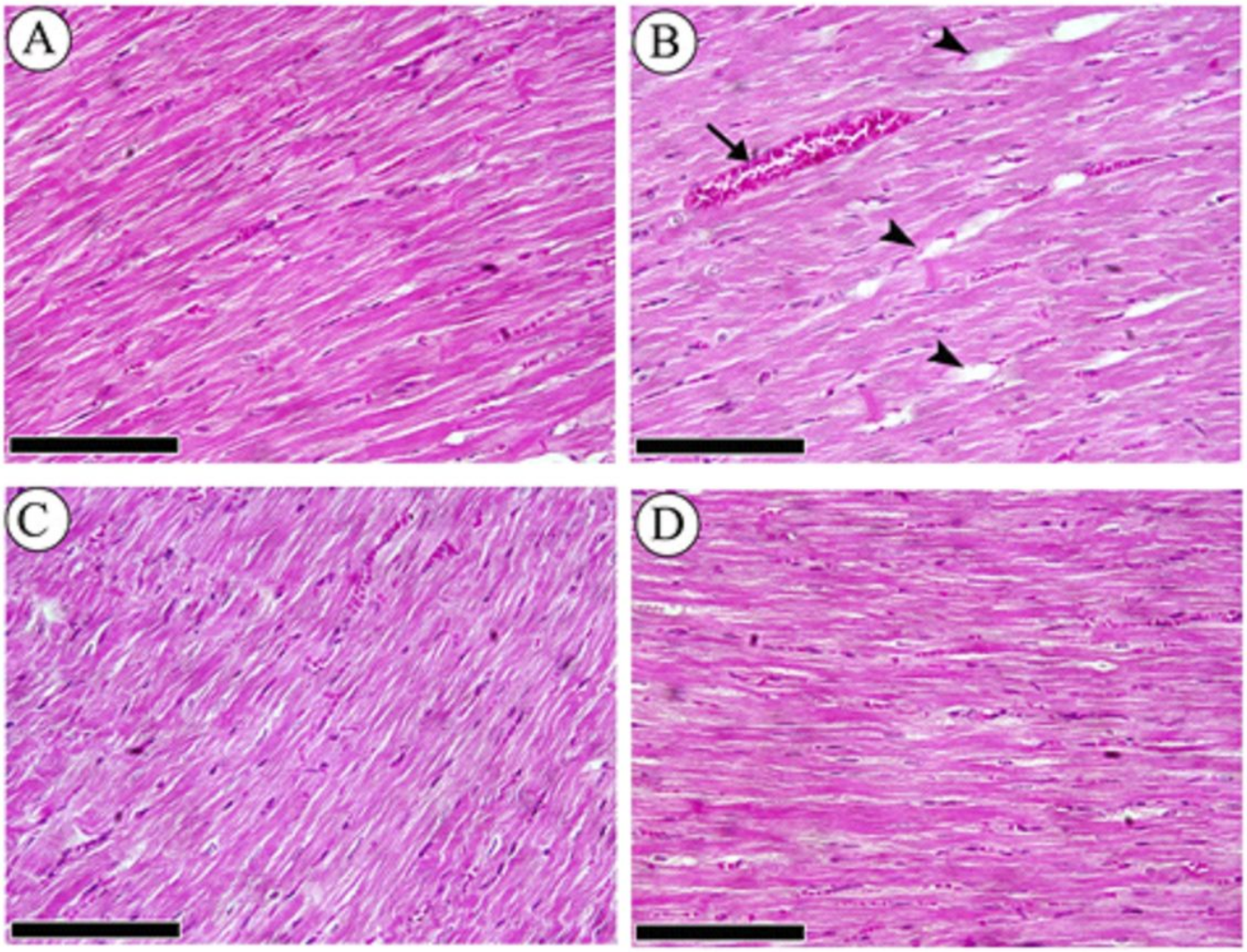

Figure 5

Effects of BL (25 and 50 mg/kg) supplementation on hypercholesterolemia-induced histopathological changes in cardiac tissues (X400). (A) Section from control group, (B) Section from HCD group with multi-focal vacuolar degeneration (heads- arrow) and congestion of blood capillaries (arrow), (C) Section from BL(25) group showing moderate myocardial cell morphology and (D) Section from BL(50) group showing normal looking myocardial cell morphology. Scale bar = 50 μm.

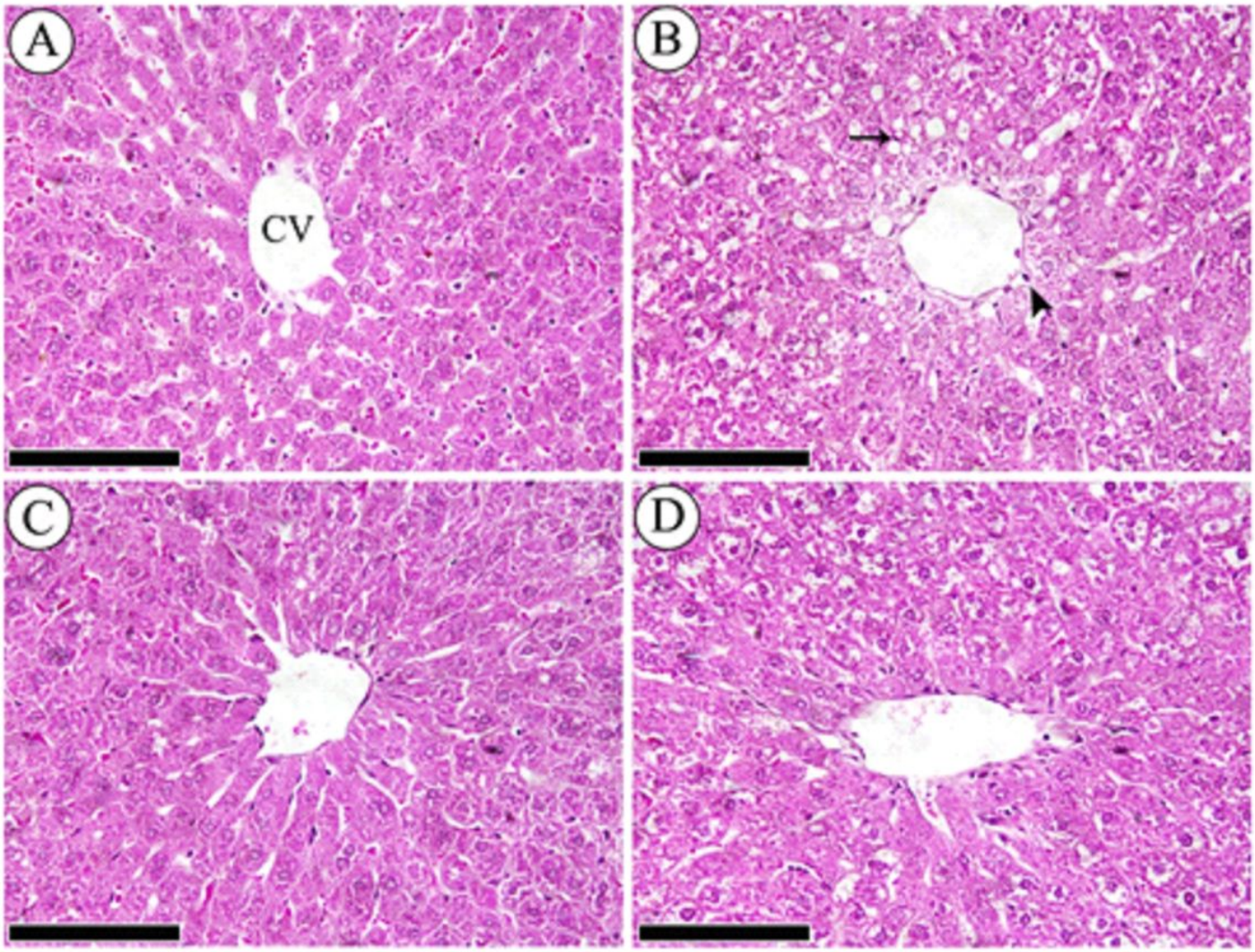

Figure 6

Effects of BL (25 and 50 mg/kg) supplementation on hypercholesterolemia-induced histopathological changes in hepatic tissues (X400). (A) Section from control group, (B) Section from HCD group with marked fat deposition (arrow), dilated sinusoids and pyknotic nuclei (head arrow), (C) Section from BL(25) group showing injury in hepatocytes and less fat deposition and (D) Section from BL(50) group showing moderate injury in hepatocytes and less fat deposition. Scale bar = 50 μm.

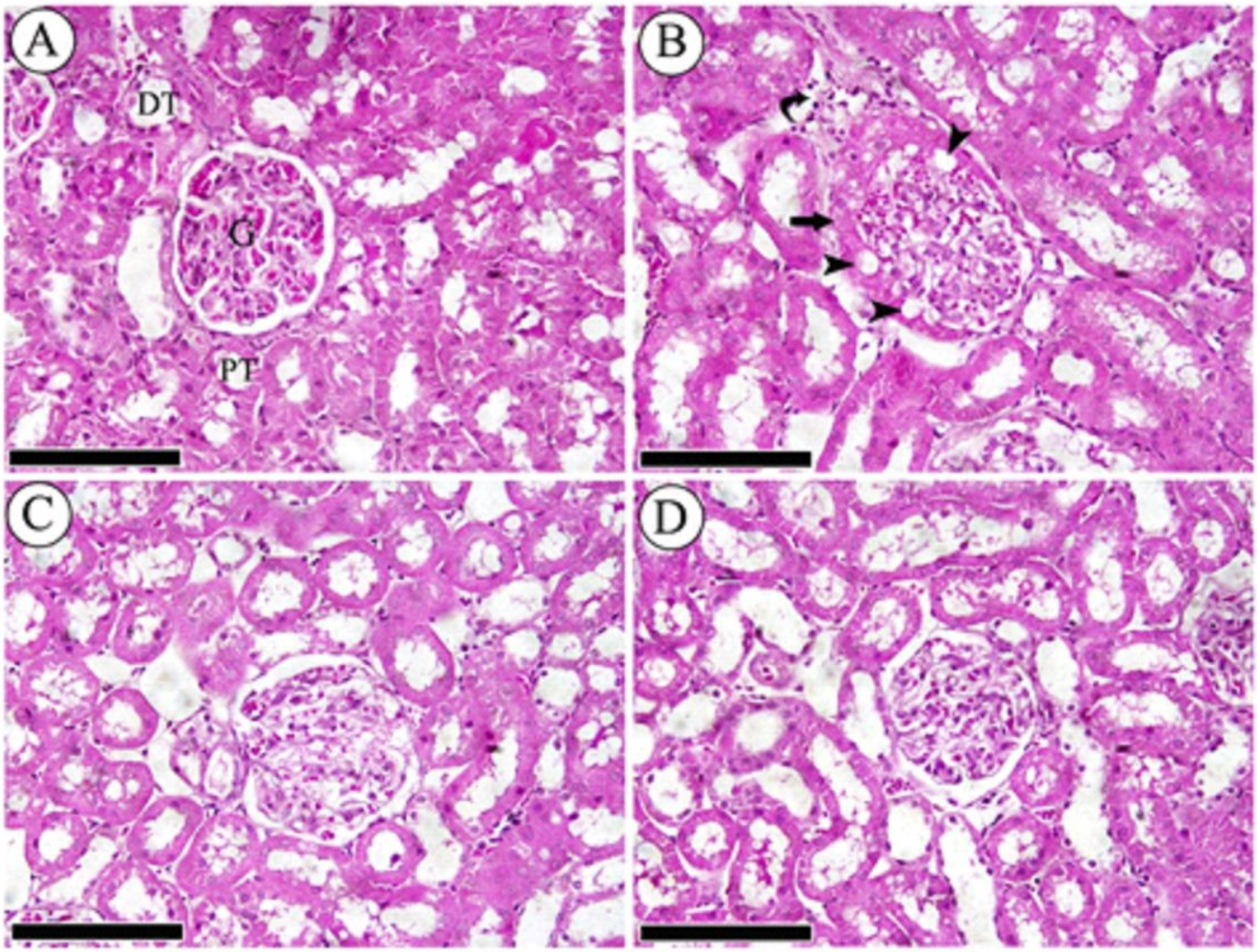

Figure 7

Light micrographs of renal cortex of rats fed high cholesterol diet and administered orally with two doses of Baicalein (25 and 50 mg / Kg bwt.). Section from the renal cortex of the control group reveals the normal appearance of the proximal convoluted tubules (PT), distal convoluted tubules (DT), Bowman's capsule and glomerulus (G) (A). Renal cortex of rats fed high cholesterol showed dilatation in glomerular capillaries (head arrow), thickening in basal membrane of glomerulus (arrow) and mononuclear cell infiltration was seen (curved arrow) (B). Renal cortex of high cholesterol diet treated with (25 mg /Kg bwt, C) and (50 mg / Kg bwt., D) of Baicalein showed reduced injury in glomeruli and renal tubules. H&E, scale bar = 50 µm.

Supplementary Files

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- ARRIVE.pdf