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Original Article

Herniarin Mitigates High-Fat Diet-Induced Atherosclerosis in Rats via Regulation of Hyperlipidemia, Endothelial Dysfunction, Inflammation and Oxidative Stress

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ABSTRACT

Background: Atherosclerosis is a complex and multifactorial disease characterized by the buildup of plaque within the blood vessels, which can result in severe cardiovascular problems. **Objectives:** The present study attempted to elucidate the beneficial activities of herniarin against High-Fat Diet (HFD)-induced atherosclerosis in a rat model. **Materials and Methods:** The HFD-induced atherosclerosis rat model was utilized in the present study and treated with the 50 mg/kg of herniarin for 10 weeks. The simvastatin was utilized as standard drug. The body weight of the animals was assessed in weekly manner during the study. The concentrations of lipid markers, HMG-CoA reductase, collagen and calcium in the rats were assessed using kits. The concentrations of oxidative stress-related markers, inflammation-related markers, endothelial dysfunction markers and liver function enzyme activities were investigated using kits. The heart tissues were excised and employed for the histopathological studies. **Results:** The present results have proved that herniarin treatment at 50 mg/kg concentration successfully decreased body weight and increased heart weight in the rats. The levels of lipid markers, HMG-CoA reductase, collagen and calcium levels were successfully decreased by the herniarin treatment in the rats with HFD-fed atherosclerosis. Furthermore, it also increased the antioxidant concentrations, reduced inflammatory markers, elevated vasodilator and reduced vasoconstrictor levels in the rats with atherosclerosis. These findings are also corroborated by the results of histopathological analysis. **Conclusion:** In conclusion, our research shows that herniarin treatment may inhibit atherosclerosis development, lower blood lipids, pro-inflammatory cytokines and oxidative stress and ameliorate HFD-induced atherogenic condition in the rats. Therefore, it was clear that herniarin may produce a favorable therapeutic effect against atherosclerosis caused by a HFD.

Keywords: Atherosclerosis, Dyslipidemia, Endothelial Dysfunction, Herniarin, HMG-CoA reductase.

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INTRODUCTION

Atherosclerosis is a chronic and progressive disease characterized by accumulation of plaque within the blood vessels, resulting in narrowing and stiffening of the blood vessels. The mechanisms of accumulation of atherogenic plaques is multifaceted that involves a combination of factors; including lipid retention, oxidation and chronic inflammation.[1] The prevalence of atherosclerosis is widespread, affecting a significant portion of the population worldwide. Atherosclerosis is a leading cause of cardiovascular complications, contributing to the development of conditions such as coronary artery disease, stroke and peripheral artery disease. The exact pathophysiology of atherosclerosis involves a multifaceted process that begins with endothelial dysfunction and progresses through various stages.[2] The initial stage of atherosclerosis involves the retention and oxidation of low-density lipoprotein cholesterol within the sub-endothelial space of the blood vessels. This triggers an inflammatory response, leading to the recruitment and activation of immune cells, such as monocytes, which differentiate into macrophages. The macrophages then internalize the oxidized Low-Density Lipoprotein (LDL), forming foam cells that gather within the arterial wall, creating the fatty streaks characteristic of early atherosclerotic lesions.[3] As the disease progresses, the inflammatory reaction continues and the accumulation of foam cells and other cellular components, such as Smooth Muscle Cells (SMCs), results in the development of a more complex atherosclerotic plaque.[4]

The specific pathophysiology of atherosclerosis comprises an interplay between various cellular and molecular mechanisms. Endothelial dysfunction, lipid retention and oxidation, inflammation and the proliferation of SMCs all contribute to

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the development of atherosclerotic plaques.[5] Rodent models, particularly rats, have been widely used to study the pathogenesis and potential therapeutic interventions for atherosclerosis. One of the commonly used models is the HFD induced atherosclerosis model in rats.[6] This model involves feeding the animals a diet rich in saturated and trans-fats, leading to the development of atherosclerotic lesions over time. This model is advantageous as it mimics the dietary and metabolic factors that contribute to atherosclerosis in humans, such as obesity and insulin resistance.[7] The HFD-fed atherosclerosis model in rats has been extensively utilized to investigate the underlying mechanisms driving the progression of atherosclerosis. This model has been valuable for evaluating the potential of therapeutic candidates for atherosclerosis.[8]

Researchers have used the model to investigate the potentials of numerous pharmacological candidates, dietary modifications and lifestyle interventions on the severity and progression of atherosclerotic lesions. Over the years, researchers have been exploring the potential of natural plant-derived compounds as an alternative or complementary approach to the treatment of atherosclerosis. It has been already explored the potential of plant-based bioactive compounds as therapeutic interventions for atherosclerosis. These bioactive compounds, derived from various plant sources, have shown promise in targeting the underlying mechanisms of atherosclerosis development, such as inflammation, cholesterol accumulation and oxidative stress.[9] These compounds have been found to improve endothelial function, reduce inflammation and inhibit the growth of SMCs, which are critical in the progression of atherosclerotic plaque development. While the potential of plant bioactive compounds in the treatment of atherosclerosis is promising, it is crucial to further comprehend their underlying mechanisms, as well as their safety and efficacy in clinical settings.[10]

Herniarin is the well-known bioactive coumarin compound found extensively in several medicinal plants that belong to the Caryophyllaceae, Moraceae, Rutaceae and Compositae families. Furthermore, it has been documented to exhibit various pharmacological properties, including antigenotoxic, anticancer; anxiolytic, antidepressive and neuroprotective properties.[11-14] In addition to these biological activities, the advantageous benefits of herniarin on atherosclerosis have not yet been scientifically addressed. Thus, the current study attempted to elucidate the salutary properties of herniarin against HFD-fed atherosclerosis in a rat model.

MATERIALS AND METHODS

Chemicals

The major chemicals and reagents such as herniarin, simvastatin, etc., utilized in this study was commercially procured from Sigma Aldrich, USA. The assay kits for the determination of biochemical markers were procured from Elabscience, USA.

Experimental rats

The present study utilized healthy, 6-8-week-old male Wistar rats weighing approximately 160±50 g. The protocols for the animal experimentations were verified and approved by institutional animal ethical committee. The rats were acclimated in a controlled laboratory environment for 7 days. The rats were accommodated in sterilized polypropylene cages. Stringent hygienic standards were upheld over the period of the experiments and cages and bedding were regularly replaced. Standard temperature and relative humidity were maintained continuously and 12 hr light/dark cycle was maintained. All rats were provided with unrestricted contact to a regular pellet diet and drinking water.

Induction of atherosclerosis

The experimental rats were experimentally developed with atherosclerosis by administering a high-fat diet including sucrose (25%), casein protein (20%) and beef tallow (40%), in addition to the regular laboratory pellet diet, for 10 weeks. The confirmation of atherosclerosis induction was achieved by biochemical and histological investigations.

Treatment groups

The rats were arbitrarily allocated into four groups, with each group consisting six rats (n=6). In Group I, rats are designated as normal control rats and are exclusively fed with normal pellet diets, which consisting casein protein (20%), corn oil (5%) and starch (60%). Atherosclerosis-induced rats in Group II were exclusively fed a HFD for 10 weeks. Group III rats were subjected to atherosclerosis-induction by HFD and subsequently treated with herniarin (50 mg/kg) through oral gavage route for 10 weeks. The rats in Group IV were induced with atherosclerosis and administered a standard drug simvastatin at 10 mg/kg concentration for 10 weeks. The body weight of the animals was weighed regularly. Following the conclusion of the treatment schedule, rats were anesthetized, sacrificed and blood samples were obtained using non-anticoagulated centrifuge tubes. Cardiac tissues were obtained from the rats, weighed precisely and preserved at -80°C for biochemical and histological examinations. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Xi 'an Jiaotong University (protocol number 2024-096).

Analysis of lipid markers

The serum samples from the rats were analysed to quantify the lipid biomarker levels, including Total cholesterol (TCh), LDL, Very-Low-Density Lipoprotein (VLDL) and High-Density Lipoprotein (HDL) using the commercial assay kits (Elabscience, USA). Triglycerides (TG) concentrations were quantified using kits obtained from Elabscience, USA. All the assays were conducted with three replicates using the methodology provided by the manufacturer.

Analysis of Hydroxymethylglutaryl-CoA (HMG-CoA) reductase, collagen, calcium and total protein levels in the liver

The concentrations of HMG-CoA reductase, collagen, calcium and total protein in the liver tissue homogenates from rats were investigated using the commercially procured kits. All the assays were conducted with three replicates using the described protocols of the manufacturer (Elabscience, USA).

Analysis of oxidative stress marker levels in the serum

The levels of oxidative stress-related biomarkers such as Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) in the serum of both control and experimental rats were evaluated using commercially available assay kits. All assays were completed in triplicate as per the manufacturer's protocols (LSBio, USA).

Analysis of Liver Function Marker Enzymes

The concentrations of liver biomarker enzymes like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) in the serum of rats were evaluated by using the commercially available kits. Each assay was conducted with three replicates using the described protocols of the manufacturer (Cusabio, USA).

Analysis of inflammatory cytokine levels

Pro-inflammatory cytokine indicators Interleukin-8 (IL-8), IL-18, IL-1β and C-Reactive Protein (CRP) concentrations were quantified in the serum using the commercially available kits. The respective kits were obtained from Abbexa, UK and the tests were conducted in triplicate according to the instructions outlined in the kit's manual.

Analysis of 6-keto-prostaglandin F 1α (6-keto-PGF 1α) and Endothelin (ET) levels

The 6-keto-PGF 1α and ET concentrations were quantified in the serum of both control and experimental rats using the commercially available kits. The respective kits were purchased from Cayman Chemical, UK and the tests were conducted in triplicates as per the instructions outlined in the kit's manual.

Histopathological analysis

The heart tissues were surgically removed from the experimental animals and the tissues were preserved in 10% formalin. The tissues were then subsequently dehydrated and treated using a gradient mixture of ethyl alcohol, water and xylene. The treated tissues were fixed in paraffin wax and cut into sections of 5 μM in thickness. The dissected tissues were subsequently stained with eosin and hematoxylin and analyzed histopathologically in a blinded manner using a light microscope.

Statistical analysis

The results are studied using GraphPad Prism software and the outcomes were presented as Mean±SD of 3 replicates. The one-way ANOVA method was employed for intergroup comparison, followed by the Tukey's *post hoc* test for multiple group comparisons with $p<0.05$ as significant.

RESULTS

Effect of herniarin on the body weight and heart weight of the experimental rats

The findings highlighted that HFD consumption resulted in substantial impairments in the rats, as indicated by a notable elevation in both body weight as well as heart eight in comparison with rats fed with normal diet (Figure 1). Interestingly, the oral treatment of 50 mg/kg of herniarin markedly effectively reduced the body weight gain as well as heart weight of atherosclerosis rats. The results of the herniarin treatment further supported by the standard drug simvastatin treatment, which also effectively reduced the heart and body weight of the rats with atherosclerosis (Figure 1).

Effect of herniarin on the lipid biomarker levels in the serum of experimental rats

The present results indicated that an HFD administration caused significant impairments in the lipid profiles of the rats, as indicated by marked increase in TC, TGs, LDL, VLDL and FFAs, along with a substantial decrease in HDL in comparison with rats on a standard diet (Figure 2). Whereas, the herniarin at 50 mg/kg concentrations, markedly diminished the elevated serum concentrations of TC, TGs, LDL, VLDL and FFAs, while remarkably increasing the diminished level of HDL in comparison to rats fed an HFD. Similarly, the standard drug simvastatin was also markedly lowered the TC, TGs, LDL, VLDL and FFAs levels while boosted the HDL concentrations, which further supports the anticholesteremic properties of the herniarin (Figure 2).

Effect of herniarin on the HMG-CoA reductase, collagen, calcium and total protein levels in the experimental rats

The present findings indicated that HMG-CoA reductase, collagen, calcium and total protein concentrations were substantially elevated in the liver tissues of HFD-fed rats (Figure 3). Contrastingly, the oral treatment of herniarin at 50 mg/kg concentration exhibited a considerable reduction in the concentrations of HMG-CoA reductase, collagen, calcium and total protein in the liver tissues when compared to rats on a normal diet. Concurrently, simvastatin treatment also demonstrated a considerable reduction in these marker levels, which supports the activity of herniarin (Figure 3).

Effect of herniarin on the oxidative stress-related marker levels in the serum of experimental rats

The impact of herniarin on oxidative stress response was assessed by evaluating oxidative stress-related marker levels in the experimental rats (Figure 4). The present results indicated a decreased in SOD, CAT and GSH concentrations and subsequent elevation in MDA concentration in the rats with HFD-fed atherosclerosis when compared with rats fed with normal diet. Notably, the 50 mg/kg Od herniarin treatment was successfully boosted the antioxidants such as CAT, SOD and GSH and effectively diminished the MDA levels in the rats with HFD-fed atherosclerosis. In similar manner, the simvastatin treatment also resulted in an increased antioxidant levels and reduced MDA levels in the rats with atherosclerosis, which proves the antioxidant properties of the herniarin (Figure 4).

Effect of herniarin on the hepatic marker enzymes in the serum of experimental rats

Concerning liver function biomarkers, the present results indicated a drastic augmentation in the ALT, AST, ALP and LDH enzyme activities in the rats with HFD-fed atherosclerosis. In contrast, the treatment with 50 mg/kg of herniarin treatment markedly reduced the ALT, AST, ALP and LDH activities in the rats with atherosclerosis, demonstrating considerable improvement in liver functions (Figure 5). The similar outcomes are also noted in the simvastatin-treated atherosclerotic rats, which also decreased these marker enzyme activities.

Effect of herniarin on the inflammatory cytokine levels in the serum of experimental rats

The current findings demonstrated the drastic elevation in the concentrations of IL-8, IL-18, IL-1β and CRP in the serum of HFD-fed rats with atherosclerosis compared to the control group (Figure 6). In contrast, the oral treatment of herniarin at

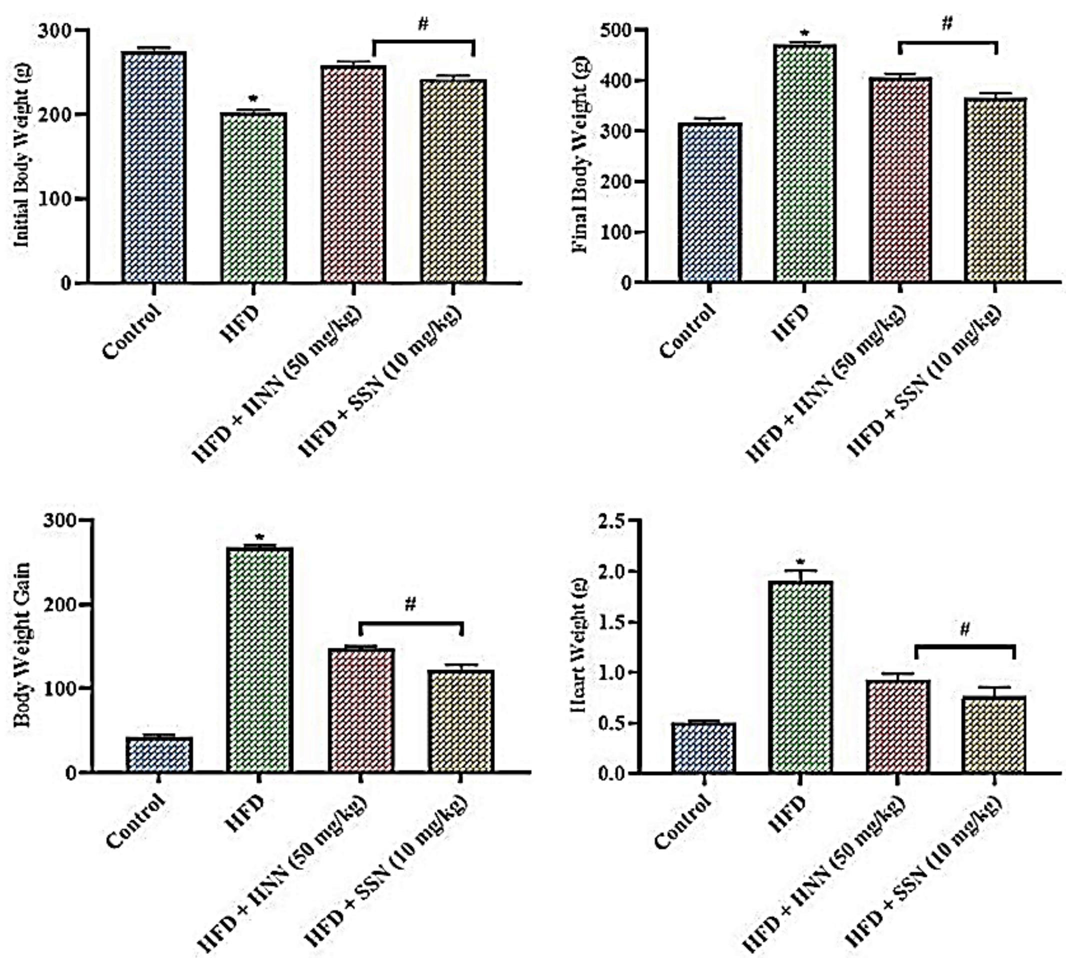

Figure 1: Effect of herniarin on the body weight and heart weight of the experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of three replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

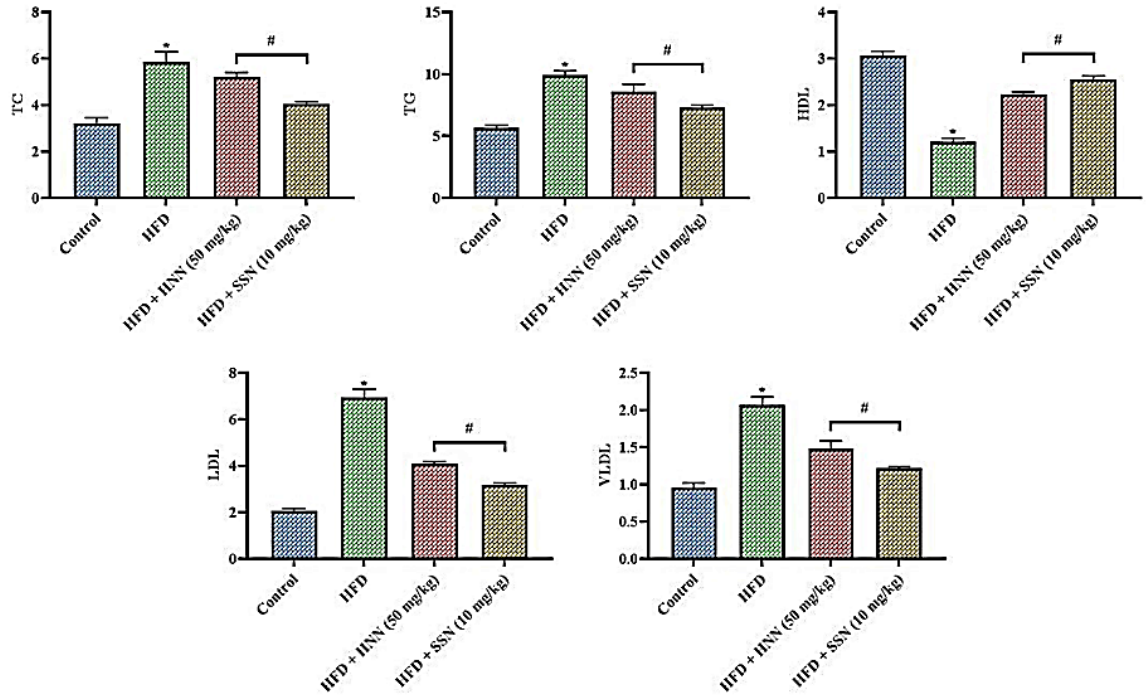

Figure 2: Effect of herniarin on the lipid biomarker levels in the serum of experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of three replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

a concentration of 50 mg/kg resulted in a notable diminution in the IL-8, IL-18, IL-1β and CRP concentrations in the serum of rats with HFD-fed atherosclerosis compared to rats with standard diet (Figure 6). Simvastatin treatment concurrently has shown a significant reduction in these inflammatory cytokine levels, hence corroborating the anti-inflammatory efficacy of herniarin.

Effect of herniarin on the 6-keto-PGF 1α and ET levels in the serum of experimental rats

The current findings revealed that the HFD consumption drastically elevated the ET and subsequent reduction in 6-keto-PGF 1α concentrations in their serum in comparison to rats fed with normal diet. However, the herniarin at 50 mg/kg dosage significantly decreased the ET levels, while substantially increasing the 6-keto-PGF 1α levels compared to HFD-fed rat (Figure 7). Likewise, the simvastatin treatment also diminished the ET concentration and elevated the 6-keto-PGF 1α levels, thereby supporting the activity of herniarin.

Effect of herniarin on the heart histopathology of the experimental rats

The histopathological investigation of heart tissue from rats on a normal diet revealed a typical cardiac architecture characterized by striated muscle fibers and centrally positioned nuclei (Figure 8). Rats administered a HFD exhibited numerous pathological alterations, including degenerated cardiac muscle with intracytoplasmic fat vacuoles, inflammatory cell infiltrations, arterial walls with fat deposition, degeneration of congested artery walls and myocytes exhibiting loss of striations and pyknotic nuclei. Interestingly, the treatment of herniarin at 50 mg/kg concentrations to rats on a HFD resulted in significant amelioration of the observed pathological alterations in their heart tissues. Furthermore, the simvastatin treatment also efficiently ameliorated the histological alterations in the HFD-fed rats, which is similar to the herniarin treatment. Overall, these findings proved that the histological alterations in HFD-fed rats were remarkably ameliorated by the herniarin (Figure 8).

DISCUSSION

Atherosclerosis is a complex and multifactorial disease characterized by the buildup of plaque within the arteries, which can result in severe cardiovascular complication.[15] To better comprehend the pathogenesis and progression of this condition, researchers often utilize animal models, such as the HFD-fed atherosclerosis in rats, to investigate the underlying mechanisms and potential therapeutic interventions. In accordance, the current study was undertaken to assess the beneficial roles of the herniarin against HFD-fed atherosclerosis in rats. In this context, the analysis of body weight gain and heart weight in the herniarin-treated HFD-induced atherosclerosis rat model can provide valuable insights into the overall metabolic and cardiovascular changes associated with the disease. Body weight gain are important parameters to consider in the HFD-induced

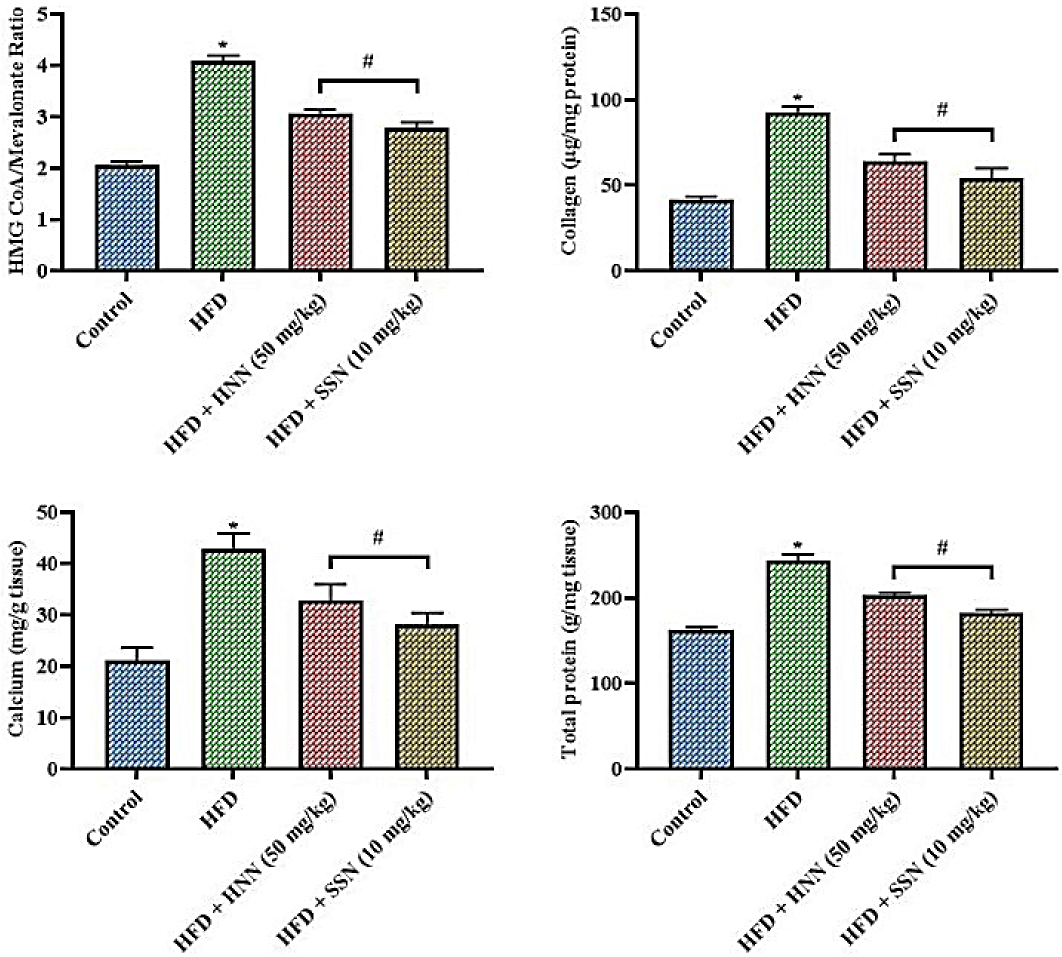

Figure 3: Effect of herniarin on the HMG-CoA reductase, collagen, calcium and total protein levels in the liver tissues of the experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of three replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

atherosclerosis rat model, as they can reflect the overall metabolic changes and the impact of the diet and potential drug treatments on the animals. HFDs are known to induce weight gain and obesity, which are major risk factors for the atherosclerosis.[16] By monitoring the body weight and weight gain of the animals, researchers can assess the efficacy of the HFD in inducing the desired metabolic alterations and the potential ameliorating effects of the drug treatment. The analysis of heart weight in the HFD-fed atherosclerosis in rats is crucial, as it can provide insights into the cardiovascular changes associated with the disease. Atherosclerosis can result in the thickening of the blood vessel walls, which can in turn increase the workload on the heart, potentially resulting in cardiac hypertrophy.[17] By measuring the heart weight of the animals, researchers can evaluate the impact of the HFD and the drug treatment on the heart's structural and functional changes, which may be indicative of the progression or regression of the atherosclerotic disease. In line with these statements, the present evaluated the body weight gain and heart weight of the experimental rats. The present findings clearly proved the significant elevation in the body weight and subsequent diminution in heart weight of the HFD-fed atherosclerosis rats. Interestingly, the herniarin treatment successfully reduced the body weight and reduced heart weight of the rats with atherosclerosis.

The pathogenesis of atherosclerosis is multifaceted that comprises several cellular and molecular events. Lipid metabolism plays a central role in the pathogenesis of atherosclerosis.[18] Understanding the role of lipid biomarkers in the pathophysiology of this condition is crucial for effective prevention, diagnosis and management strategies. Dysregulation of lipoprotein metabolism, characterized by increased TC, TGs, LDL and VLDL concentrations and decreased HDL levels, has been consistently connected with the advancement of atherosclerotic lesions. Elevated levels of LDL, in particular, have been known as a primary cause for the initiation and progression of atherosclerosis, as these markers are known to transport cholesterol and other lipids into the arterial wall, leading to the formation of fatty streaks and plaque buildup.[19] Inflammation and oxidative stress play essential roles in the conversion of

LDLs to their oxidized form, which is highly atherogenic.[20] The role of TGs in atherosclerosis is also well-recognized, as they are embedded in VLDL and chylomicrons that circulate in the bloodstream. Lipoprotein lipase, an enzyme responsible for the hydrolysis of these TG-rich lipoproteins, plays a significant role in the pathophysiology of certain familial dyslipidemias and the management of high serum TG.[21] In contrast, HDL have been shown to exert a protective effect against atherosclerosis, as they are involved in the reverse cholesterol transport process, which facilitates the removal of cholesterol from peripheral tissues, including the arterial wall and its subsequent transport to the liver for excretion.[22] The present findings proved that the rats with HFD-induced atherosclerosis demonstrated an elevated TC, TG, LDL and VLDL concentrations while reduced their HDL concentrations. Though, the herniarin treatment successfully reduced the TC, TG, LDL and VLDL concentrations and elevated the HDL concentration in the rats with atherosclerosis, which proves it lipids lowering effects.

HMG-CoA reductase is an essential enzyme in the cholesterol biosynthesis mechanisms and its activity is closely linked to the onset of atherosclerosis. Increased concentrations of this enzyme contribute to the excess production of cholesterol, which can then be modified and oxidized, resulting in the accumulation of foam cells and the initiation of the atherosclerotic cascade. The dysregulation of macrophage activation, driven by modified lipids, cholesterol crystals and other mediators, plays a central role in this process, contributing to the proinflammatory and anti-inflammatory phenotypes observed in atherosclerotic plaques.[23] Collagen, a structural protein found in the extracellular matrix of the arterial wall, also plays a crucial role in the pathogenesis of atherosclerosis. The deposition of collagen within the plaque, coupled with the SMCs growth, results in the development of a fibrous cap that can ultimately become unstable and rupture, triggering thrombotic events and the progression of the disease.[24] Calcium, a key mineral involved in various cellular processes, has also been participated in the advancement of atherosclerosis. The formation of calcium-containing deposits, or calcifications, within the arterial wall can facilitate the hardening and stiffening of the vessel, further compromising its function and increasing the risk of cardiovascular problems.[25] Total protein levels, which reflect the overall protein content in the body, can provide insight into the inflammatory status and metabolic disturbances associated with atherosclerosis. Alterations in total protein levels, particularly in combination with other biochemical markers, can help elucidate the interplay of factors involved in the pathophysiology of this disease.[26] The observed findings in this study highlighted that the levels of HMG-CoA reductase, collagen, calcium and total proteins were remarkably elevated in the atherosclerosis rats. Interestingly, the treatment with the herniarin successfully reduced these biomarker levels in the rats with atherosclerosis.

Oxidative stress is a condition, which is characterized by an disproportion between the excess generation of ROS and inadequate antioxidant mechanisms. This oxidative stress plays a central role in the onset of atherosclerosis, leading to the conversion of LDLs to their oxidized form (ox-LDL), which is highly atherogenic.[27] Oxidative stress can instigate endothelial dysfunction and inflammation, affecting various cells within the vascular wall. MDA, a marker of lipid peroxidation, has been consistently elevated in patients with atherosclerosis, reflecting the increased generation of ROS.[28] SOD and CAT, two key antioxidant enzymes, have been found to have diminished activities in atherosclerosis, indicating an impaired antioxidant defense system. Similarly, reduced levels of GSH, a crucial intracellular non-enzymatic antioxidant, have been observed in atherosclerotic conditions.[29] The excess of oxidative stresses and the imbalance in these oxidative stress-related markers contribute to the sub-endothelial deposition of oxidized LDL, the

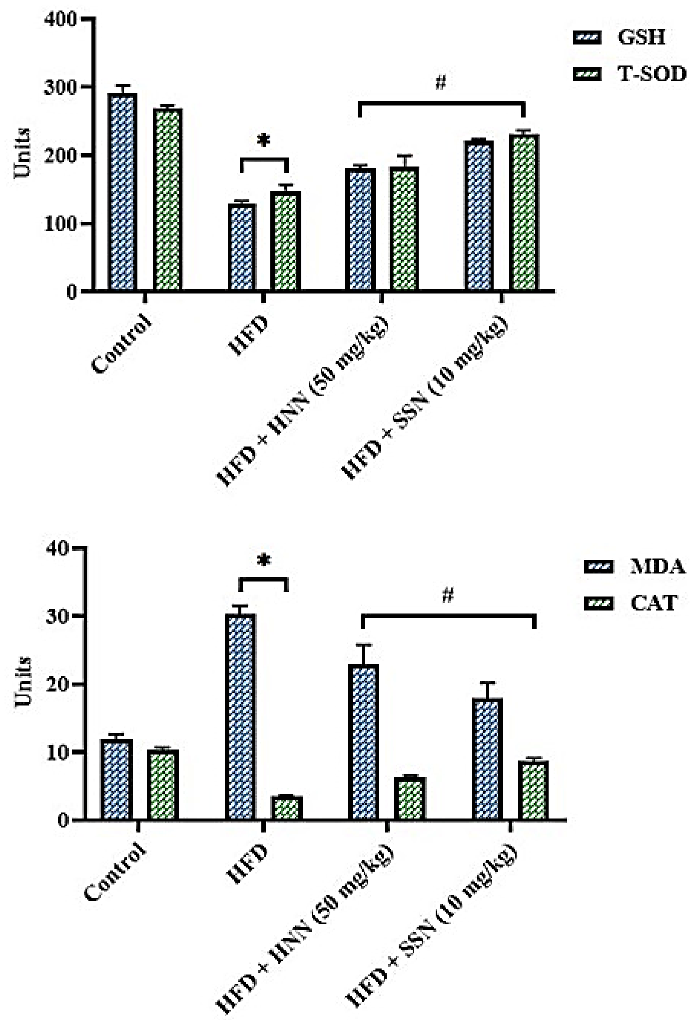

Figure 4: Effect of herniarin on the oxidative stress-related marker levels in the serum of experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of three replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

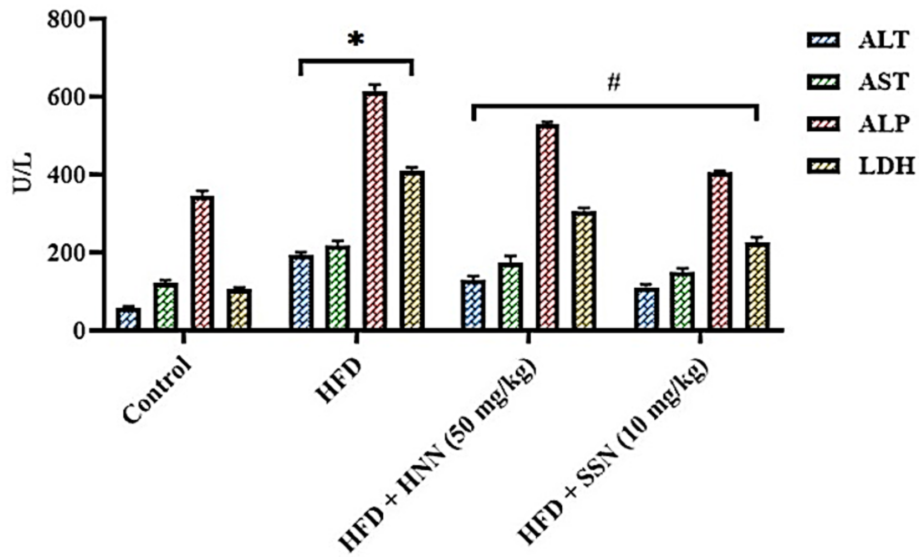

Figure 5: Effect of herniarin on the hepatic marker enzymes in the serum of experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of three replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

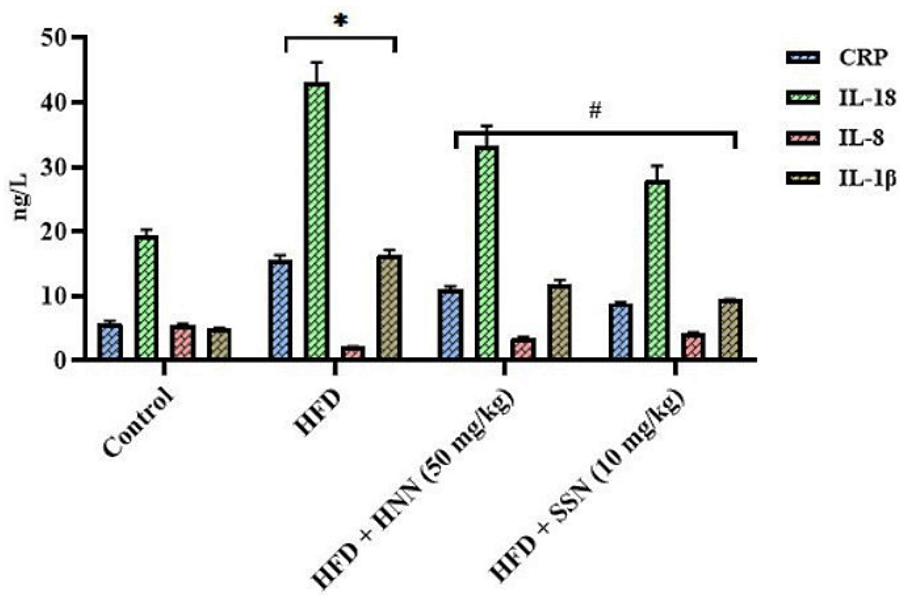

Figure 6: Effect of herniarin on the inflammatory cytokine levels in the serum of experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of three replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

formation of lipid-laden foam cells, vascular SMCs growth and the collagen deposition-all of which are key to the pathogenesis of atherosclerosis.[30] Targeting oxidative stress-related markers may offer promising therapeutic strategies to prevent and manage atherosclerosis. The present results highlighted that occurrence of increased TC, TG, LDL and VLDL and subsequent reduction in the HDL levels in the rats with HFD-fed atherosclerosis. Whereas, the herniarin treatment substantially decreased the TC, TG, LDL and VLDL concentrations and subsequently boosted the HDL concentration in the rats with atherosclerosis.

Liver function biomarker enzymes, like ALT, AST, ALP and LDH, have been implicated in the pathophysiology of HFD-induced atherosclerosis. These enzymes are typically elevated in individuals with fatty liver disease, which is a common comorbidity in individuals with atherosclerosis.[31] The role of liver marker enzymes in this process is multifaceted. Elevated levels of these enzymes, particularly ALT and AST, have been connected with high risk of atherosclerosis. These enzymes are participated in the metabolism of various substances, including lipids and glucose and their dysregulation can lead to alterations in the lipid profile and inflammation, both of which are key drivers of atherosclerosis.[32] Furthermore, liver marker enzymes can also serve as indicators of liver dysfunction, which is often comorbid with metabolic disorders. These conditions are known to participate in the onset of atherosclerosis through their effects on lipid metabolism and inflammatory pathways.[33] The findings of this work has highlighted that rats with HFD-fed atherosclerosis revealed drastic elevation in the AST, ALT, ALP and LDH activities than normal diet-fed rats. Whereas, the herniarin treatment successfully reduced these liver function marker enzyme activities in the rats with atherosclerosis. These findings proved that herniarin may facilitate liver function and lipid metabolism in atherosclerosis rats.

The onset of atherosclerotic lesions is characterized by interplay of various inflammatory markers, including IL-8, IL-18, IL-1β and CRP.[34] IL-8 is a potent chemokine that plays an essential role in the recruitment and neutrophils activation, which are key players in the inflammatory process. High concentrations of IL-8 have been associated with the onset of atherosclerosis, as it promotes the migration and adhesion of immune cells to the vascular endothelium, leading to the onset of inflammation.[35] In addition, IL-18 is a pro-inflammatory cytokine that has been linked to the pathogenesis of atherosclerosis. IL-18 can stimulate the accumulation of other inflammatory markers, like IFN-γ and promote the upregulation of adhesion molecules, further exacerbating the inflammatory state.[36] IL-1β, another key cytokine, plays a central role in the inflammation. IL-1β can induce the expressions of adhesion molecules, chemokines and other proinflammatory markers, contributing to the recruitment of immune cells within the vascular wall.[37,38] CRP, a well-established marker of systemic inflammation, has also been implicated in the onset of atherosclerosis. Increased CRP levels are connected with a high risk of cardiovascular events, as it can promote endothelial dysfunction, foam cell development and plaque destabilization.[39] The interplay of these inflammatory markers is crucial in the progression of atherosclerosis. Atherosclerotic patients have higher concentrations of these markers, highlighting their potential as diagnostic and prognostic tools.[40] The outcomes of this study has demonstrated an elevated IL-8, IL-18, IL-1β and CRP concentrations in the rats with HFD-fed atherosclerosis. Whereas, the concentrations of these inflammatory markers were effectively reduced by the herniarin treatment, which highlights its anti-inflammatory activities.

It has been well known that endothelial dysfunction has paying delicate role in the onset of atherosclerosis.[41] One crucial mediator in the development of atherosclerosis is 6-keto-PGF 1α, a metabolite of prostacyclin. Prostacyclin is an essential vasodilator and inhibitor of platelet aggregation and its levels are typically reduced in atherosclerosis. The decreased generation of 6-keto-PGF 1α can lead to an imbalance in the homeostatic mechanisms that regulate vascular tone and platelet function, contributing to the progression of atherosclerotic lesions.[42] Another key player in the pathogenesis of atherosclerosis is ET, a potent vasoconstrictor. ET has been shown to promote vascular SMCs growth and extracellular matrix deposition, which are all integral to the onset of atherosclerosis. The overexpression of ET can also lead to endothelial dysfunction, further exacerbating the atherosclerotic

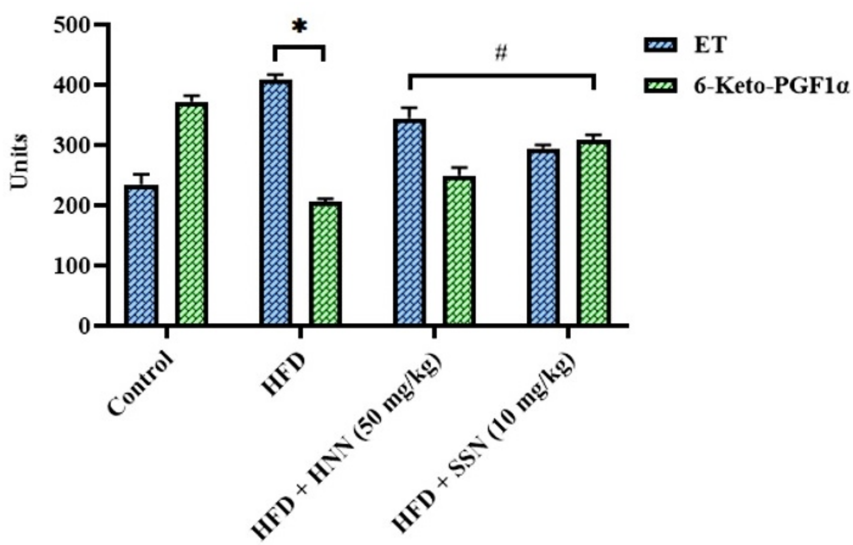

Figure 7: Effect of herniarin on the 6-keto-PGF 1α and ET levels in the serum of experimental rats. The results are studied using GraphPad Prism software and the outcomes were presented as mean±SD of 3 replicates. The statistical significant level between treatment groups were fixed as '*' $p<0.01$ when compared with control vs HFD-induced atherosclerosis group; '#' $p<0.05$ when compared with HFD-fed group vs HFD+herniarin and/or simvastatin-treated group.

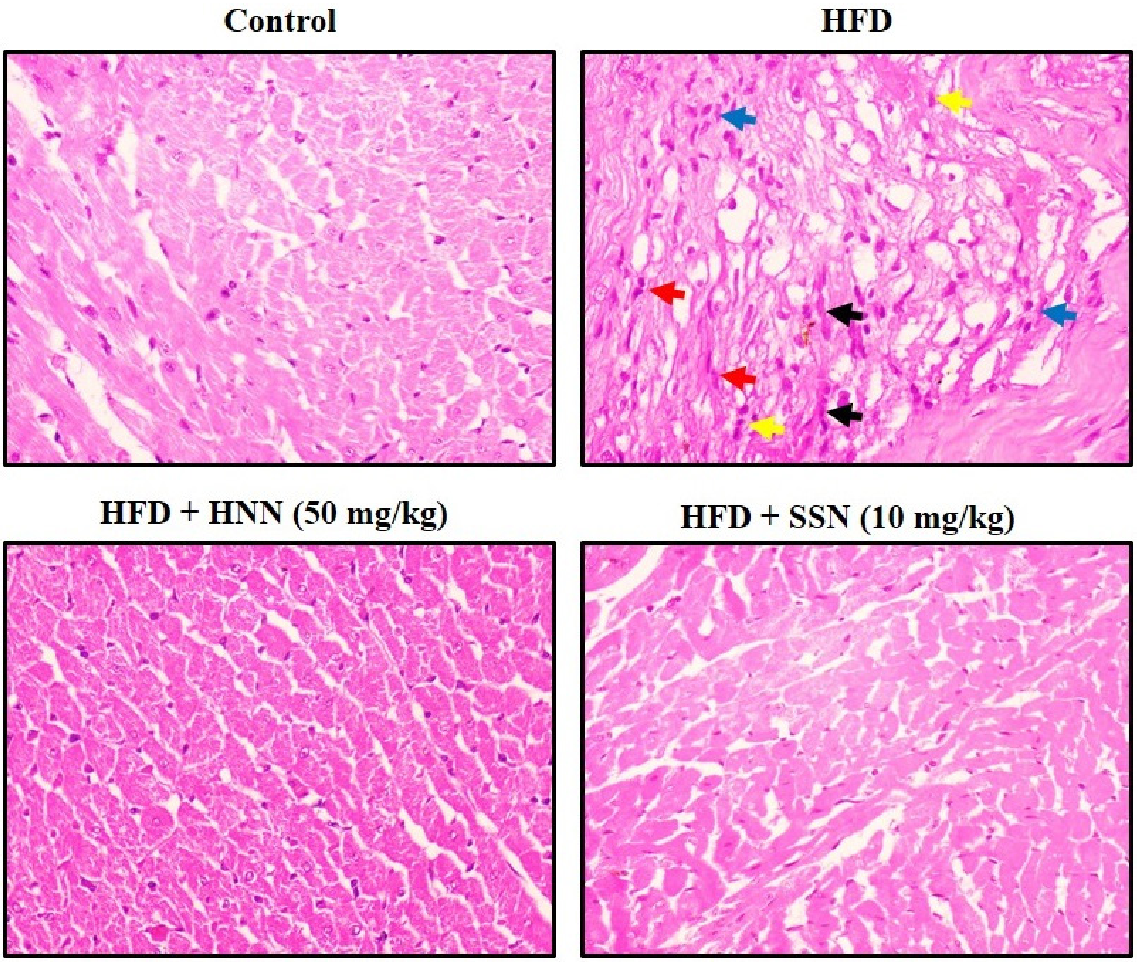

Figure 8: Effect of herniarin on the heart histopathology of the experimental rats. Group I: Control rats fed with normal diet; Group II: HFD-induced atherosclerosis rats; Group III: HFD+Herniarin (50 mg/kg)-treated group; Group IV: HFD+Simvastatin (10 mg/kg)-treated group. Black arrows: Degenerated cardiac muscle with intracytoplasmic fat vacuoles; Blue arrows: Inflammatory cell infiltrations; Yellow arrows: Myocytes exhibiting loss of striations; Red arrows: Pyknotic nuclei.

process.[43] Oxidative stress, a hallmark of atherosclerosis, is also closely linked to the dysregulation of 6-keto-PGF 1α and ET. Oxidized LDL can induce the expression of ET and downregulate the production of prostacyclin, resulting in a pro-atherogenic environment.[44] In the current work, the findings are revealed an increased ET level and subsequently reduced 6-keto-PGF 1α levels in the atherosclerosis rats when compared with the normal diet-induced rats. Remarkably, the treatment with the herniarin successfully reduced the ET level and subsequently boosted the 6-keto-PGF 1α concentrations in the rats with atherosclerosis.

CONCLUSION

In conclusion, our research shows that herniarin treatment may inhibit atherosclerosis development, lower blood lipids, proinflammatory cytokines and oxidative stress and ameliorate HFD-induced atherogenic condition in the rats. Furthermore, herniarin treatment elevated vasodilator levels and reduced vasoconstrictor levels, hence preventing endothelial impairment, as corroborated by the histopathological findings. Therefore, it was clear that herniarin may produce a favorable therapeutic effect against atherosclerosis caused by a HFD through the control of hyperlipidemia, oxidative stress, endothelial dysfunction and inflammation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HFD: High-fat diet; **SMCs:** Smooth muscle cells; **LDL:** Low-density lipoprotein; **VLDL:** Very-low-density lipoprotein; **HDL:** High-density lipoprotein; **TG:** Triglycerides; **HMG-CoA:** Hydroxymethylglutaryl-CoA; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **CAT:** Catalase; **MDA:** Malondialdehyde; **ALT:** Alanine aminotransferase; **ALP:** Aspartate aminotransferase; **LDH:** Lactate dehydrogenase; **ET:** Endothelin.

ETHICAL STATEMENTS

The study was approved by the biomedical ethics committee of Health Science Centre of Xi'an Jiaotong University (XJTUAE2024-2621).

SUMMARY

The present study attempted to elucidate the beneficial activities of herniarin against High-Fat Diet (HFD)-induced atherosclerosis in a rat model. The HFD-induced atherosclerosis rat model was utilized in the present study and treated with the 50 mg/kg of herniarin for 10 weeks. The concentrations of lipid markers, HMG-CoA reductase, collagen and calcium in the rats were assessed using kits. The concentrations of oxidative stress-related markers, inflammation-related markers, endothelial dysfunction markers and liver function enzyme activities were investigated using kits. Herniarin treatment may inhibit atherosclerosis development, lower blood lipids, proinflammatory cytokines and oxidative stress and ameliorate HFD-induced atherogenic condition in the rats. Furthermore, herniarin treatment elevated vasodilator levels and reduced vasoconstrictor levels, hence preventing endothelial impairment, as corroborated by the histopathological findings. Therefore, it was clear that herniarin may produce a favorable therapeutic effect against atherosclerosis caused by a HFD.

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