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Original Article

Anti-Atherosclerotic Potency of Lichen Metabolite Usnic Acid against High Fat Diet Induced Atherosclerosis Rat Model

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ABSTRACT

Background: Cardiovascular diseases and its associated chronic disorders are was and tend to be a global threat for centuries. 80% of cardiovascular diseases were reported in the lower and middle income countries. Chronic atherosclerosis, a chronic circulatory disorder occurs due to the platelet aggregation, thrombosis formation and eventually rupture of atherosclerotic plaque causes excessive mortalities and morbidities worldwide. Previously elderly people were at risk of cardiovascular diseases whereas at present due to life style changes rise in premature chronic atherosclerosis mortality was observed. Since atherosclerosis is mostly asymptomatic diagnosis and treating the disease is a challenging task for the physicians. Lipids lowering drugs, anticoagulants, antiplatelet drugs, diuretics etc were prescribed to treat cardiovascular disease. This medication on long term usage causes enormous side effects which affects the quality of life of the patients. Natural drug are potential alternative for the allopathic drugs to various chronic diseases. **Objectives:** In our study we examined the potency of lichen metabolite usnic acid potency to treat atherosclerosis induction in rats. Atherosclerosis was induced in Wistar rats with high fat diet model. **Materials and Methods:** Food intake and body weight gain in experimental animals were monitored regularly. To confirm the induction of atherosclerosis in rats the complete lipid profile was assessed with commercially available kits. Atherogenic index, TC/HDLc and LDLc/HDLc were also calculated to further confirm the high fat diet induced hyperlipidemic condition. Cardiac profile was quantified to evaluate the ameliorative potency of usnic acid against high fat diet induced atherosclerosis. Inflammation the prime initiator of atherosclerosis hence pro-inflammatory cytokines and C-reactive protein were measured in the experimental rats. To assess the impact of usnic acid on high fat diet induced endothelial dysfunction the levels of NO, ET, 6-keto-PGF1α and TXB2 in serum of experimental rats. Finally, to confirm the anti-atherosclerotic property of usnic acid histological analysis of aorta was performed. **Results:** Usnic acid significantly prevented weight gain, dysregulation of lipid and cardiac profile in high fat diet induced rats. It also inhibited the inflammatory response via decreasing the levels of pro-inflammatory cytokines. Usnic acid potentially increased the NO, 6-keto-PGF1α and decreased ET, TXB2 thereby prevented high fat diet induced endothelial dysfunction in experimental rats. Histopathological analysis endorses the ameliorative potency of usnic acid against high fat diet induced atherosclerosis in rats. **Conclusion:** Altogether our study reveals usnic acid can be a potent drug to treat high fat diet induced atherosclerosis and can be subjected to further research.

Keywords: Atherosclerosis, High fat diet, Inflammation, Dyslipidemia, Usnic acid.

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INTRODUCTION

On the current era one of the most challenging diseases treated by the global physicians is Cardiovascular Disease (CVD). Even though a sustained reduction in the morbidity and mortality rate of cardiovascular disease was observed in past few decades the CVD tends to be the foremost causes of global pre mature mortality.[1] Cardiovascular disease is an umbrella term which comprises group disorders related to blood vessels and heart such as stroke, coronary heart disease, rheumatic heart disease etc., Out of these disorders four out five mortality occurs in cardiovascular patients are due to stroke and coronary heart disease.[2] Each year about 17.9 million deaths were reported globally out of which 1.16 million deaths were reported in lower and middle income countries are due to hypertension.[3] It has been estimated by the year 2030 about 23.6 million deaths per year be likely to be reported worldwide.[4] Coronary atherosclerosis is fatal chronic

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cardiovascular disease which is more prevalent among aged population.

Atherosclerosis is a multifactorial chronic inflammatory disease characterized by the deviant lipid metabolism, calcification and plaque deposition within the arteries, foaminess of macrophages.[5] Further aggregation of platelet, thrombosis formation and rupture of unstable atherosclerotic plaque results in acute cardiovascular disease.[6] Numerous factors were involved in initiation of atherosclerosis such as age, gender and genetic which are unchangeable but some factors such as sedentary life style, hypercholesterolemia, hyperglycemia, hypertension, obesity are tends to be the prime causative factor at present.[7] Due to these changeable risk factors premature atherosclerosis incidence have been increased and it accounts about 10% of the atherosclerosis patients.[8,9]

Oxidative stress, hyperlipidemia followed by initiation of inflammatory signaling cascades are the prime cause of atherosclerosis initiation.[10] Activation of endothelium induces the fat deposition in arteries, fibrous matrix eventually leads to the formation of atheroma plaques causing narrowing of arteries.[11] Lipid lowering drugs such as statins, fibrates etc., were at present prescribed to treat atherosclerosis.[12] Long term medications of these statin drugs in causes various side effects rhadomyolysis.[13] peripheral neuropathy, hepatotoxicity,[14] mood disorders, increases the risk of diabetes mellitus,[15] myalgia.[16] It also causes withdrawal symptoms, allergic reaction and gastrointestinal disturbance.[17] World Health Organization (WHO) had reported large quantity consumption of fruits, vegetables and sea foods had decreased the incidence of cardiovascular disease.[18] These natural substance possess immense antioxidants and functional nutrients which combats the oxidative stress prime cause of cardiovascular diseases. These antioxidants present phytochemicals proven to reduce the risk of cardiovascular disease and also prescribed in treating these disorders.[19,20]

At present bioactive compounds from lichens are focused by researchers to be utilized in pharma industries since they possess immense medicinal values but often underestimated.[21] One of the promising metabolite obtained from lichens is usnic acid. Usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzofurandione) is obtained from Usnea, Alectoria, Cladonia, Lecanora, Ramalina and Flavocetraria possess antioxidant, anti-inflammatory, antibacterial and antimycotic properties.[22,23] In this study we assessed the therapeutic potency of the usnic acid against the high fat diet-induced atherosclerosis in rats.

MATERIALS AND METHODS

Drug and Chemicals

Drugs such as usnic acid and simvastatin used to treat animals were procured from Sigma Aldrich, USA. Each and every chemical used in the study were of standard analytical research grade chemicals.

Animals

Healthy young 6-8 weeks old male Wistar albino rats weighing about 170±40 were used for the experiment. The procedures to be followed on the rats were clearly explained before the ethical committee members and proper approval for rat's procurement was obtained. The rats were acclimatized in laboratory condition for a period of 7 days. The rats were housed in a hygienic polypropylene cages bedded with rice husk. Strict hygiene is maintained throughout the experiment period and cages, bedding were changed in regular intervals. 12 hr light/dark cycle standard temperature and relative humidity prescribed by IAEC for maintaining rats were followed. The rats were fed with standard laboratory pellet diet and water *ad libitum*.

Atherosclerosis induction

Atherosclerosis was induced in Wistar rats by feeding the rats with high fat diet consisting of cholesterol, cholic acid, propylthiouracil and salad oil along with the standard laboratory pellet diet for 30 days. The induction of atherosclerosis was confirmed with biochemical and histopathological analysis.

Treatment regimen

Acclimatized animals were randomly divided into five groups each group consisting of 6 rats. Group I rats are control rats fed with standard pellet diets alone. Group II atherosclerosis induced rats were fed only with high fat diet alone. Group III, IV and V were induced atherosclerosis through high fat diet and treated with drugs. Group III and IV atherosclerosis induced rats were treated intragastrically with 25 and 50 mg/kg b.wt usnic acid respectively. Group V are standard control rats which were induced atherosclerosis and treated with 10 mg/kg b.wt simvastatin for 30 days.

After completion of treatment regimen blood samples were collected from the anesthetize rats in non-anticoagulated centrifuge tubes. The blood samples were kept undisturbed for 60 min and centrifuged at 3000 rpm for 20 min at 4°C for the separation of serum samples. Heart tissue was collected from the rats and stored at -80°C for histopathological analysis.

Feed Consumption and Body weight

The rats were fed with 400 g of diet every day and on the next day the feed consumed by the rats were measured and noted. The body weights of the rats were monitored regularly and the values were recorded.

Quantification of Lipid profile

The mandatory lipid profile test such as total, low density, very low density and high-density cholesterol levels were quantified in the

serum of the experimental rats. Triglycerides and free fatty acid levels were also measured using commercially available research kits procured from Crystal Chem, USA. The assay was performed according the manufacturer's protocol and it was performed in triplicates.

Assessment of atherogenic index

A key predictor of atherosclerosis is atherogenic index hence we calculated the atherogenic index in the experimental rats using the formula

AI=log [Triglycerides/HDL-Cholestrol] (Niroumand *et al*.,2015).24

Assessment of Cardiac profile

Cardiac markers serum lactate dehydrogenase and creatinine phosphokinase were quantified in the high fat diet fed untreated and usnic acid treated rats. The levels were quantified using the research laboratory ELISA kits procured from My BioSource, USA. The final absorbances of the samples were measured at 450 nm using microplate reader. Standard curve was plotted with OD of standard concentration. The concentrations of LDH and CPK in test samples were interpolated from the standard curve.

Quantification of Inflammatory cytokines

Proinflammatory cytokine markers C-reactive protein, IL-18, IL-8 and IL-1β were measured in the test samples using the commercially available ELISA kits (Abcam, USA). The ELISA kits were procured from Abcam and the test were performed as per the guidelines provided in the kit manual. The assay was performed in test and standard triplicates samples. Standard curve was plotted and the concentrations of test samples were interpolated with the standard curve plot.

Assessment of endothelial dysfunction

Endothelial dysfunction in high fat diet fed untreated and usnic acid treated animals were assessed by quantifying the levels of nitric oxide, endothelin, 6-keto-prostagladin F1α and thromboxane B2 in the serum. Commercially available ELISA kits purchased from ElabScience, USA were used for the estimation.

Histopathological Examination

Aorta was excised from the experimental animals and the tissue was fixed in 10% neutral buffered formalin. The fixed tissue was then dehydrated and processed with gradient mixture of ethyl alcohol, water and xylene. The processed tissue was embedded in paraffin wax and subjected to tissue sections of 5-micron thickness. The sectioned tissue was then stained with hematoxylin and eosin stains and subjected to histopathological examination in a blind folded method using light microscope.

Data Analysis

Obtained results were analyzed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. One Way ANOVA method was used to compare between groups and to compare among groups Student's Newman Keul test was performed. The statistically significance was considered to be $p<0.05$.

RESULTS

Impact of usnic acid on feed in take and weight gain

Figure 1 depicts the results of feed intake and weight gain in rats fed with high fat diet untreated and usnic acid treated. Initially first ten days a significant decrease in the feed intake was observed in the high fat diet fed rats compared to the standard laboratory rat pellets fed rats. On mid of the treatment period the feed intake high fat diet fed untreated rats was significantly increased compared to the control and usnic acid treated rats. The feed intake was further gradually increased in high fat diet fed rats during the final 21-30th day of treatment (Figure 1A).

The weight gained by rats for every seven days was measured throughout the treatment period and the results were illustrated in Figure 1B. Initially during first week of treatment period a significant decrease weight was observed in the high fat diet fed rats. Both untreated and usnic acid treated rats shown significant decrease in body weight compared to the control. During subsequent weeks compared to usnic acid, simvastatin treated rats the untreated rats shown significant weight gain. High dose usnic acid, simvastatin treated rats shown significant decrease in the body weight compared to the low usnic acid and untreated rats.

Hypocholesterolemic effect of usnic acid

The hypocholesterolemic effect of usnic acid in high fat diet fed rats was assessed by measuring the lipid profile and the results were depicted in Figure 2. The levels of total cholesterol, triglycerides and free fatty acids were significantly increased in high fat diet fed untreated rats compared to the control and usnic, simvastatin treated rats. Both usnic and simvastatin treatment significantly lowered the levels of cholesterol, triglycerides and free fatty induced due to high fat diet. The decrease in the cholesterol levels observed in usnic acid treated rats were dose dependent.

Apart from total cholesterol levels, the levels of LDL, VLDL and HDL were observed separately in the serum of high fat diet fed untreated and drugs treated rats. The levels of both LDL and VLDL were significantly increased in the high fat diet fed untreated compared to the control rats. Both the doses of usnic acid significantly reduced the levels of LDL, VLDL and increased the level of HDL cholesterol. The levels of LDL, VLDL and HDL cholesterol observed in the high dose usnic treated rats

is comparatively similar to the levels observed in the standard hypocholesterolemic drug simvastatin treated group (Figure 3).

Influence of usnic acid on atherogenic index of high fat diet fed rats

Atherogenic index, basic indicator of atherosclerosis initiation was calculated with the obtained cholesterol values and the results represented in Figure 4. Atherogenic index was significantly increased to 2.8 in drugs untreated rats whereas it is only 0.5 in control rats. Usnic acid treatment significantly decreased the atherogenic index in 1.6 in low dose treated rats and 1.2 in high dose treated rats. Simvastatin treated rats shown significantly lower level of 0.8 atherogenic index compared to the usnic acid treated rats.

Usnic acid treated rats shown significantly decreased Tc/HDLc and LDLc/HDLc ratio compared to drug untreated rats. Tc/HDLc and LDLc/HDLc ratio were comparatively similar between simvastatin and high usnic acid treated rats.

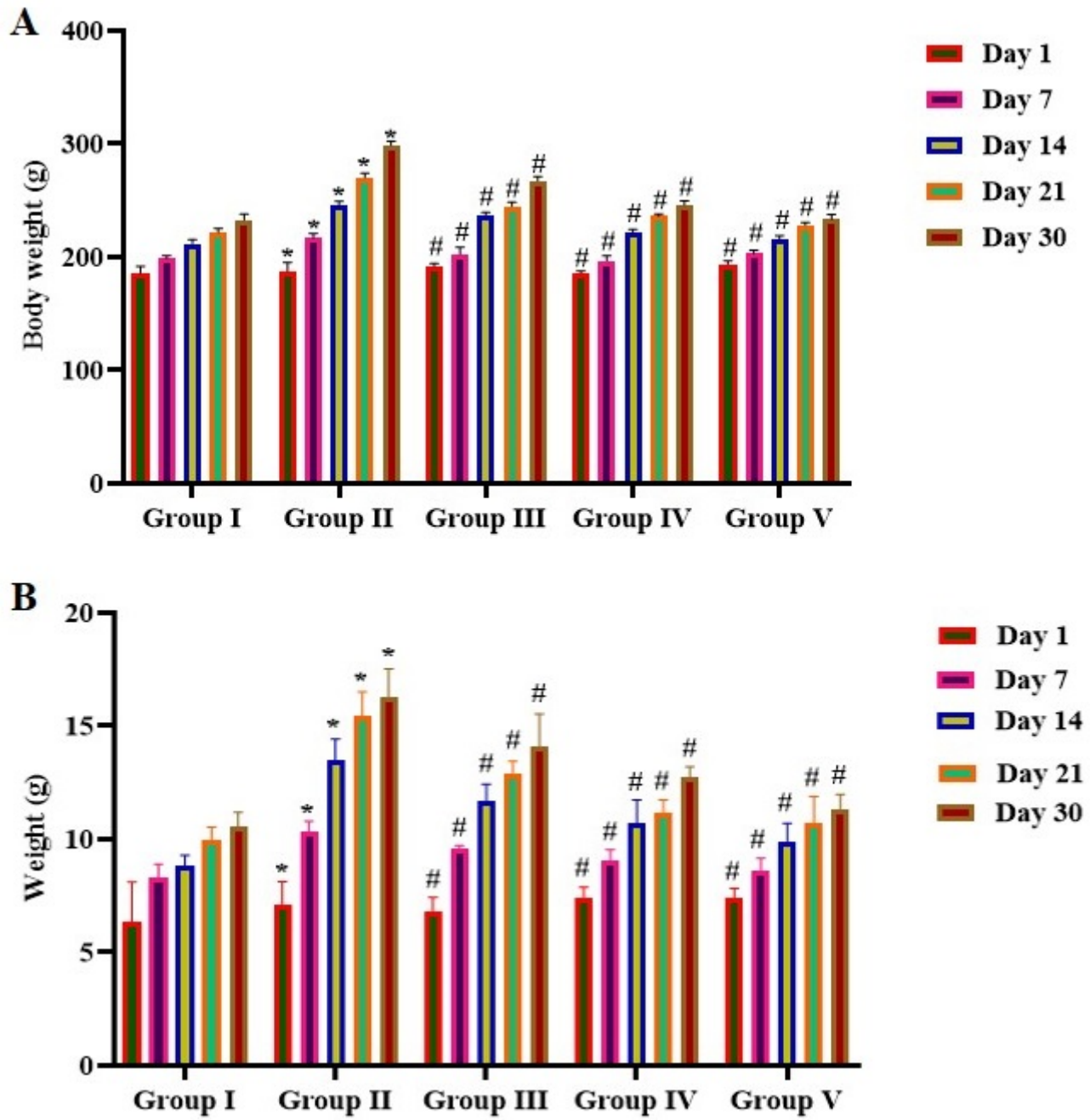

Figure 1: Impact of usnic acid on feed intake and weight gain. Change in body weight of control and experimental rats for every seven days (A).

Feed intake graph of control and experimental rats for every ten days (B). Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

Influence of usnic acid on cardiac markers of high fat diet fed rats

To assess the impact of usnic acid on the atherosclerosis induction in high fat diet fed rat's cardiac markers were quantified and the levels were depicted in Figure 5. Lactate metabolism plays a prime role in atherosclerosis induction hence we assessed the lactate catalyzing enzyme lactate dehydrogenase in high fat diet fed rats. Usnic acid treatment significantly decreased the levels of lactate dehydrogenase in high fat diet fed rats in dose dependent manner compared to the untreated rats.

Increased level of serum creatinine phosphokinase was observed in the drug-untreated rats compared to the other groups confirming the occurrence of myocardial infraction. Usnic acid and simvastatin treatment significantly decreased the levels of serum creatinine phosphokinase in high-fat diet-fed rats. The decrease in serum creatinine phosphokinase in usnic acid treated were observed in dose-dependent manner (Figure 5).

Anti-inflammatory potency of usnic acid

To analyze the anti-inflammatory potency of usnic acid in rats fed with high-fat feed the levels of pro-inflammatory cytokines IL-18, IL-8 and IL-1β were quantified (Figure 6). Proinflammatory cytokines were significantly increased in the drug-untreated rats compared to the control and the drug-treated rats. IL-1β and IL-18 were significantly increased in the high fat diet-fed rats compared to the IL-8 cytokine. Usnic acid treatment significantly decreased the levels of all the three proinflammatory cytokines and the reduction was observed in a dose-dependent manner.

C-Reactive protein, a key regulator of inflammation was also quantified and the levels were depicted in Figure 6. The induction of myocardial inflammation in the high fat diet fed rats was confirmed with the marked increase C-RP levels. Both the drugs treated and untreated rats shown increase in the levels of C-RP compared to control. Whereas compared to drug untreated rats the usnic and simvastatin treated rats shown significant decrease in the levels of C-RP.

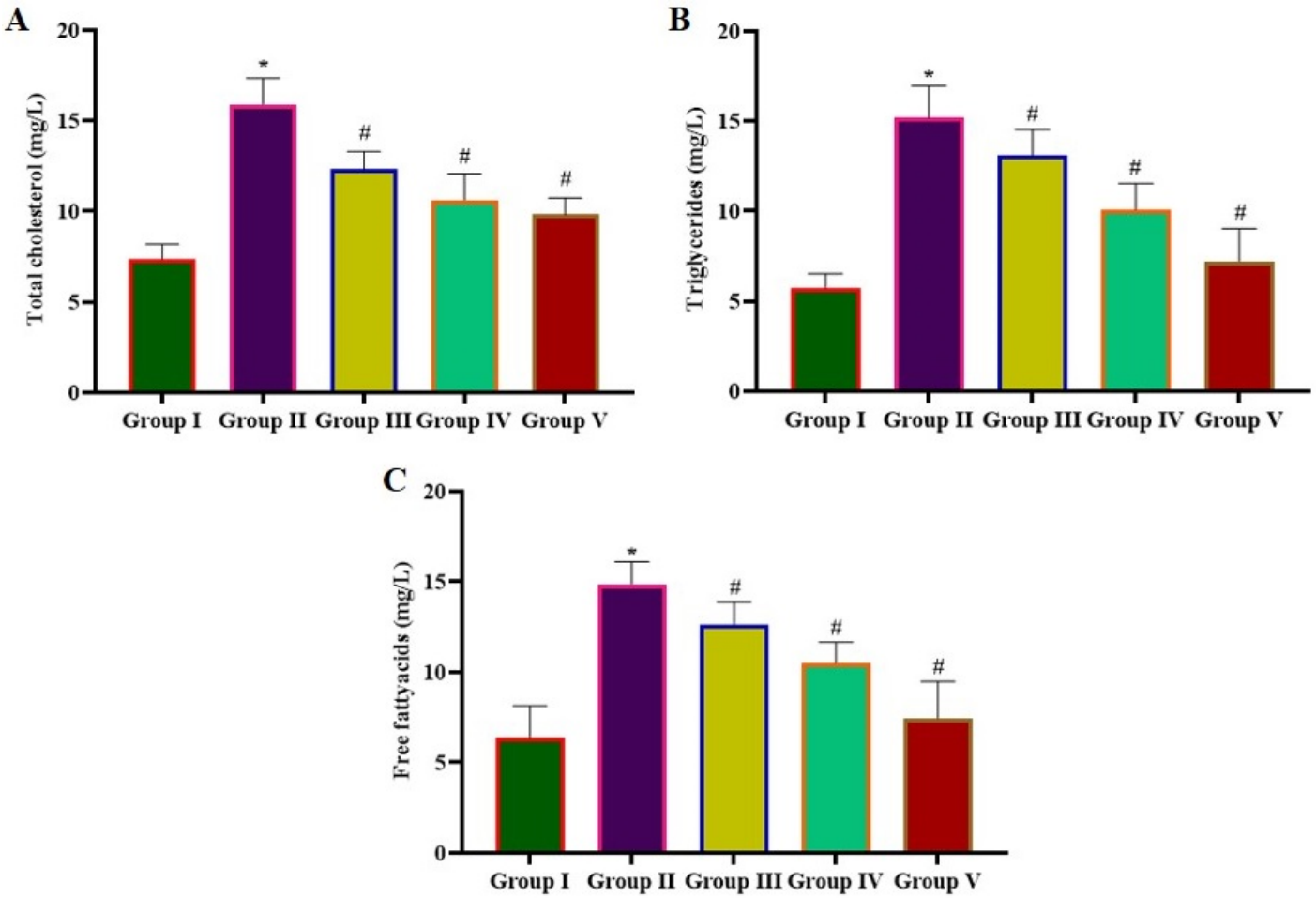

Figure 2: Hypocholesterolemic effect of usnic acid.

Lipid profile was quantified in control and experimental rats. A) Total Cholestrol B) Triglycerides C) Free fatty acid. Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean ±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

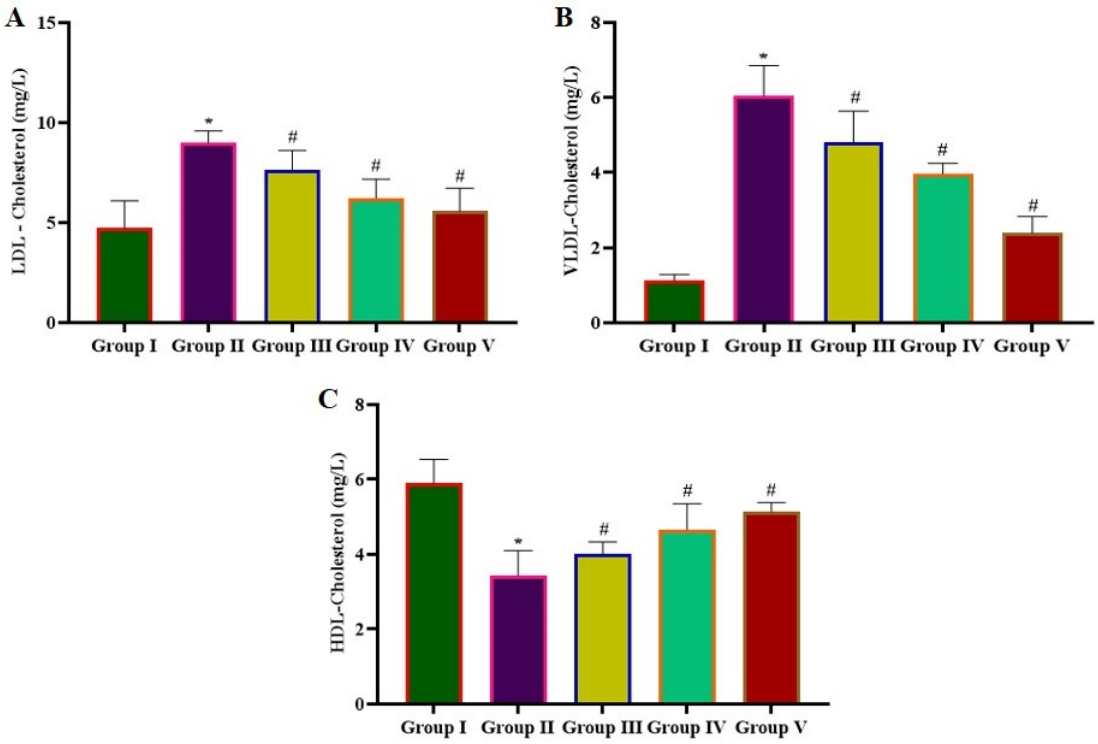

Figure 3: Hypocholesterolemic effect of usnic acid.

Lipid profile was quantified in control and experimental rats. A) Low density lipoprotein cholesterol B) Very low density lipoprotein cholesterol C) High density lipoprotein cholesterol. Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

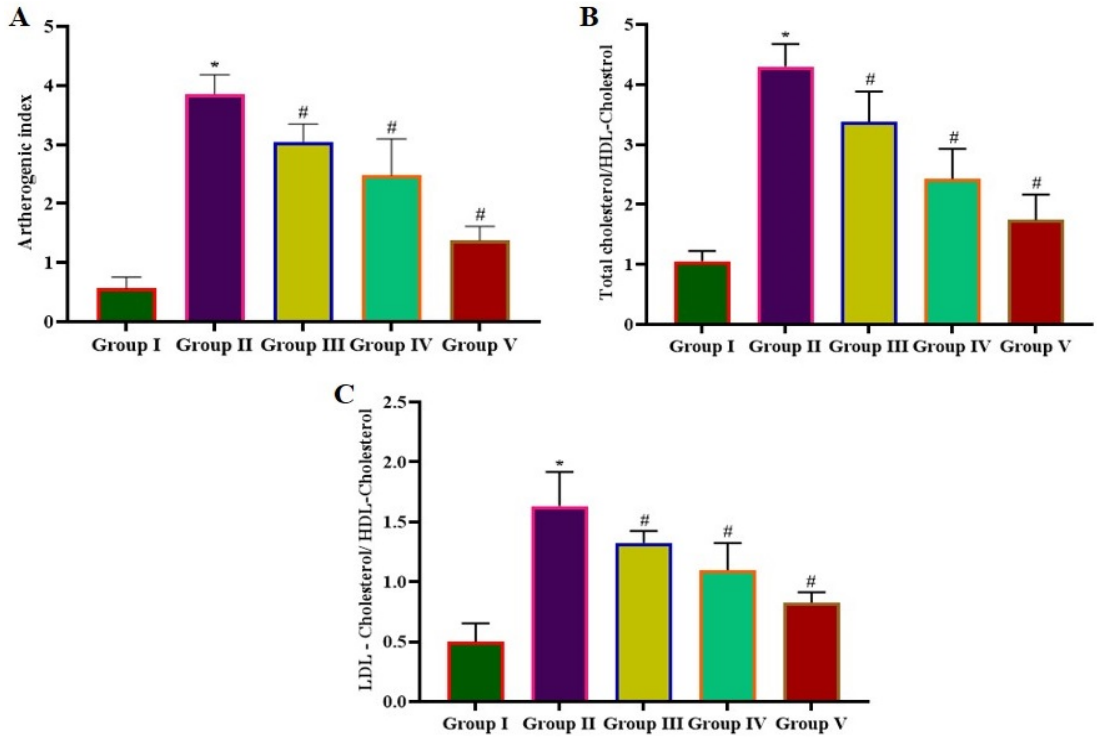

Figure 4: Influence of usnic acid on Atherogenic index of high fat diet fed rats. A) Atherogenic index B) Total cholesterol vs High density lipoprotein cholesterol C) Low density lipoprotein cholesterol vs High density lipoprotein cholesterol ratio of control and experimental rats.

Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

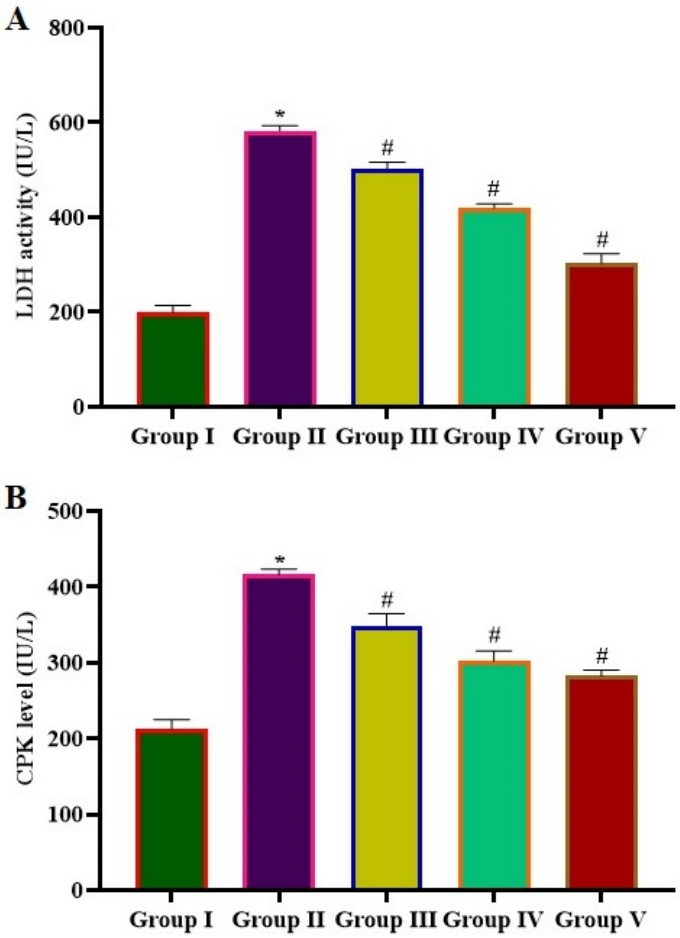

Figure 5: Influence of usnic acid on cardiac markers of high fat diet fed rats. A) Lactate Dehydrogenase B) Creatinine phosphokinase of control and experimental rats.

Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

Ameliorative role of usnic acid against endothelial dysfunction

High fat diet induced endothelial dysfunction in cardiac muscles were assessed by quantifying endothelial functional markers such as nitric oxide, 6-keto-prostaglandin F1α and thromboxane B (Figure 7). Decrease in nitric oxide and increase in endothelin considered to be the initiator event of atherosclerosis induction. Usnic acid treatment significantly increased the nitric oxide and decreased the endothelin levels in the high fat diet fed rats compared to the drugs untreated rats. Usnic acid also increased the levels of 6-keto- prostaglandin F1α and decreased the levels of thromboxane B which also observed in the standard lipid lowering drug simvastatin.

Cardioprotective effect of usnic acid

Atherosclerosis induction and the protective effect of usnic acid against the high fat diet in rats were assessed with histological analysis. The representative images of H and E stained cardiac tissue sections were represented in Figure 8.

Microscopic examination of H and E stained cardiac tissue sections of control, drug treated and untreated high fat diet fed rats. Control (Group I) and simvastatin (Group V) treated rats shown normal cardiac histological structure whereas the drug untreated rats shown degeneration of granules and vacuoles. Degenerated myofibril with necrotic cells and edema was observed in the high-fat diet-fed rats (Group II). Usnic acid treated rats shown comparatively decreased number of necrotic cells and the inter-muscular edema was reduced (Group III). Vacuolar and granular degeneration was reduced in usnic acid high dose treated group compared to the low-dose treated group (Group IV).

DISCUSSION

In spite of sustained decrease in the incidence of cardiovascular disease related mortalities it tends to be the foremost cause for global disease induced mortalities of last four decades.[1] This may be due to the increase in population and geriatric medications but the increase in artherosclerotic mortalities in young population is distressing factor of present decade.[25,26] Coronary atherosclerosis and stroke are the prime contributors for the increase in disability adjusted life years of the cardiovascular patients.[1] Hypertension, diabetes mellitus, hyperlipidemia, cigarette smoking are the major causes for formation of atherosclerotic plaques apart from the ageing factor, among these hypertension ranks first.[27] The dysregulated lipid metabolism causes fat deposition in arteries eventually development of smooth muscle cells, platelet aggregation and finally atherosclerotic plaques.[28] Inflammation induced local endothelial dysfunction induces rupture of atherosclerotic plaque causing instability in blood in the long run causing cardiovascular diseases.[6,29,30] Hence we hypothesized our study to evaluate the role of usnic acid, lichen metabolite on lipids, proinflammatory cytokines and endothelial functional markers of high fat diet induced atherosclerosis rats.

One of the predominant conditions of cardiovascular disease is dyslipidemia. It is responsible for about 4 million related global cardiovascular disease mortality.[31,32] 18% of ischemic heart disease and 56% of stroke mortalities were reported by WHO and the hyperlipidemic patients were twice risky to develop cardiovascular disease.[33] The other major global concern is premature mortality due to cardiovascular disease which also linked to dyslipidemia.[34] Therefore in our study we induced dyslipidemia condition in young rats with high fat diet and assessed the lipid lowering potency of usnic acid. Successfully dyslipidemia was induced in the rats with high fat diet which was evidenced with the results of our lipid profile examination in experimental rats.

Dyslipidemia is a condition which enhances the arterial fat deposition causing narrowing of the lumen leading to obstructed blood supply eventually stroke, ischemic heart attack, pulmonary

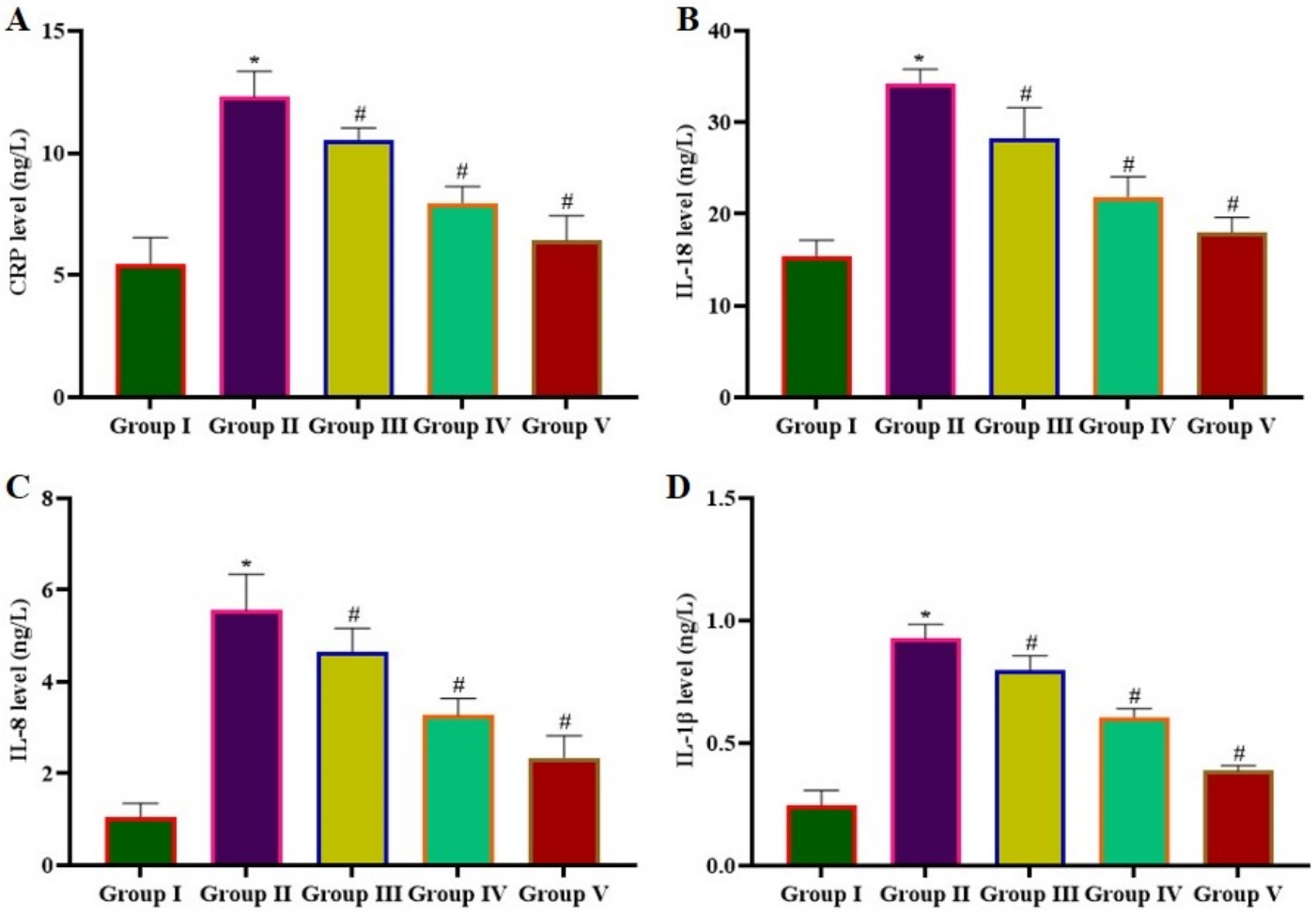

Figure 6: Anti-inflammatory potency of usnic acid. A) C-reactive protein, B) Interleukin-18, C) Interleukin-8, D) Interleukin-1β of control and experimental rats.

Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

embolism etc.,[35] The occurrence of thrombo-embolic events are unpredictable since the formation of atherosclerotic plaque and the thickening of intima-media were silent process and asymptomatic.[36,37] The high level of circulating triglycerides, total cholesterol, low-density lipoprotein particles and decrease in high-density lipoprotein particles were observed in the dyslipidemia condition.[38] An indispensable tool to diagnose cardiovascular disease is the assessment of lipid profile. Quantifying total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density and very low-density lipoprotein cholesterol, free fatty acids.[39] High fat diet in rats had increased the total cholesterol, triglycerides, free fatty acids, low-density lipoprotein cholesterol and decreased the levels of high-density lipoprotein cholesterol confirming the occurrence of dyslipidemia. Increased weight gain also observed in the high fat diet-fed rats which further supports the results of lipid profile.

Removal of triglycerides from VLDL and IDL cholesterol mediated by LPL/Apo C-II form LDL cholesterol, these LDL cholesterol transports cholesterol to the peripheral tissue and it is directly involved in the formation of atherosclerotic plaques.[40,41] Whereas the HDL are responsible for the uptake and delivery of peripheral cholesterol to the liver and other organs producing lipid derived hormones. It is also involved in anti-inflammatory and antioxidant action which inhibits the atherosclerotic plaque formation.[42] Usnic acid treatment in high fat diet fed rats significantly decreased the levels of LDL and HDL this may be the reason for reduction to total cholesterol and triglycerides. Herbs which possess antioxidant property effectively reduces the circulating lipid levels thereby prevent atherosclerosis.[43] This correlates with usnic acid it effectively decreased the levels of LDL, VLDL, triglycerides and total cholesterol thereby decreased the atherogenic index and prevented atherosclerosis induction in rats.

Lactate dehydrogenase is cytoplasmic enzyme ubiquitously present in varied tissues acts as checkpoint of anaerobic glycolysis and DNA metabolism.[44,45] The levels of serum LDH is found to be increased upon cell injury hence it is suggested to be marker enzyme to detect myocardial infraction.[46-48]

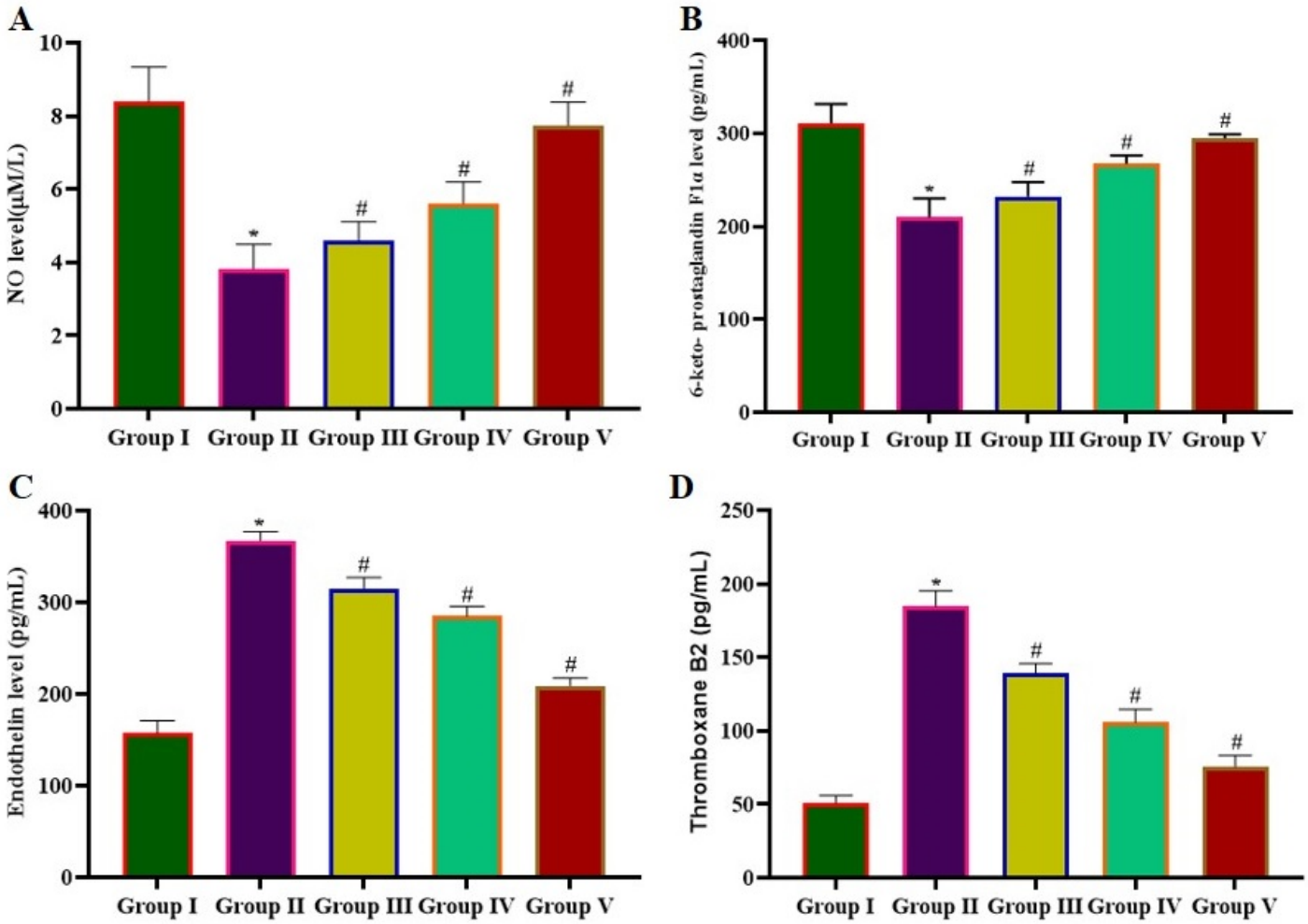

Figure 7: Ameliorative role of usnic acid against endothelial dysfunction. A) Nitric oxide B) 6-keto-prostagladin F1α C) endothelin D) thromboxane B2 in the serum of control and experimental rats.

Results were analysed statistically with software GraphPad Prism and the final results were illustrated as mean±SEM. The statistically significance was considered to be $p<0.05$. # Control vs High fat diet fed untreated group, * High fat diet fed untreated group vs Usnic acid treated and simvastatin treated group.

Usnic acid treatment significantly decreased the levels of LDH confirming the cardioprotective role against the high fat diet induced atherosclerosis. Prolonged intake of antidepressant, statins, antipsychotics, fibrates, antihistamine drugs leads to rhabdomyolysis in patients.[49] Lipid lowering medications prescribed to treat cardiovascular patients also reported to induce rhabdomyolysis, myalgias.[50,51] The induction of rhabdomyolysis, myalgias were detected via quantifying creatine phosphokinase in serum.[52] Therefore we evaluated the impact of usnic acid on high fat diet fed rats, usnic acid treatment decreased the levels of creatinine phosphokinase which proves usnic acid doesn't render any side effect in experimental rats.

Cholesterol crystals activate inflammasome present macrophage cytoplasm in the intima of artery. These infalmmasome protein complex on external stimuli cleaves pro-interleukin 1β and 18 which are secreted as inflammatory cytokines.[53,54] The secreted inflammatory cytokines interact with the cognate receptors in the extracellular space causing oxidative stress, activation of T-cells and stimulates further cytokine production. IL-1 signalling also triggers the synthesis of acute phase pentraxin protein often elevated during inflammatory diseases is C-reactive protein. It is also found to be increased in atherosclerosis patients and considered to potent biomarker for detecting cardiovascular disease.[55,56] IL-1β mediated inflammatory signaling are the potent inducers of atherosclerosis and has been targeted to formulate anti-atherosclerotic drug.[57] Usnic acid treatment significantly decreased the levels of proinflammatory cytokines IL-1β, IL-8, IL-18 and c-reactive protein in high fat diet fed rats proving the anti-inflammatory role against dyslipidemia induced inflammation.

Endothelium dysfunction is characterized by the imbalance in vasodilation to vasoconstriction ratio which is the primary function of endothelial cells. This impairment occurs due to the decreased levels of nitric oxide and increase in endothelin vasoconstrictor levels.[58] In atherosclerosis patients decreased levels of nitric oxide was estimated which causes damage to

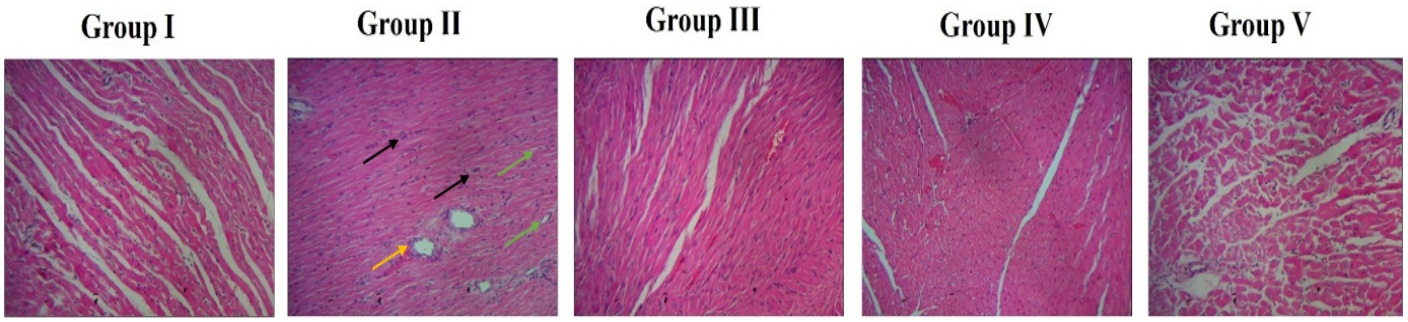

Figure 8: Cardioprotective effect of usnic acid. Representative images of H andE stained cardiac tissue sections of control and experimental rats. Group I) Control, Group II) High fat diet fed untreated rats Group III) High fat diet fed low dose usnic acid treated rats.Group IV) High fat diet fed high dose usnic acid treated rats Group V) High fat diet fed simvastatin treated rats.

Note: Yellow arrow: vacuoles and granules; black arrows: degenerated myofibrils with necrotic cells; green arrows: edema.

the endothelial junctions thereby led to the accumulation of cholesterol containing lipoproteins in the subendothelium.[59] Thromboxane a vasoconstrictor upon activation of TP receptors leads to endothelial dysfunction in cardiovascular disorders.[60] A stable metabolite of prostacyclin is 6-keto prostaglandin F1α (6-keto PGF1α) is a biomarker which is observed to be decreased in coagulation abnormal patients and aged individuals.[61] Significant increase in the vasodilators nitric oxide, 6-keto prostaglandin F1α and decrease in vasoconstrictors endothelin, thromboxane B2 were observed in the usnic acid treated high fat diet fed rats which tends to be due to the antioxidant and anti-inflammatory property of usnic acid. Further the histopathological analysis of aorta H and E stained confirms the anti-atherosclerotic property of usnic acid.

CONCLUSION

To conclude, in the present study we evaluated the potency of lichen metabolite usnic acid potency to ameliorate high fat diet induced atherosclerosis in rat model. Usnic acid significantly prevented the excess weight gain and regulated the dyslipidemia induced by high fat diet. It decreased the lactate dehydrogenase and creatinine phosphokinase levels. The pro-inflammatory markers IL-1β, IL-8, IL-18 and C-reactive protein were also significantly decreased with usnic acid treatment in high fat diet fed rats. Usnic acid increased the levels of vasodilators and decreased the levels of vasoconstrictors thereby prevented endothelial dysfunction which was also evidenced with our histopathological analysis. Overall usnic acid is a potent anti-atherosclerotic agent which can be subjected to further research to be formulated as anti-atherosclerotic drug. However, the further studies still need to be performed in the future to clearly understand the therapeutic potentials of the usnic acid.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

This work has approved by the institutional animal ethical committee by the Xi'an Gaoxin Hospital, Xi'an, China, 710000.

ABBREVIATIONS

CVD: Cardiovascular disease; **WHO:** World Health Organisation; **LDH:** lactate dehydrogenase; **CPK:** Creatine Phosphokinase; **LDL:** Low-density lipoproteins; **HDL:** high-density lipoprotein; **VLDL:** Very-low-density lipoprotein

SUMMARY

Atherosclerosis is a multifactorial chronic inflammatory disease characterized by the deviant lipid metabolism, calcification and plaque deposition within the arteries, foaminess of macrophages; Usnic acid significantly prevented weight gain, dysregulation of lipid and cardiac profile in high fat diet induced rats. It also inhibited the inflammatory response via decreasing the levels of pro-inflammatory cytokines. Usnic acid potentially increased the NO, 6-keto-PGF1α and decreased ET, TXB2 thereby prevented high-fat diet-induced endothelial dysfunction in experimental rats.

REFERENCES

1. GBD. Diseases and injuries collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2019;396(10258). 2020: 1204-22. doi: 10.1016/S0140-6736(20)30925-9. Erratum in: Lancet. 2020;396(10262):1562. doi: 10.1016/S0140-6736(20)32319-9, PMID 33189177.
2. WHO; 2021. Cardiovascular Diseases. Available from: http://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
3. Lu WL, Yuan JH, Liu ZY, Su ZH, Shen YC, Li SJ, *et al.* Worldwide trends in mortality for hypertensive heart disease from 1990 to 2019 with projection to 2034: data from the Global Burden of Disease 2019 study. Eur J Prev Cardiol. 2024;31(1):23-37. doi: 10.1093/eurjpc/zwad262, PMID 37665956.
4. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. heart disease and stroke Statistics-2021 update: A report from the American Heart Association. Circulation. 2021;143(8):254-743. doi: 10.1161/CIR.0000000000000950, PMID 33501848.
5. Libby P. The changing landscape of atherosclerosis. Nature. 2021;592(7855):524-33. doi: 10.1038/s41586-021-03392-8, PMID 33883728.

6. Meng H, Ruan J, Yan Z, Chen Y, Liu J, Li X, *et al.* new progress in early diagnosis of atherosclerosis. Int J Mol Sci. 2022;23(16):8939. doi: 10.3390/ijms23168939, PMID 36012202.
7. Gallucci G, Tartarone A, Lerose R, Lalinga AV, Capobianco AM. Cardiovascular risk of smoking and benefits of smoking cessation. J Thorac Dis. 2020;12(7):3866-76. doi: 10.21037/jtd.2020.02.47, PMID 32802468.
8. Arora S, Stouffer GA, Kucharska-Newton AM, Qamar A, Vaduganathan M, Pandey A, *et al.* Twenty-year trends and sex differences in young adults hospitalized with acute myocardial infarction. Circulation. 2019;139(8):1047-56. doi: 10.1161/CIRCULATIONAHA.118.037137, PMID 30586725.
9. Neifert AR, Su D, Ochoa Chaar CIO, Sumpio BE. Premature atherosclerosis: a review of current literature. JVS-Vasc Insights. 2023;1:100013. doi: 10.1016/j.jvsvi.2023.100013.
10. Theofilis P, Oikonomou E, Chasikidis C, Tsioufis K, Tousoulis D. Inflammasomes in atherosclerosis-from pathophysiology to treatment. Pharmaceuticals (Basel). 2023;16(9):1211. doi: 10.3390/ph16091211, PMID 37765019.
11. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, *et al.* Pathophysiology of atherosclerosis. Int J Mol Sci. 2022;23(6):3346. doi: 10.3390/ijms23063346, PMID 35328769.
12. Zhao X, Zhu J, Wang L, Li Y, Zhao T, Chen X, et al. *U. diffracta* extract mitigates high fat diet and VD3-induced atherosclerosis and biochemical changes in the serum liver and aorta of rats. Biomed Pharmacother. 2019;120:109446. doi: 10.1016/j.biopha.2019.109446, PMID 31542617.
13. Brown WV. Safety of statins. Curr Opin Lipidol. 2008;19(6):558-62. doi: 10.1097/MOL.0b013e328319baba, PMID 18957877.
14. Russo MW, Hoofnagle JH, Gu J. Spectrum of statin hepatotoxicity: experience of the drug-induced liver injury network. Hepatol Baltim Md. 2014;60(2):679-86.
15. Bang CN, Okin PM. Statin treatment, new-onset diabetes and other adverse effects: a systematic review. Curr Cardiol Rep. 2014;16(3):461. doi: 10.1007/s11886-013-0461-4, PMID 24464306.
16. Pedro-Botet J, Núñez-Cortés JM, Flores JA, Rius J. Muscle symptoms related with statin therapy in general practice. Atherosclerosis. 2015;241(1):197. doi: 10.1016/j.atherosclerosis.2015.04.957.
17. Florentin M, Liberopoulos EN, Moutzouri E, Rizos CV, Tselepis AD, Elisaf MS. The effect of simvastatin alone versus simvastatin plus ezetimibe on the concentration of small dense low-density lipoprotein cholesterol in subjects with primary hypercholesterolemia. Curr Med Res Opin. 2011;27(3):685-92. doi: 10.1185/03007995.2010.546394, PMID 21271793.
18. Meier T, Gräfe K, Senn F, Sur P, Stangl GI, Dawczynski C, *et al.* Cardiovascular mortality attributable to dietary risk factors in 51 countries in the WHO European Region from 1990 to 2016: a systematic analysis of the Global Burden of Disease Study. Eur J Epidemiol. 2019;34(1):37-55. doi: 10.1007/s10654-018-0473-x, PMID 30547256.
19. Liu K, Daviglus ML, Loria CM, Colangelo LA, Spring B, Moller AC, *et al.* Healthy lifestyle through young adulthood and the presence of low cardiovascular disease risk profile in middle age: the coronary artery Risk Development in (Young) Adults (CARDIA) study. Circulation. 2012;125(8):996-1004. doi: 10.1161/CIRCULATIONAHA.111.060681, PMID 22291127.
20. Granato D, Barba FJ, Bursać Kovačević D, Lorenzo JM, Cruz AG, Putnik P. Functional foods: product development, technological trends, efficacy testing and safety. Annu Rev Food Sci Technol. 2020;11:93-118. doi: 10.1146/annurev-food-032519-051708, PMID 31905019.
21. Luzina OA, Salakhutdinov NF. Usnic acid and its derivatives for pharmaceutical use: a patent review (2000-2017). Expert Opin Ther Pat. 2018;28(6):477-91. doi: 10.1080/13543776.2018.1472239, PMID 29718734.
22. Cocchietto M, Skert N, Nimis PL, Sava G. A review on usnic acid, an interesting natural compound. Naturwissenschaften. 2002;89(4):137-46. doi: 10.1007/s00114-002-0305-3, PMID 12061397.
23. Paździora W, Podolak I, Grudzińska M, Paśko P, Grabowska K, Galanty A. Critical assessment of the anti-inflammatory potential of usnic acid and its derivatives-A review. Life (Basel). 2023;13(4):1046. doi: 10.3390/life13041046, PMID 37109575.
24. Niroumand S, Khajedaluee M, Khadem-Rezaiyan M, Abrishami M, Juya M, Khodaee G, *et al.* Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. Med J Islam Repub Iran. 2015;29:240. PMID 26793631.
25. An J, Zhang Y, Zhou H, Zhou M, Safford MM, Muntner P, *et al.* Incidence of atherosclerotic cardiovascular disease in young adults at low short-term but high long-term risk. J Am Coll Cardiol. 2023;81(7):623-32. doi: 10.1016/j.jacc.2022.11.051, PMID 36792277.
26. Nedkoff L, Briffa T, Zemedikun D, Herrington S, Wright FL. Global trends in atherosclerotic cardiovascular disease. Clin Ther. 2023;45(11):1087-91. doi: 10.1016/j.clinthera.2023.09.020, PMID 37914585.
27. Stewart J, Manmathan G, Wilkinson P. Primary prevention of cardiovascular disease: a review of contemporary guidance and literature. JRSM Cardiovasc Dis. 2017;6:2048004016687211. doi: 10.1177/2048004016687211, PMID 28286646.
28. Taleb S. Inflammation in atherosclerosis. Arch Cardiovasc Dis. 2016;109(12):708-15. doi: 10.1016/j.acvd.2016.04.002, PMID 27595467.
29. Gusev E, Sarapultsev A. Atherosclerosis and inflammation: insights from the theory of general pathological processes. Int J Mol Sci. 2023;24(9):7910. doi: 10.3390/ijms24097910, PMID 37175617.
30. Kanter JE, Kramer F, Barnhart S, Averill MM, Vivekanandan-Giri A, Vickery T, *et al.* Diabetes promotes an inflammatory macrophage phenotype and atherosclerosis through acyl-CoA synthetase 1. Proc Natl Acad Sci U S A. 2012;109(12):715-24. doi: 10.1073/pnas.1111600109, PMID 22308341.
31. Gebreegziabiher G, Belachew T, Mehari K, Tamiru D. Prevalence of dyslipidemia and associated risk factors among adult residents of Mekelle City, Northern Ethiopia. PLOS ONE. 2021;16(2):0243103. doi: 10.1371/journal.pone.0243103, PMID 33561153.
32. Thongtang N, Sukmawan R, Llanes EJ, Lee ZV. Dyslipidemia management for primary prevention of cardiovascular events: best in-clinic practices. Prev Med Rep. 2022;27:101819. doi: 10.1016/j.pmedr.2022.101819, PMID 35656215.
33. Yao YS, Di LT, Zeng ZH. Mechanisms underlying direct actions of hyperlipidemia on myocardium: an updated review. Lipids Health Dis. 2020;19(1):1.
34. Addisu B, Bekele S, Wube TB, Hirigo AT, Cheneke W. Dyslipidemia and its associated factors among adult cardiac patients at Ambo university referral hospital, Oromia region, west Ethiopia. BMC Cardiovasc Disord. 2023;23(1):321. doi: 10.1186/s12872-023-03348-y, PMID 37355585.
35. Goff DC Jr, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB Sr, Gibbons R, *et al.* ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013; 2014;63(25 Pt B):2935-59. doi: 10.1016/j.jacc.2013.11.005. Erratum in: J Am Coll Cardiol. 2014; 63;25(B):3026.
36. Takase M, Nakaya N, Nakamura T, Kogure M, Hatanaka R, Nakaya K, *et al.* Carotid intima media thickness and risk factor for atherosclerosis: Tohoku medical Megabank community-based cohort study. J Atheroscler Thromb. 2023;30(10):1471-82. doi: 10.5551/jat.64039, PMID 36775331.
37. Garg R, Aggarwal S, Kumar R, Sharma G. Association of atherosclerosis with dyslipidemia and co-morbid conditions: A descriptive study. J Nat Sci Biol Med. 2015;6(1):163-8. doi: 10.4103/0976-9668.149117, PMID 25810656.
38. Hasheminasabgorji E, Jha JC. Dyslipidemia, diabetes and atherosclerosis: role of inflammation and ROS-redox-sensitive factors. Biomedicines. 2021;9(11):1602. doi: 10.3390/biomedicines9111602, PMID 34829831.
39. Fernández-Macías JC, Ochoa-Martínez AC, Varela-Silva JA, Pérez-Maldonado IN. Atherogenic index of plasma: novel predictive biomarker for cardiovascular illnesses. Arch Med Res. 2019;50(5):285-94. doi: 10.1016/j.arcmed.2019.08.009, PMID 31593853.
40. Helkin A, Stein JJ, Lin S, Siddiqui S, Maier KG, Gahtan V. Dyslipidemia. Part I-Review of lipid metabolism and vascular cell physiology. Vasc Endovasc Surg. 2016;50(2):107-18. doi: 10.1177/1538574416628654, PMID 26983667.
41. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic and clinical studies. A consensus statement from the European atherosclerosis society consensus panel. Eur Heart J. 2017;38(32):2459-72. doi: 10.1093/eurheartj/ehx144, PMID 28444290.
42. Bandeali S, Farmer J. High-density lipoprotein and atherosclerosis: the role of antioxidant activity. Curr Atheroscler Rep. 2012;14(2):101-7. doi: 10.1007/s11883-012-0235-2, PMID 22441969.
43. Behradmanesh S, Nasri P. Serum cholesterol and LDL-C in association with level of diastolic blood pressure in type 2 diabetic patients. J Ren Inj Prev. 2012;1(1):23-6. doi: 10.12861/jrip.2012.09, PMID 25340098.
44. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harb Protoc. 2018;2018(6). doi: 10.1101/pdb.prot095497, PMID 29858337.
45. Farhana A, Lappin SL. Biochemistry, lactate dehydrogenase. In: Treasure Island, (FL): StatPearls Publishing. Jan; 2023 May 1. StatPearls [Internet]. p. 2024.
46. Szunerits S, Mishyn V, Grabowska I, Boukherroub R. Electrochemical cardiovascular platforms: current state of the art and beyond. Biosens Bioelectron. 2019;131(131):287-98. doi: 10.1016/j.bios.2019.02.010, PMID 30851492.
47. Agarkov NM, Markelov MY, Markelova EA, Lutai YA, Volkov PS. The information content and predictive value of cardiac markers in myocardial infarction in the elderly. Adv Gerontol. 2020;33(1):82-6. PMID 32362088.
48. Cai X, Wang T, Xie L. Lactate dehydrogenase is associated with flow-mediated dilation in hypertensive patients. Sci Rep. 2023;13(1):768. doi: 10.1038/s41598-023-27614-3, PMID 36641500.
49. Eizadi-Mood N, Sabzghabaee AM, Gheshlaghi F, Mehrzad F, Fallah Z. Admission creatine phosphokinase in acute poisoning: is it a predictive factor for the treatment outcome? JPMA J Pak Med Assoc. 2012; 62(3); Suppl 2:S67-70. PMID 22768464.
50. Kammoun R, Charfi O, Lakhoua G, Zaiem A, Daghfous R, Kastalli S, *et al.* Statin associated muscular adverse effects. Curr Drug Saf. 2024;19(1):114-6. doi: 10.2174/1574886318666230227143627, PMID 36847228.
51. Safitri N, Alaina MF, Pitaloka DA, Abdulah R. A narrative review of statin-induced rhabdomyolysis: molecular mechanism, risk factors and management. Drug Healthc Patient Saf. 2021;13:211-9. doi: 10.2147/DHPS.S333738, PMID 34795533.
52. Dadpour B, Tajoddini S, Shaarbaf Eidgahi E, Shokouhizadeh M, Shafahi A. Role of serum creatinine phosphokinase in outcome prediction of intoxicated patients; a brief report. Emerg (Tehran). 2017;5(1):63. PMID 28894778.
53. Sheedy FJ, Moore KJ. IL-1 signaling in atherosclerosis: sibling rivalry. Nat Immunol. 2013;14(10):1030-2. doi: 10.1038/ni.2711, PMID 24048132.
54. Soehnlein O, Libby P. Targeting inflammation in atherosclerosis - from experimental insights to the clinic. Nat Rev Drug Discov. 2021;20(8):589-610. doi: 10.1038/s41573-021-00198-1, PMID 33976384.

55. Amezcua-Castillo E, González-Pacheco H, Sáenz-San Martín A, Méndez-Ocampo P, Gutierrez-Moctezuma I, Massó F, *et al.* C-reactive protein: the quintessential marker of systemic inflammation in coronary artery disease-advancing toward precision medicine. Biomedicines. 2023;11(9):2444. doi: 10.3390/biomedicines11092444, PMID 37760885.
56. Moriya J. Critical roles of inflammation in atherosclerosis. J Cardiol. 2019;73(1):22-7. doi: 10.1016/j.jjcc.2018.05.010, PMID 29907363.
57. Raggi P, Genest J, Giles JT, Rayner KJ, Dwivedi G, Beanlands RS, *et al.* Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. Atherosclerosis. 2018;276:98-108. doi: 10.1016/j.atherosclerosis.2018.07.014, PMID 30055326.
58. Tabas I, García-Cardeña G, Owens GK. Recent insights into the cellular biology of atherosclerosis. J Cell Biol. 2015;209(1):13-22. doi: 10.1083/jcb.201412052, PMID 25869663.
59. Henein MY, Vancheri S, Longo G, Vancheri F. The role of inflammation in cardiovascular disease. Int J Mol Sci. 2022;23(21):12906. doi: 10.3390/ijms232112906, PMID 36361701.
60. Ciavarella C, Motta I, Capri M, Gargiulo M, Pasquinelli G. Heterogeneity and differentiation of the human arterial tree: focus on microRNA expression in vascular disease. Biomolecules. 2024;14(3):343. doi: 10.3390/biom14030343, PMID 38540762.
61. Gallo G, Savoia C. New insights into endothelial dysfunction in cardiometabolic diseases: potential mechanisms and clinical implications. Int J Mol Sci. 2024;25(5):2973. doi: 10.3390/ijms25052973, PMID 38474219.

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