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Original Article

Preclinical Studies of *Euphorbia lactea* Aerial Extract on Atherogenic-Diet-Induced Atherosclerosis

Zubair Khan[1], Nazia Siddiqui[1], Garima Agarwal[1], Anurag Chaudhary[1], Syed Salman Ali[2], Abdel-Nasser El-Shorbagi[3], Sachin Chaudhary[3], Prabhash Nath Tripathi[1,*]

[1]Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH-58, Baghpat Road Crossing, Bypass Road, Meerut, Uttar Pradesh, INDIA.
[2]Department of Pharmaceutical Technology, Lloyd Institute of Management and Technology, Greater Noida, Uttar Pradesh, INDIA.
[3]Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah, UNITED ARAB EMIRATES.

ABSTRACT

Background: Atherosclerosis is a complex progressive pathological process that produces lesions in the inner wall of an artery because of the abnormal lipid metabolism, and dysregulation of inflammatory processes. Increased levels of Low-Density Lipoprotein (LDL) and Total Cholesterol (TC) lead to hyperlipidaemia which is a major risk factor for atherosclerosis. **Materials and Methods:** This research comprises the assessment of hydro-alcoholic extract of the aerial part of *Euphorbia lactea* on high-fat diet-induced atherosclerosis in rats. The hydroalcoholic extract with doses (200 and 400 mg/kg body mass, *p.o.*) was assessed for hypolipidaemic activity. The blood samples were collected in heparinized tubes at the end of the experiment, and then centrifuged and collected the supernatant to determine Triglyceride (TG), High-Density Lipoprotein (HDL), LDL, TC, and Very Low-Density Lipoprotein (VLDL). The atherosclerotic markers including Creatine Phosphokinase (CPK), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), and Aspartate Transaminase (AST) section of heart aorta were examined by histopathology. **Results:** The results exhibited significantly reduced TG, LDL, TC, and VLDL levels. However, the HDL level was significantly increased as compared with induced rats. This current research demonstrated the anti-atherogenic potential of hydroalcoholic extract of the aerial part of *E. lactea*. **Conclusion:** Overall, the results revealed that plant extract at different doses lowered LDL and TC levels significantly. Hence it could be used to benefit from atherosclerosis and its associated risk factors inclusive of heart attack, and stroke. Furthermore, it might be used as a traditional medicine against CVD and beneficial in the treatment of hyperlipidemia-induced atherosclerosis and diabetes mellitus.

Keywords: Atherosclerosis, High-fat diet, Hypolipidemic, *E. lactea*, Heart attack, Stroke.

Correspondence:
Dr. Prabhash Nath Tripathi
Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH-58, Baghpat Road Crossing, Bypass Road, Meerut-250005, Uttar Pradesh, INDIA.
Email: prabhash.tripathi@miet.ac.in
ORCID ID: 0000-0002-1558-4964

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INTRODUCTION

Atherosclerosis is a complex progressive pathological process that produces lesions in the inner wall of an artery because of the abnormal lipid metabolism, and dysregulation of inflammatory processes. In addition, the abnormal metabolism of fat leads to disturbing the inflammation pathways and hormones that cause pancreatic cancer and diabetes mellitus.[1,2] It is the major global leading morbidity and death in various illnesses, including cardiovascular disease, coronary artery disease, and stroke.[3] According to World Health Organisation (WHO) assessments, 17.9 million people died worldwide in 2019, with 85% of those deaths occurring in developed countries.[4] The growth and development of atheroma in the artery walls because of ingestion of saturated fat to develop atherosclerotic plaque. The atheroma plaque causes a narrow lumen of arteries and restricts the blood flow in the arteries and other organs.[5] Hypercholesterolemia is a common risk factor, which raises TC, LDL, and VLDL levels while lowering HDL levels.[6,7] Lipid-enriched diets are frequently employed in animal models of atherosclerosis to promote or expedite the development of atherosclerotic plaques. Therefore, adopting a high-cholesterol diet to speed up atherosclerosis using the rat model to determine how treatments affect the disease.[8-10]

Alternative systems of medicine such as Unani, Siddha, Ayurveda, and tribal folklore medicines are used to control human health in India. Currently, these methods are used to treat a variety of disorders due to their varied pharmacological activities.[11,12] There are over 2000 species of flowering plants in the genus *Euphorbia* (Euphorbiaceae).[13,14] Additionally, ancient texts mention that it was utilized in the Chinese and Ayurvedic medical systems to cure rheumatism, liver ailments, respiratory problems, asthma, and

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stings from scorpions and snakes, as well as other conditions.[15] The presence of several chemical components, including phenols, flavonoids, diterpenes, triterpenoids, and essential oils, has been linked to the medical uses of these species.[16-18]

One of the Euphorbia species, *E. lactea,* has been used in India to cure a wide range of illnesses. It is reported to treat vomiting, constipation, digestive disorders, respiratory and skin infections, migraine, gonorrhea, intestinal and parasitic infections, and cancer.[19-22] Since it is known that *E. lactea's* aerial portion contains a variety of phenolic chemicals, including flavonoids, it is frequently investigated. The most common flavonoids include anthocyanidins, chalcones, flavonols, flavanone, and flavanol. These flavonoids include isoflavonoids (3-phenylbenzopyrans), neoflavonoids (4-phenylbenzopyrans), and others.[23-28] The current study proposed to examine the anti-atherosclerotic activity of the hydroalcoholic extract against HFD-induced atherosclerosis in rats and then evaluated the lipid profiles and lipid proteins (TG, HDL, LDL, TC, and VLDL) as well as atherosclerotic markers including CPK, ALT, ALP, LDH, and AST. The thoracic aortae of the heart were examined by histopathological studies.

MATERIALS AND METHODS

Chemicals

The chemicals, reagents, and solvents were acquired from Central Drug House in New Delhi, India, and were of laboratory reagent quality.

Plant collection and authentication

The fresh and matured aerial parts of *E. lactea* were collected from the herbal garden of the Department of Pharmacognosy, MIET, NH-58, Meerut, India. The plant was categorized and authenticated by a taxonomist from Chaudhary Charan Singh University, Meerut, India.

Extraction

The freshly collected aerial parts of *E. lactea* (500 g) were dried for a week and powdered at room temperature (24°C-28°C). This powder was macerated with petroleum ether (60-80°C temperature) and marc was thoroughly extracted with 20:80 (water: ethanol) solution for three days. The extract was desiccated and dried using a rotatory evaporator (Buchi, United States of America) at lower pressure before being used in additional pharmacological investigation.

Qualitative chemical examination of *E. lactea* extract

The hydroalcoholic extract of aerial parts of *E. lactea* was evaluated for preliminary phytochemical investigation to identify the phytochemical constituents.[29-32]

Animals

For the atherosclerosis study, 30 female Wistar rats (200-250 g) were obtained from the Meerut Institute of Engineering and Technology animal house facility, Meerut. Rats were kept in an institutional animal facility for a week before and after the studies in an optimum cross-flow ventilated room at 22°C and 12 hr cycles of light and dark. The experimental procedure and modules were approved by the Institutional Animal Ethical Committee, India (Reg. No. IAEC/MIET/2022/52).

Acute oral toxicity

The Organisation for Economic Co-operation and Development (OECD) guideline 423 was applied for conducting the acute toxicity experiments. 12 Swiss Albino mice (25-30 g) were grouped into 4 groups of 3 animals each. Group I was administered 5 mg/kg body weight, group II 50 mg/kg body weight, group III 300 mg/kg body weight, and group IV 2000 mg/kg body weight, with *E. lactea* extract. The route of administration of doses of the extract is per oral (*p.o.*) in the form of suspension to experimental animals.[33,34] All the animals were examined critically for the first 4 hours and then every 24 hours for 14 days for signs of toxicity and cholinergic effects.[35-39]

E. lactea extract dose selection

An acute toxicity study was conducted as per the OECD guidelines 423 to calculate the dose for pharmacological investigations. The acute oral toxicity study of hydroethanolic extract of *E. lactea* resulted in no symptoms of acute toxicity at the highest dose (2000 mg/kg). As a result, 1/5th (200 mg/kg, body weight) and 1/10th (400 mg/kg, body weight) were chosen as the dosage for pharmacological research using the fixed-dose approach.[40]

High-Fat Diet (HFD)-induced atherosclerosis

HFD or atherogenic diet consisted of BYCALVIT-500, procured from Biochem Pharmaceutical Industries Ltd., India) containing cholesterol (2 g), saturated groundnut fat (8 g), and calcium (0.1 g) with suitable nutrients, mixed with powdered standard pellet (90 g).[41] Five groups of 6 rats each were made by randomly selecting female rats from the population. Each animal was marked on the tails for identification. For 28 days, group I was kept on a pellet diet, and group II was kept on HFD. Groups III and IV were given HFD and *E. lactea* extract (200 and 400 mg/kg, *p.o.*, respectively). Further, Group-V was administered atorvastatin (30 mg/kg, *p.o.).* On the 29th day, Water was provided *ad libitum* to all animals. The rats in all groups were anesthetized and blood was drawn through the retro-orbital plexus and collected in EDTA tubes. Then centrifugation was used to separate the serum. Using standard kits, serum was examined for lipid profile and liver function test parameters. After collecting blood samples, all rats were sacrificed using an overdose of anaesthesia, and their thoracic aorta was removed, rinsed with saline, and stored in 10% formalin for histopathological studies.

Estimation of biochemical parameters

ELISA kits were used to estimate the biochemical parameters. The collected plasma supernatant was tested for biochemical estimations of lipid profile such as Free Fatty Acids (FFA), TC, TG, and lipoproteins status, HDL, VLDL, LDL as well as atherosclerotic markers such as AST, ALT, ALP, LDH, and CPK. The Atherogenic Index (AI) was determined by using the mentioned equation.[42]

$$\text{Atherogenic Index} = [\text{Log}\,(\text{TG/HDL})]$$

Histopathology

A histopathological study was done to determine the grading of atherosclerotic plaque formation in all groups of rodents. On completion of the experimental study, all animals of corresponding groups were sacrificed, and a thoracic aorta was isolated. Thoracic aortae from experimental groups were placed in 10% formalin, dehydrated alcohol, and then fixed in paraffin wax. Each thoracic aortae sample was cut into microtome sections (5 µm thick). For deparaffinization, silane-coated slides were rinsed with xylene and rehydrated with ethanol. The sections of tissues were marked with hematoxylin and eosin for photo-microscopic study. All the slides were investigated under a Meiji fluorescent microscope for any histological ruination and protection.[43]

Table 1: Phytochemical analysis of *E. Lactea* extract.

Phytoconstituents analysis	Test	Result
Carbohydrates	Benedict's test	Positive
	Fehling's test	
Phenols	Ferric chloride test	Positive
Flavonoids	Alkaline reagent test	Positive
	Shinoda test	Positive
Tannins	Gelatin test	Positive

Statistical analysis

Statistical analysis was performed through the utilization of the Graph Pad Prism 5.0 for all the analyses. The outcome of the study was shown as mean±SEM (n=6). Statistical significance (***p<0.0001; **p<0.001; **p<0.01; *p<0.05) was applied to evaluate and compare the means of the treatment groups with the negative control group.

RESULTS

Phytochemicals analysis

Phytochemical analysis of *E. lactea* determined the presence of carbohydrates, tannins, phenols, and flavonoids, as shown in Table 1.

Acute oral toxicity

The acute oral toxicity studies of hydroalcoholic *E. lactea* extract results reported no toxic reactions or cholinergic effects on the rats at doses of 5, 50, 300, and 2000 mg/kg, *p.o.* No mortality was observed in any of the groups during the acute oral toxicity studies. The entire study also showed that both doses had a substantial margin of safety up to 2000 mg/kg, the maximum dose. 1/5th and 1/10th of the dosage is considered therapeutic doses based on the safety at the maximal dose. Therefore, to test the anti-atherosclerotic activity, dosages of 200 and 400 mg/kg body weight were used, respectively.

Effect of *E. lactea* extract on body weight

The experimental animals in all groups showed an increase in their body weight as tabulated in Table 2. In group I, the body weight was increased by 30.99% at the end of the fourth week compared to day one. The group-II displayed a linear elevation in body weight of 60.34% as compared to day one. The group-III and IV treated with *E. lactea* extract exhibited weight loss (29.45% and 28.22%) in comparison to group-I and II. Group V displayed weight loss in comparison to group II (60.34%). The experimental

Table 2: Effect of *E. lactea* extract on the body weight of experimental animals.

Group	1st Day	7th Day	14th Day	21st Day	28th Day
Group I	116.70±8.17	122.94±2.61 (5.35) [a]	140.02±4.21 (19.99) [a]	146.01±5.69 (25.12) [a]	152.87±5.92 (30.99) [a]
Group II	118.78±3.31	136.78±5.57 (15.16) [a]	158.05±6.59 (33.16) [a]	173.64±3.38 (46.19) [a]	190.45±7.87 (60.34) [a]
Group III	120.18±2.62	132.34±7.92 (10.11) [a]	142.16±4.77 (18.28) [a]	149.69±7.44 (24.56) [a]	155.58±6.99 (29.45) [a]
Group IV	119.21±2.74	132.72±6.14 (10.72) [a]	143.39±5.66 (20.29) [a]	150.64±4.25 (26.37) [a]	152.86±5.77 (28.22) [a]
Group V	118.53±3.95	131.14±6.80 (10.63) [a]	142.10±5.85 (19.89) [a]	153.15±6.20 (29.21) [a]	155.01±5.44 (30.77) [a]

Values are mean±SEM of the corresponding group.[a]Variation in body weight as a percentage from day one.

rats (group-III and IV) treated with *E. lactea* extract (dose of 200 and 400 mg/kg) showed a decrease in their body weights due to the anti-obesity potential of *E. lactea* extract in a dose-dependent manner. Additionally, *E. lactea* was administered in experimental animals for its anti-atherosclerosis action which also contributes to the reduction of body weight dose-dependently.

Lipid profile

The results are expressed in Figure 1, the outcome illustrated that the HFD group represented significantly higher plasma levels of lipids (TC, TG, and FFA) in comparison to group I. However, administration of *E. lactea* extract (200 and 400 mg/kg; body weight), group-III and IV respectively, exhibited significantly lower levels of blood lipids (TC, TG, and FFA) compared to group-II. Animals in *E. lactea* group-III and V also displayed an improvement in the induced atherosclerosis in a dose-dependent manner. Moreover, the atorvastatin-treated group also displayed in marked decline in lipid profile (TC, FFA, and TG) in comparison to group-I.

Lipoprotein status

The results in Figure 2 expressed that the HFD group showed increased levels of (LDL; Figure 2a; $^{***}p<0.001$ and VLDL; Figure 2b; $^{***}p<0.001$) and reduced level of (HDL; Figure 2c; $^{***}p<0.001$) significantly in comparison to group-I. The rich cholesterol diet is reported to increase LDL and VLDL levels which are responsible for the generation of atherogenic form and subsequently cause atherosclerosis and leads to cardiovascular impairment. However, *E. lactea* extract (200 and 400 mg/kg, body weight) displayed a statistically significant decline in levels of (LDL; Figure 2a; $^{\#\#}p<0.01$ and VLDL; Figure 2b; $^{\#}p<0.05$, $^{\#\#}p<0.01$) and elevated the level of (HDL; Figure 2c; $^{\#}p<0.05$, $^{\#\#}p<0.01$) at a dose-dependent manner in comparison to group-II. Additionally, atorvastatin demonstrated downfall in levels of (LDL; Figure 2a; $^{\#\#\#}p<0.001$ and VLDL; Figure 2b; $^{\#\#\#}p<0.01$) and elevation in level of HDL (Figure 2C; $^{\#\#\#}p<0.001$) in comparison to group-II. It is broadly accepted that elevated levels (LDL and VLDL) and reduced levels of HDL are the most common factors for the development of atherosclerosis that augments the progression of ischemic heart and coronary heart diseases.

Estimation of Atherogenic index (AI), TC/HDL, and LDL/HDL

The results in (Figure 3) HFD-induced atherosclerosis showed a strong connection with vascular necrosis and oxidative stress which ultimately leads to the pathogenesis of diseases. The group-II represented significantly higher AI, TC/HDL, and LDL/HDL levels (Figure 3a, 3b, and 3c; $^{***}p<0.001$) in comparison to group-I. These atherogenic parameters provide the best direction to identify the irregularity in the lipid metabolism processes. The *E. lactea* extract-treated groups (III and IV) displayed significantly lower levels of AI, TC/HDL, and LDL/HDL (Figure 3a $^{\#\#\#}p<0.001$; 3b $^{\#\#\#}p<0.001$; 3c $^{\#\#\#}p<0.001$) in comparison to group-II. However, group-V results demonstrated significantly lower levels of AI, TC/HDL, and LDL/HDL (Figure 3a $^{\#\#\#}p<0.001$; 3b $^{\#\#\#}p<0.001$; 3c $^{\#\#\#}p<0.001$) in comparison to group II. These findings indicated that greater AI is among the chief key parameters for atherosclerosis. The results also demonstrated that *E. lactea* extract has the potential to reduce AI, TC/HDL, and LDL/HDL levels in a dose-dependent manner.

Atherosclerotic markers

The results depicted in Figure 4 expressed the effects of *E. Lactea* extract on atherosclerotic markers (ALT, AST, ALP, LDH, and CPK enzymes) in comparison to the groups-I, II, and V. The estimation of AST and LDH has been used as a diagnostic marker specifically for myocardial infection and other cardiovascular diseases. During the pathological situation, these markers outflow from necrotic cardiac cells to blood, so these markers are used as an alert for cardiac injury. The results in Figure 4a, 4b and 4c showed that marker levels (AST, ALT, and ALP) were marked elevated in group-II (HFD)in comparison to group-I (control) (Figure 4a $^{***}p<0.001$; 4b $^{***}p<0.001$; 4c $^{***}p<0.001$). Further, *E. lactea* extract treated groups-III and IV (200 and 400 mg/kg, body weight) marked declined these markers levels (Figure 4a $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$; 4b $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$; 4c $^{\#}p<0.05$ $^{\#\#}p<0.01$) in compared to group-II. In comparison to group-II,

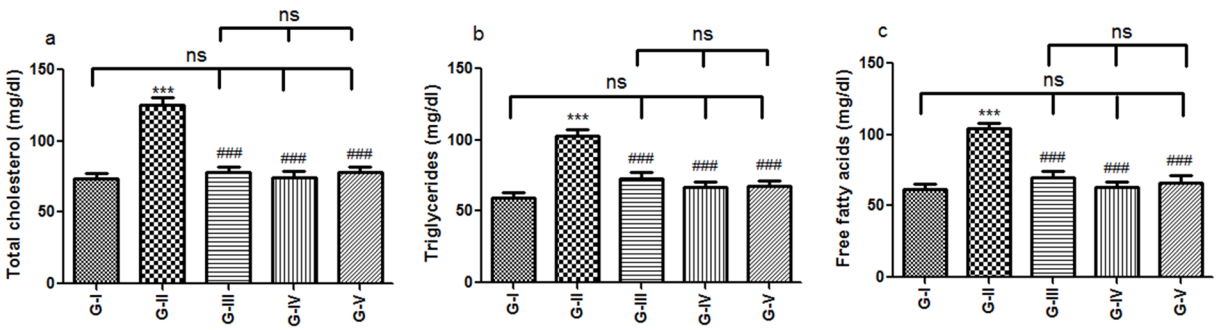

Figure 1: Effects of *E. lactea* extract on HFD-induced atherosclerosis in Wistar rats on levels of lipid profile status. All the values presented as mean±SEM (n=6), significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. group-II by one-way ANOVA used for multiple comparisons and ns=non-significant.

group-V which received atorvastatin (30 mg/kg, body weight) also displayed a marked decrease in the levels of ALT, AST, and ALP (Figure 4a $^{###}p<0.001$; 4b $^{###}p<0.001$; 4c; $^{##}p<0.01$).

The results in Figures 4d and 4e expressed that the atherosclerotic markers of serum LDH and CPK were marked higher in group-II in comparison to group-I (Figure 4d$^{***}p<0.001$; 4e$^{***}p<0.001$). A dose-dependent reduction in LDH and CPK was observed in *E. lactea* extract (200 and 400 mg/kg) groups in comparison to group II (Figure 4d $^{##}p<0.01$, $^{###}p<0.001$; 4e $^{###}p<0.001$, $^{###}p<0.001$). Group-V, atorvastatin (30 mg/kg) expressed similar results as *E. lactea* extract treated groups (Figure 4d $^{###}p<0.001$; 4e $^{###}p<0.001$).

Histopathological studies

The results of histopathological studies of thoracic aortae, Figure 5 demonstrated that control (Group-I) rats that were put on the pellets diet showed no changes in the aorta architecture. The rats that were put on HFD (Group-II) had minor mineralization and localized rupture in the aortic intima and media. An increase in the gap was also observed between the tunica media and intima. The thickness was also increased due to the slight build-up of atherosclerotic plaque in the artery intima. The increased space between the layers may be the result of the production of free radicals. These free radicals are likely to harm the genetic material and they may also cause harm to the membrane of endothelial cells in the tunica intima and of the smooth muscle cells of tunica media. Rats in group II that were given HFD had deposits of lipid content in their aortae. Rats in groups III and IV were administered *E. lactea* extract at doses of 200 and 400 mg/kg, body weight and group V received atorvastatin (30 mg/kg, body weight). Groups-III, IV, and V showed a substantial reduction in the gap between the tunica intima and tunica media within the intimal thickness.

DISCUSSION

Atherosclerosis is a coronary arterial disease that results in the cholesterol plug formation in the lumen of the artery that causes blood flow restriction. This is a leading risk factor for Coronary Artery Disease (CAD) like angina, and myocardial infarction. Elevated cholesterol specifically LDL and VLDL are major reasons that are responsible for cardiovascular disease.[44-52] *E. lactea* is a medicinal plant that is used to treat atherosclerosis traditionally. This plant is well known for diverse biological and pharmacological uses for the treatment of digestive disorders like, jaundice, diarrhea, and constipation. Moreover, it was also used against skin disorders like itching, rashes, ulcers, and respiratory disorders like bronchial complaints and breathlessness.[53-60] In this current study, the anti-atherosclerosis effect of *E. lactea* was investigated in experimental Wistar rats. The anti-atherosclerotic

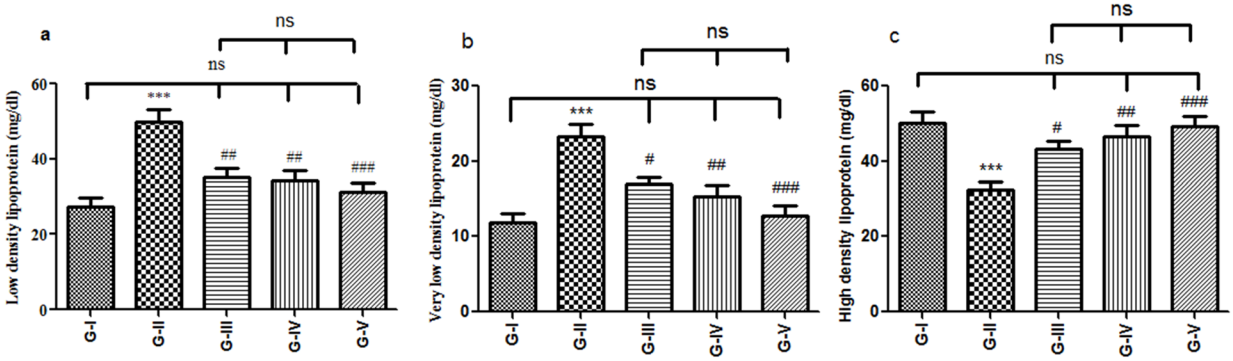

Figure 2: Effects of *E. lactea* extract on HFD-induced atherosclerosis in Wistar rats on levels of lipoprotein profile. All the values presented as mean±SEM (*n*=6), significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. group-II by one-way ANOVA used for multiple comparisons and ns=non-significant.

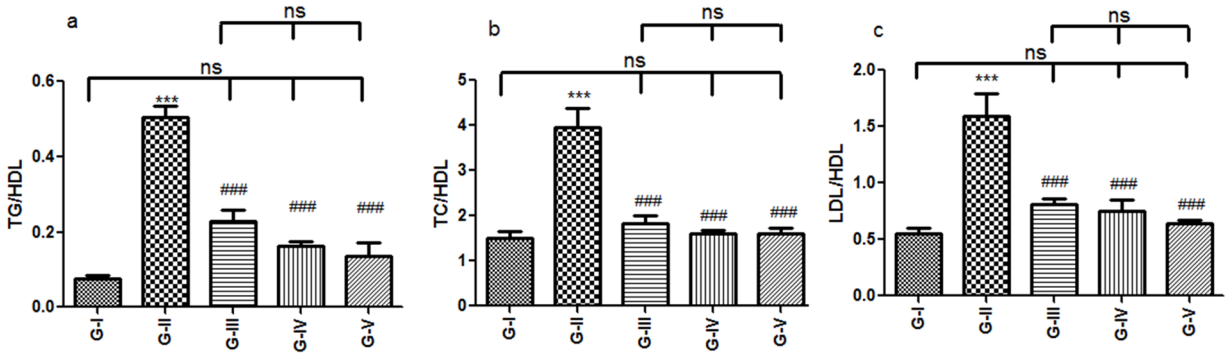

Figure 3: Atherogenic index of *E. lactea* extracts on HFD-induced atherosclerosis in Wistar rats on levels of AI, TC/HDL, LDL/HDL. All the values presented as mean±SEM (*n*=6), significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. group-II by one-way ANOVA used to multiple comparison and ns=non-significant.

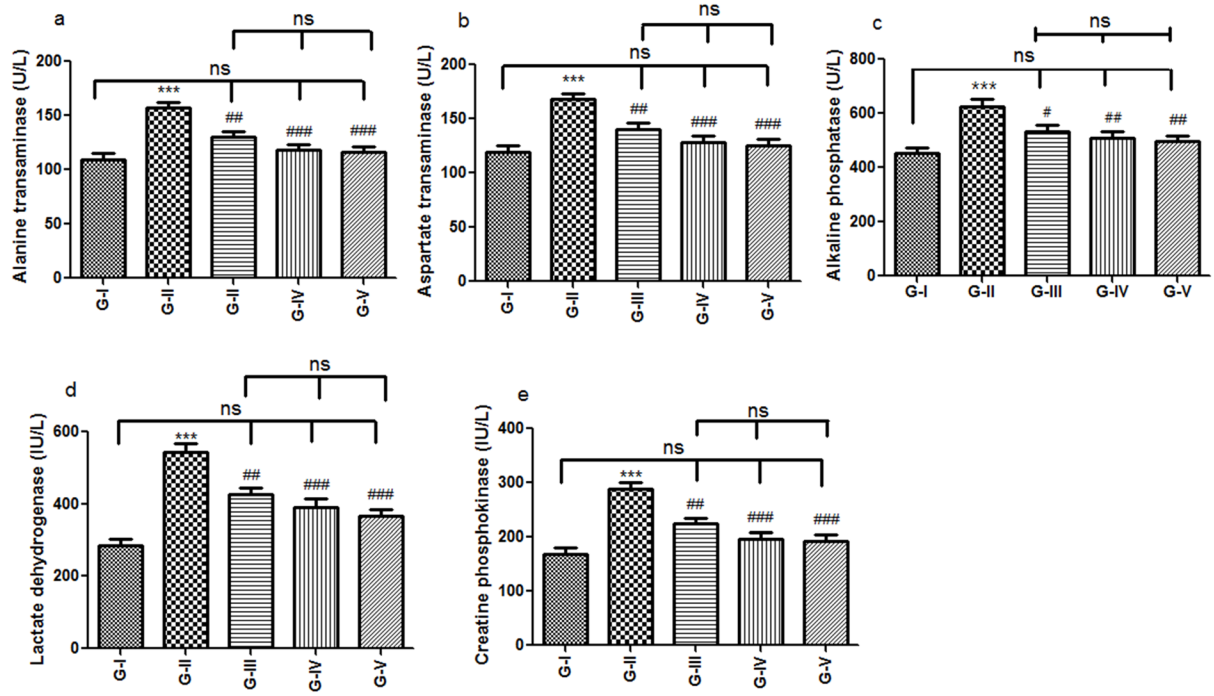

Figure 4: Effect of *E. lactea* extract on atherosclerotic markers ALT, AST, ALP, LDH, and CPK on HFD-induced atherosclerosis in Wistar rats. All the values presented as mean±SEM (n=6), significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. group-II by one-way ANOVA used to multiple comparisons and ns=non-significant.

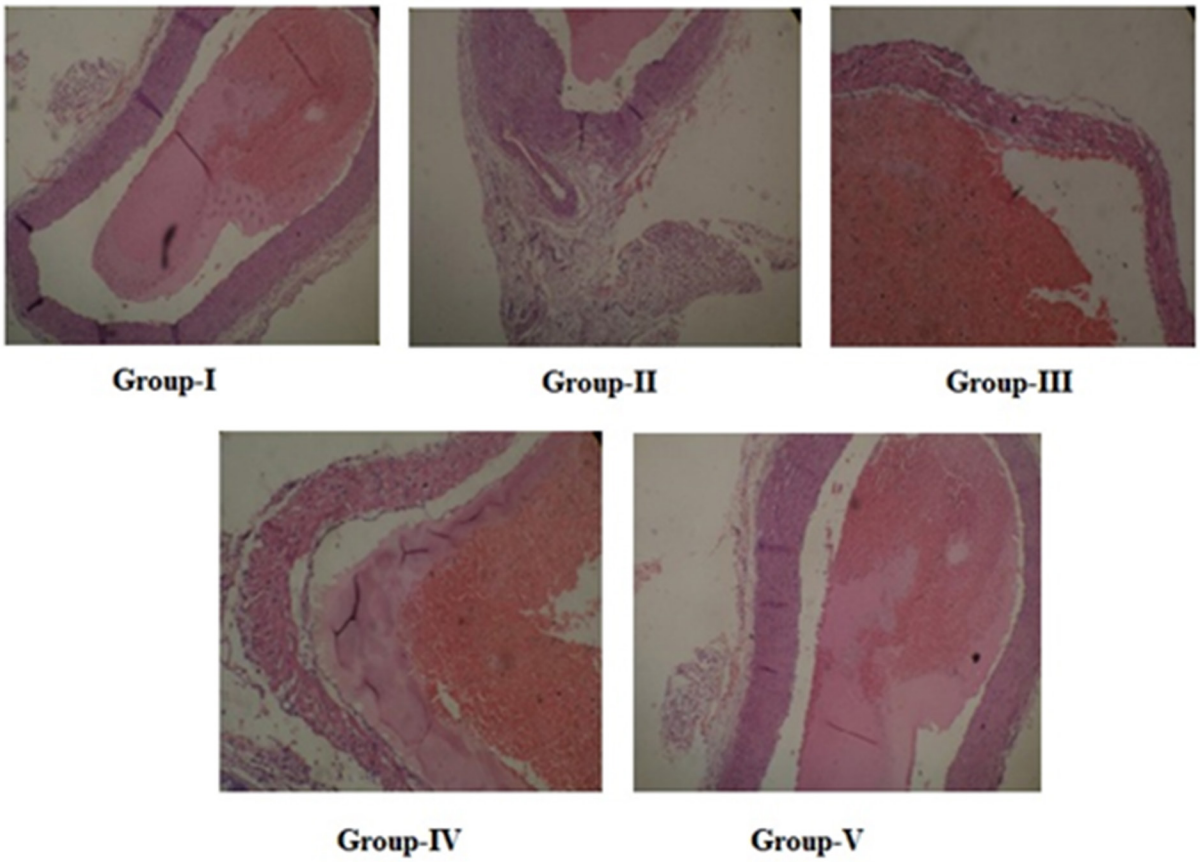

Figure 5: Photomicrographs of hematoxylin and eosin-stained sections of thoracic aortae at 250X.

activity of *E. lactea* extract may be due to phytoconstituents like phenolic compounds and flavonoids. This study affirms reduction in TC and improvement in lipids profile in a dose-dependent manner.

Different parameters were used to evaluate the anti-atherosclerotic activity of *E. lactea* including estimation of biochemical parameters such as lipid profile (TC, TG, and FFA), lipoprotein status (HDL, LDL, and VLDL), atherosclerotic markers (ALT, AST, ALP, LDH, and CPK) and histopathological analysis of aorta.

Atherosclerosis causes high levels of TC, TG, and LDL and the level of HDL decreases in blood. *E. lactea* decreases levels of TC, TG, and FFA reducing the further progression of atherosclerosis, an arterial disease. The lipid profile significantly improved after two weeks of treatment with *E. lactea* extract (200 mg/kg and 400 mg/kg, body weight) in a dose-dependent manner. *E. lactea* showed anti-hyperlipidemia properties, as evidenced by lipid profile activity in serum, lowering the risk of atherosclerosis.

The Lipoprotein status (LDL, VLDL, and HDL) was also measured in all the experimental groups. In comparison to HFD group II, treatment groups III, IV, and V showed lower levels of LDL and VLDL and higher levels of HDL. LDL is directly involved in the steps of atherosclerosis and is considered the main risk factor for atherosclerosis. LDL lipoprotein accumulates in the coronary artery and forms the plug that creates the restriction in the blood flow in arteries. The VLDL is hydrolyzed by lipoprotein, resulting in the larger particles being rich in triglyceride and apolipoprotein-C.[60-61] In the current investigation, HFD rats had considerably higher serum TG levels. Excessive fat intake raises TG levels, one of the factors in artery hardening. The reserve cholesterol transport mechanism enables the removal of extra tissue cholesterol for onward transportation to the liver for further metabolism. This mechanism is responsible for the production of HDL that prevents arterial plugs.

The Atherogenic Index (AI) is a biomarker of dyslipidemia, atherosclerosis, and coronary and metabolic syndrome to find the lipid levels as well as alert the risk of Cardiovascular Disorders (CVD) progression. These atherogenic parameters provide the best direction to identify the irregularity in the lipid metabolism processes. The experimental endpoints exhibited a marked higher level of AI, TC/HDL, and LDL/HDL in group-II than group I. However, the treatment with *E. lactea* at doses of 200 and 400 mg/kg, body weight) displayed a marked reduction in levels of atherogenic parameters (AI, TC/HDL, and LDL/HDL) as compared to group-II. Additionally, atorvastatin-treated rats (Group-V) significantly reduced the levels of atherogenic parameters as compared to group-II rats. The atherogenic parameters have greater AI is the most important key parameter for indicating atherosclerosis. These experimental data also demonstrated that the extract of EL has the potential to reduce AI, TC/HDL, and LDL/HDL.

The atherosclerotic markers (ALT, AST, ALP, LDH, and CPK) were measured to detect myocardial injury in experimental rats. In the pathological condition, there is an out flux of CPK, LDH, AST, and ALT from cardiomyocytes to blood indicating myocardial injury. The atherosclerosis markers (LDH, ALT, AST, ALP, and CPK) were expressed more in the treatment group than control. Whereas treatment with *E. lactea* extract in group-III, and IV, and group-V treated with atorvastatin showed a marked reduction in levels of (LDH, ALT, AST, ALP, and CPK) in group-II rats. The remarkably lower enzyme levels of ALT, CKP, AST, and LDH confirmed the potential of *E. lactea* extract for cardio protection.

Histopathology is used for the diagnosis and study of diseases in the tissues and cells under the microscope. In comparison to the group I rats, group II rats had slight mineralization and localized damage in the intima and media of the aorta. However, group III, and IV (treated with *E. lactea* extract) and group V rats (treated with atorvastatin) results significantly exhibited restoration of intima thickness in a dose-dependent manner.

CONCLUSION

Aerial parts of *E. lactea* were subjected to extraction by using petroleum ether and hydroalcoholic solution (water: ethanol; 20: 80). The preliminary phytochemicals investigation of *E. lactea* extract showed the presence of carbohydrates, tannins, flavonoids, and phenols. Results obtained from pharmacological investigations are consistent with exhibiting the anti-atherosclerotic property of *E. lactea*. The estimation of serum TC, HDL, LDL, VLDL, and TG levels was used as a model for the evaluation of anti-atherosclerotic activity. The study showed a dose-dependent relationship in the efficacy of the extract where 400 mg/kg, b.wt. was substantially more effective than 200 mg/kg, b.wt. In addition, *E. lactea* extract showed significant properties such as, hypolipidemic and anti-atherosclerogenic with safety in the experimental study. In this way, it might be used as a traditional medicine against CVD. *E. lactea* is also very beneficial in the treatment of hyperlipidemia-induced atherosclerosis and diabetes mellitus.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LDL: Low-Density Lipoprotein; **TC:** Total Cholesterol; **TG:** Triglyceride; **HDL:** High-Density Lipoprotein; **VLDL:** very low-density lipoprotein; **CPK:** Creatine Phosphokinase; **ALT:** Alanine Transaminase; **ALP:** Alkaline Phosphatase; **LDH:** Lactate Dehydrogenase; **AST:** Aspartate Transaminase; **WHO:** World Health Organisation; **HFD:** High-fat diet; **AI:** Atherogenic index; **CVD:** Cardiovascular Disorders.

SUMMARY

- Atherosclerosis is produces lesions in the inner wall of an artery because Increased levels of LDL and TC lead to hyperlipidaemia which is a major risk factor for atherosclerosis.
- The plant extract of E. lacteal exhibited a significant reduction of TG, LDL, TC, and VLDL levels whereas HDL level was increased in induced rats.
- Also, its lowered LDL and TC levels and could be used to benefit from atherosclerosis and its associated risk factors inclusive of heart attack, and stroke.
- It might be used as a traditional medicine against CVD and beneficial in the treatment of hyperlipidemia-induced atherosclerosis.

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