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Evaluation of anti-atherosclerotic activity of stem bark of *Peltophorum pterocarpum* by high fat diet induced atherosclerosis model in experimental animals.

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Declaration of interest
Authors declares no conflict of Interest

Abstract

Introduction: An ongoing inflammatory is condition in atherosclerosis in which there is fat streak, a buildup of foam cells with lipid content in the artery's intimal layer act as precursor to atherosclerosis. *Peltophorum pterocarpum* stem bark extract found to be effective in inhibition of platelet aggregation and possess anti-inflammatory effect that may be useful in treating atherosclerosis. Therefore, the goal of the current investigation is to ascertain if the ethanolic extract of *Peltophorum pterocarpum* stem bark (EEPP) has anti-atherosclerosis effect in Wistar rats using a high fat diet in atherosclerotic induced atherosclerosis model for 60 days. Atherosclerosis was induced in male Wistar rats by giving them a high-fat diet. Following induction, oral doses of EEPP (250, 500 mg/kg) and atorvastatin (10 mg/kg) were administered orally to the rats for the following 20 days.

Methods: The literature was searched using PubMed and google scholar search Engines. A total 30 plants were mentioned through database searches. Papers from between 1999 and 2022 were included. An attempt was made to document relevant literature that is only focused on potential effect of various plants in treatment of atherosclerosis.

Results: In comparison to the control group, EEPP was able to significantly lower the raised serum levels of total cholesterol, triglyceride, LDL, and VLDL while significantly increasing the levels of HDL. When compared to the diseased control group, EEPP significantly lessened damage to the endothelial lining of the aorta and had an impact on lumen size by lowering cholesterol buildup.

Keywords: Atherosclerosis, stem bark, Atorvastatin, *Peltophorum pterocarpum*, High-fat diet.

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INTRODUCTION

Atherosclerosis is the pathology that underlies CHD (Coronary Heart Disease).Atherosclerosis or CHD is an inflammatory fibro-proliferative response to multiple forms of endothelial injury. [1] CHD is associated with increased in inflammation, deregulated lipid metabolism, fibrinogenesis, thrombogenesis and increased oxidative stress. [2] Most important risk factor is hyperlipidemia in development of atherosclerosis, and is characterised by elevation of total cholesterol and triglyceride levels. [3]

Atherosclerosis is a condition characterized by the buildup of plaque in arterial walls, leading to reduced blood flow and an elevated the risk of cardiovascular events such as heart attacks and strokes. Antiplatelet agents are drugs that prevent platelet aggregation and clot formation, thereby reducing the risk of thrombotic events in patients with atherosclerosis or other cardiovascular diseases.

The condition known as atherosclerosis is when the arterial wall hardens as a result of buildup of fatty substances like cholesterol and triglycerides. At the site of the injury, fatty deposits that are composed of lipids and another biological material grow over time, harden and constrict the arteries. The consequent lack of sufficient blood flow prevents the organs and tissues affiliating the blocked arteries from operating normally. In the end, a piece of the fat deposits can separate and enter the bloodstream; furthermore, the plaque's smooth inside may crack, releasing cholesterol and other materials into the blood. [4] This could result in a blood clot, which could stop the flow of blood to a particular area of the body, as is the case when the heart is the target of restricted blood flow, leading to a heart stroke. This blood clot may also travel to other body regions and obstruct blood flow to other organs.

Heperlipidemia and thrombosis are main causes of atherosclerosis. [5] In the world, statins, sometimes referred to as HMG-CoA reductase (HMG-CoA) inhibitors, are one of the most commonly prescribed atherosclerosis drugs. Concern has lately been raised concerning the overprescription of statin drugs due to the potential for major side effects from statin therapy.

There is a long history of using herbal therapy to treat a number of illnesses. As a result, people have used this herbal medicine to heal illnesses for a very long time and still do now. The bulk of medicinal plants utilised in traditional medicine today typically lack supporting scientific evidence, necessitating efficient and reliable assessments of their potential.

Peltophorum pterocarpum, also known as copperpod, golden flamboyant, yellow flamboyant, yellow flame tree, yellow Poinciana, and radhahura in Bangla, is a Fabceae family native to tropical Southeast Asia. It is also a well-known ornamental tree grown all over the world. *P. pterocarpum* flower extracts in petroleum ether and ethanol were discovered to have cardiotonic effects on frogs.[6]

In comparison to ethanol extract, which produced significant positive ionotropic but slightly negative chronotropic effects (as digoxin), petroleum ether extract significantly increased force of contraction

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while decreasing heart rate (as adrenaline).In comparison to ethanol extract, which produced significant positive ionotropic but slightly negative chronotropic effects (as digoxin), petroleum ether extract significantly increased force of contraction while decreasing heart rate (as adrenaline).

In order to determine if an ethanolic extract of stem bark from *Peltophorum pterocarpum*, which is used in folk medicine, is effective in the therapy of inflammatory illnesses. We undertook the current investigation

Methods

Collection of plant material and preparation of plant extract

The bark of *Peltophorum pterocarpum* was collected from the month of September 2022.The plant was identified and authenticated by botanical survey of India, Koregaon Park, Pune and a Ref. No (2023/1403230026810).The fresh *Peltophorum pterocarpum* bark were collected and dried under shade until they are completely dried. Cut into pieces and shed dried. Then dried bark pulverized into powder by electric grinder. A known weight of powder (1kg) was macerated in 3.5L absolute ethanol in macerated flask. The mixture left for 72hrs with stirring. Filter with muslin cloth .Further by whatman N0.1 filter paper. Filterate was concentrated to remove fine residues using rotary evaporator at 45^0C. Then ethanolic extract is stored in sterile screw capped bottle at 2-8^0C. [7]

Drugs and chemicals

Peltophorum pterocarpum bark was gathered in the Pimpri Chinchwad region of Pune, India. Chemicals for high-fat diets (cholesterol, choline acid, and vitamin D3) were bought from Uttam Chemicals Pune, MS (India). All additional compounds were of the analytical grade and were obtained from nearby vendors.

Experimental Animals

Healthy male Wistar rats (180-200) and obtained from crystal biological solutions Pune India. Animal were housed in a group of 6 per cage in standard polypropylene cages (32.5 x 21 x14) cm lined with rae husk. The animal house were maintains on 12 hrs light and dark cycle Approx. 22 ± 2^0C, relative humidity 60-70%.[8] All animals were provided with standard laboratory diet and water. All experimental procedures were carried out in conformity with the rules set forth by the committee responsible for controlling and overseeing animal experimentation i.e. CPSCEA (MCP/IAEC/06/2022) were approved by institutional animal ethics committee.

Induction of atherosclerosis

The study aimed to induce atherosclerosis in rats by subjecting them to a high-fat diet along with vitamin D3 administration through oesophageal injection (700,000 IU/kg) in the initial four days. The high-fat diet consisted of a lipid emulsion containing 4% cholesterol, 1% cholic acid, 0.5% PTU, and salad oil. The effectiveness of the model was evaluated after 80 days by examining gross anatomy and histology. The standard group received Atorvastatin (10 mg/kg), while the test groups were given

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EEPP (250 mg/kg & 500 mg/kg p.o.) during the last 20 days. The normal control group received distilled water, whereas the disease control and diseased groups both received a high-fat diet alongside regular chow. [9]

Animal grouping and dosing methods

The animals were grouped using simple allocation. There were five groups, each containing six rats.

Group I: This was the normal control group, where rats received the vehicle (distilled water).

Group II: The disease control group consisted of rats that were administered a high-fat diet for a duration of 80 days.

Group III: The standard group received a high-fat diet for 60 days, followed by 20 days of treatment with 10 mg/kg of the standard drug (Atorvastatin).

Group IV: Test group 1 underwent a high-fat diet for 60 days, followed by 20 days of treatment with 250 mg/kg of EEPP (a test substance).

Group V: Test group 2 followed the same high-fat diet for 60 days, followed by 20 days of treatment with 500 mg/kg of EEPP.

Fibrinolytic Activity of *Peltophorum pterocarpum*

In-vitro blood clot dissolving activity of *Peltophorum pterocarpum*, In-vitro blood clot dissolving activity of *Peltophorum pterocarpum* was studied on 4 white cotton cloth pieces (5 × 5) stained with blood (Rat blood). The following sets were prepared & studied:

1. Control set - Petriplate with D.W. (Distilled Water) (10 ml) + stain cloth
2. Standard set –Petriplate with D.W. (10 ml) + Streptokinase 5000 IU/ml + stain cloth
3. Test set I -Petriplate with D.W. (10 ml) + stain cloth + EEPP(250mg/kg)
4. Test set II -Petriplate with D.W. (10 ml) + stain cloth + EEPP(500mg/kg)

The above petriplate were incubated at 37^{o}C for 1h 20 minutes. After incubation, cloth pieces were taken out, rinsed with water and dried. Visual examination of various cloth pieces was done.[10]

Artificial blood clot degradation method

In a glass test tube with 500 mL of fresh rat blood, an artificial blood clot was created using spontaneous coagulation. The fake blood clot was cleaned numerous times an hour later. EEPP (3g/ml), EEPP (6 g/ml), and streptokinase (5000 IU/ml) were applied to the artificial blood clot at room temperature as a standard and control, respectively. Using colour development after one hour at room temperature, the clot's deterioration was visually evaluated.[11]

Biochemical analysis

Rats' retro-orbital venous plexus was used to obtain blood samples on days 60 and 80 without the use of a coagulant to separate the serum.[12] The serum was separated from the blood by centrifuging it at 2000 rpm for 15 minutes after the blood had been drawn into microcentrifuge tubes. The serum was then preserved until it could be examined for various biochemical parameters. The blood had been drawn into microcentrifuge tubes. Using an ERBA kit that is available in the lab at our institute, an autoanalyser was used to do the biochemical assay.

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Histopathology

The aorta and liver tissues were separated and preserved in 4% neutral buffer formalin for fixation. Then, they underwent a gradual dehydration process by passing through a series of ethyl alcohol and water gradients until they were dried. After the dehydration step, the samples were washed with xylene, and subsequently, they were embedded in paraffin.

To visualize the tissue sections, Hematoxylin and eosin dye (H and E) staining was employed. The sections were made approximately 5 micrometers thick.

Histological analysis of the stained tissue sections was conducted using light microscopy to examine the cellular and tissue structures.

Statistical Analysis

The results obtained were expressed as the mean ± standard error means (SEM) and statistically analyzed by applying one-way ANOVA, followed by the Dunnett method. Differences with $P \leq 0.0001$ were considered statistically beneficial.

Results

Preliminary Phytochemical analysis

The percentage yield of ethanolic extract of *Peltophorum pterocarpum* stem bark was found to be 14.2%.

Qualitative Phytochemical analysis

The qualitative phytochemical analysis of EEPP showed an occurrence of active phytochemical constituents such as Alkaloids, Tannins, Flavonoids, Phenolics, Saponins, Terpenoids.

Quantitative Phytochemical analysis

Total phenolic and flavonoid content

Quantitative test	**Ethanolic extract of PP**
Total Phenolic Content	52.09 mg/gm
Total Flavonoid Content	20.04mg/gm

Table1. Total phenolic and flavonoid content

Thin Layer Chromatography

The results of TLC analysis of ethanolic extract of *Peltophorum pterocarpum*stem bark are given in following table and fig.

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Figure1:-TLC of ethanolic extract of EEPP stem bark

Sr. No	Solvent system	Rf value
1	Petroleum ether : Chloroform	0.89

Table2. TLC solvent system and Rf values

High Performance Thin Layer Chromatography

In the present study the result of HPTLC analysis of ethanolic extract *Peltophorum pterocarpum* stem bark are given in the following data (Spectrum data at Rf 0.82 in UV 255 nm) and the standard Quarcetin is Rf 0.79 in UV 255nm).

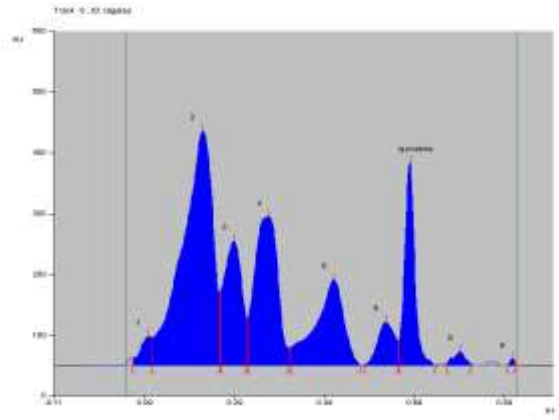

Figure 2:- HPTLC of Quarcetin Rf 0.79

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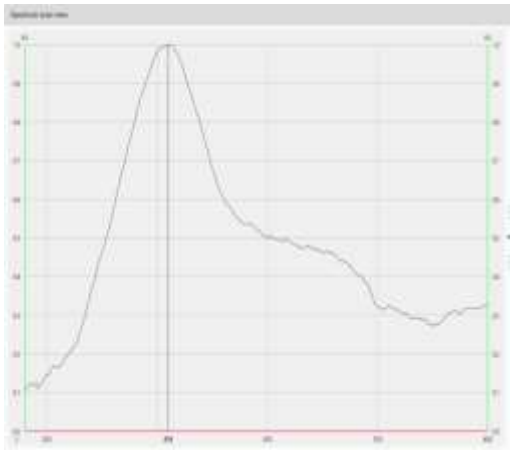

Figure 3:- HPTLC of EEPP Rf 0.82

Determination of Fibrinolytic activity

In-vitro blood clot dissolving activity of EEPP stems bark

By visual examination of blood-stained cloth, it was proved that cloth washed with EEPP stem bark and Streptokinase dissolved the blood clot as compared to distilled water. Hence they possessed fibrinolytic property (Badhe R. et al 2013). When compared to EEPP stem bark, streptokinase had a significantly higher effect on clot dissolution.

(A) (B) (C)

Figure 4:-In-vitro blood clot dissolving activity of EEPP stems bark

A- Blood stained cloth washed with water.

B- Blood stained cloth washed with streptokinase.

C- Blood stained cloth washed with EEPP stem bark.

Fibrinolytic effect by artificial blood clot degradation method

In the test tube containing EEPP stem bark, blood clot degradation was observed. Within this tube, red blood cells were found to be trapped by multiple fibrin nets, indicating that the EEPP stem bark had a clot-dissolving effect. However, in the test tube containing normal saline, no blood clot degradation was observed until 60 minutes had passed. This suggests that normal saline did not possess clot-dissolving properties during the observed time frame. However in EEPP stem bark and streptokinase test tubes, clot degradation was observed until 60 min. The colour density is higher in streptokinase containing test tube than EEPP stem bark containing test tubes.

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(A) (B) (C)

Figure 5:-Fibrinolytic activity of EEPP stem bark by degrading the blood clot within 60 min.

A – Control

B – Standard (streptokinase 5000 IU/ml)

C – EEPP stem bark

Effect on clotting time

The blood clotting time was decreased when animal fed with fat enriched diet. EEPP srem bark treated animals showed increased in blood clotting time (min)

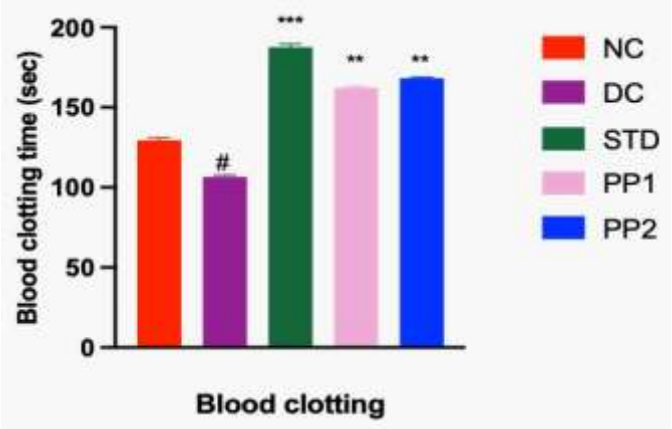

Figure 6:-Effect of EEPP on blood clotting time

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups $^{***}P<0.0001$, $^{**}P<0.001$, Standard – AVT (10 mg/kg) , EEPP - (250 mg/kg ,500mg/kg)

Evaluation of liver enzyme activity in serum

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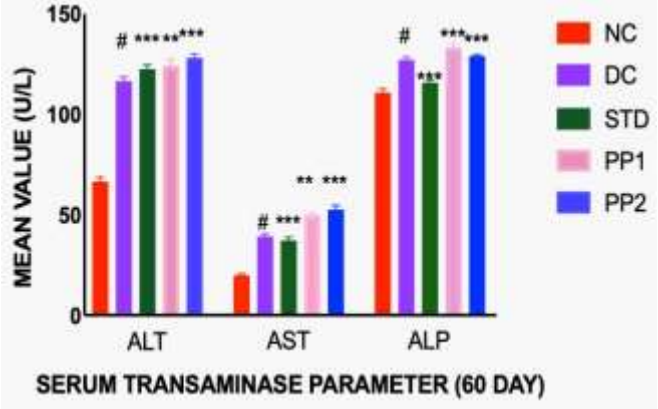

Figure 7:-Effect of EEPP on liver enzyme activity in serum on day 60

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

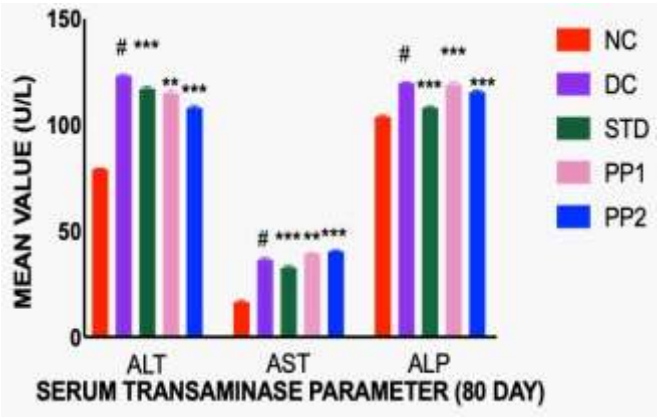

Figure 8:-Effect of EEPP on liver enzyme activity in serum on day 80

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg , 500mg/kg)

Effect of *peltophorum pterocarpum* stem bark on heamatological parameters

Groups	**Normal Control**	**Control**	**Standard**	**PP1**	**PP2**
RBCs count(10^{12}/L)	7.74± 0.04	6.47± 0.037417	7.46± 0.024495	7.63± 0.017205	7.53± 0.011576
WBCs (10^{9}/L)	11.36± 0.04	11.85± 0.013038	13.22± 0.04899	12.758± 0.01562	12.944± 0.021119

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Platelet count (10^9/L)	564.8± 1.319091	546.6± 1.208305	364.4± 1.720465	386.6± 0.678233	380.6± 0.6
Hb count (g/dl)	11.186± 0.002449	11.706± 0.051342	13.326± 0.004	13.22± 0.007071	13.298± 0.002

Table 3.Effect of EEPP on blood parameters

Va / Dunnet's test: Co rd – AVT (10 m

Determination of effect of EEPP stems bark on serum lipid levels

Effect of ethanolic extract of *Peltophorum pterocarpum* stems bark on total cholesterol in high-fat diet-induced atherosclerosis.

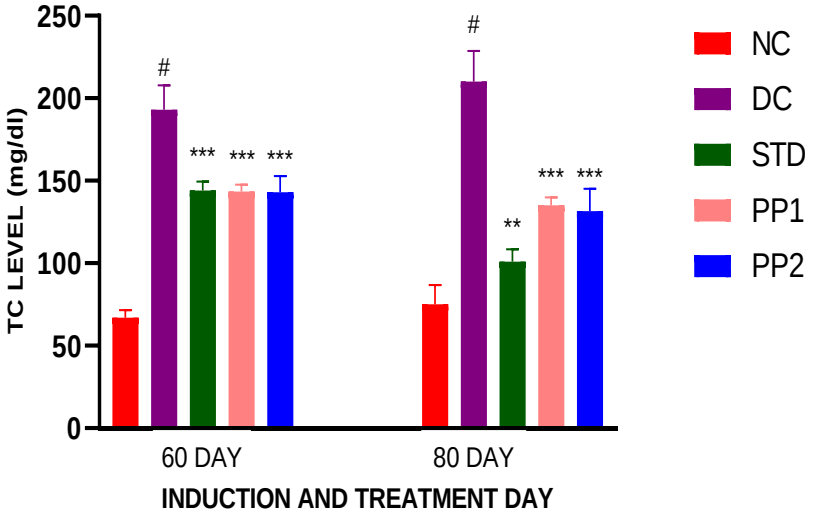

Figure 9:-Effect of EEPP on total cholesterol level

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups $^{***}P<0.0001$, $^{**}P<0.001$, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of ethanolic extract of *Peltophorum pterocarpum* stem bark on Triglyceride level in high-fat diet induced atherosclerosis.

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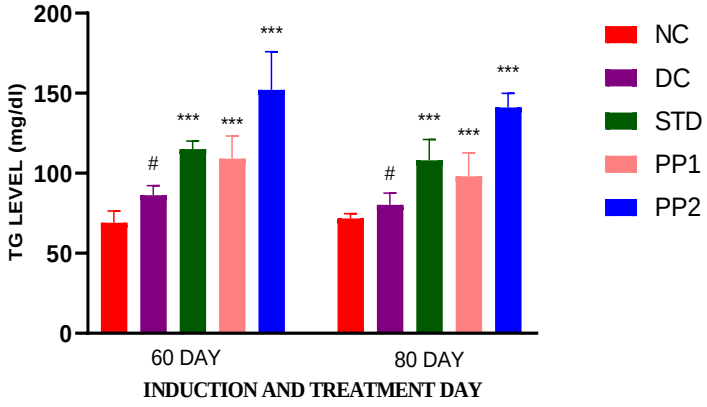

Figure 10:-Effect of EEPP on Triglyceride level

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of ethanolic extract of*Peltophorum pterocarpum* stem bark on high-density lipoprotein cholesterol in high-fat diet-induced atherosclerosis.

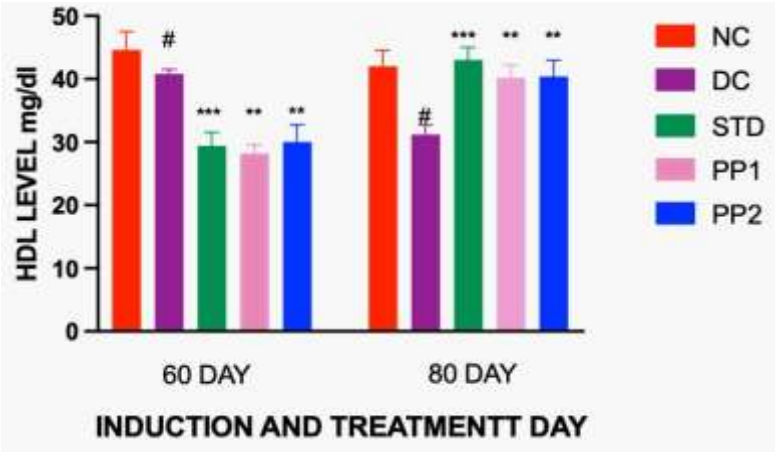

Figure 11:-Effect of EEPP on High density lipoprotein level

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of ethanolic extract of *Peltophorum pterocarpum* stem bark on low-density lipoprotein cholesterol in high-fat diet-induced atherosclerosis.

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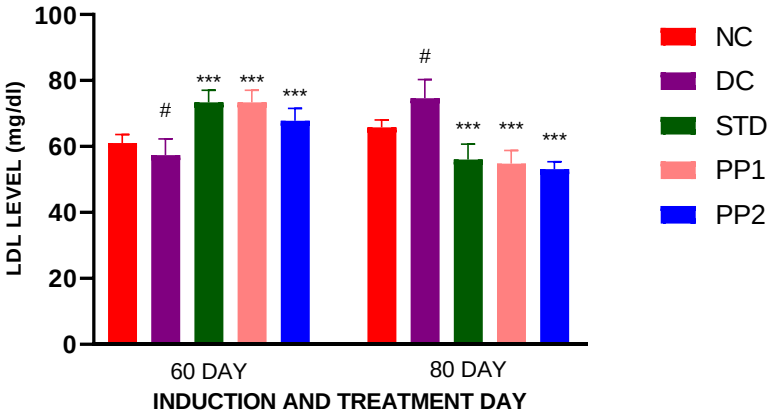

Figure 12:-Effect of EEPP on Low-density lipoprotein level

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of ethanolic extract of *Peltophorum pterocarpum* stem bark on very low-density lipoprotein cholesterol in high-fat diet-induced atherosclerosis.

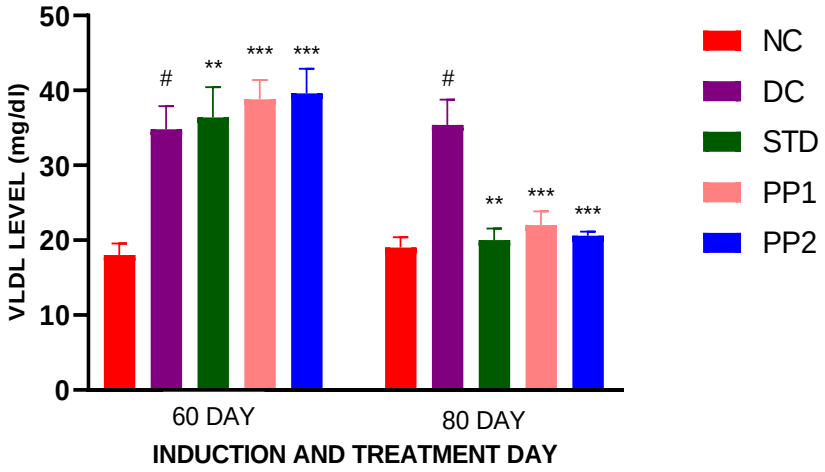

Figure 13:-Effect of EEPP on very-low density lipoprotein level

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of ethanolic extract of *Peltophorum pterocarpum* stems bark on Atherogenic index on 60th and 80th day.

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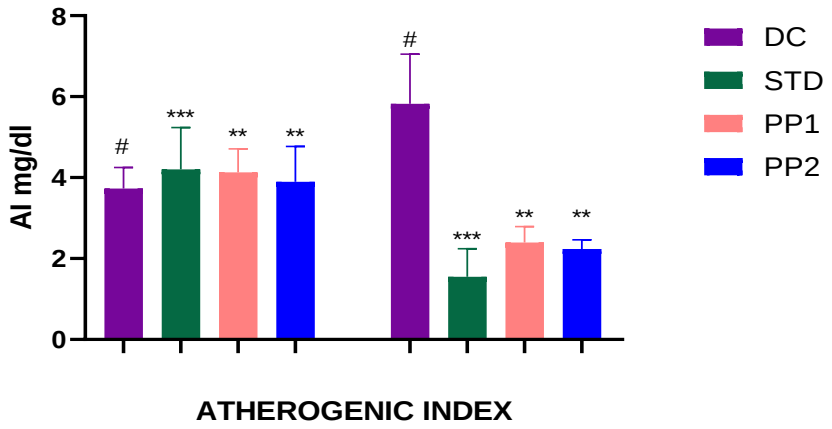

Figure 14:-Effect of EEPP on Athrogenic index

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups $^{***}P<0.0001$, $^{**}P<0.001$, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of *Peltophorum pterocarpum* stem bark on food intake in high fat diet induced atherosclerotic model.

FOOD INTAKE

Animal group	0 day	60 day	80 day
DC	29.09±0.6	15.26±0.29	19.24±0.32
STD	28.21±1.01	16.03±0.29	24.3±0.89
PP1	28.42±0.60	17.03±0.21	23.64±0.26
PP2	28.43±0.62	17.01±0,19	22.64±0.23

Table 4.Effect of EEPP on food intake

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Effect of ethanolic extract of *Peltophorum pterocarpum* stem bark on body weight

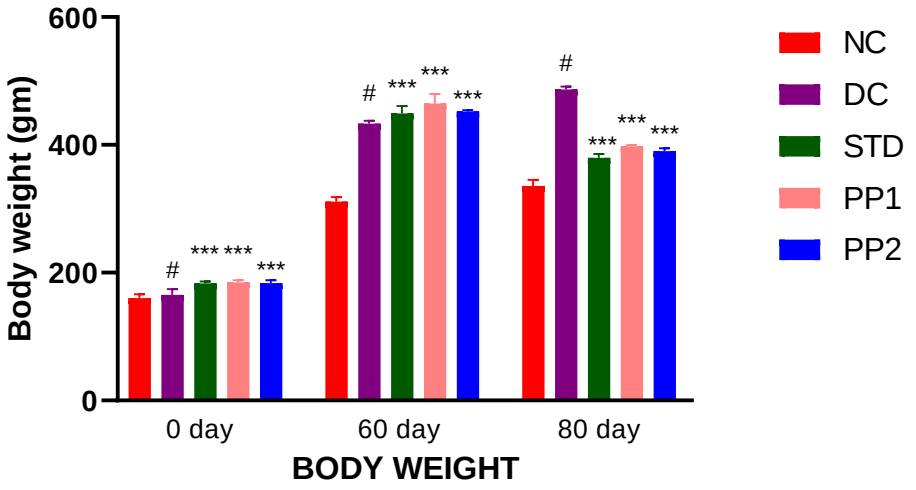

Figure 15:-Effect of EEPP on Body weight

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

Effect of ethanolic extract of *Peltophorum pterocarpum* stem bark on organ weight

GROUPS	NC	DC	STD	PP1	PP2
HEART	1.78± 0.037417	2.74 ± 0.08124	1.776 ± 0.095216	1.872 ± 0.06822	1.716 ± 0.072498
LIVER	9.16 ± 0.08124	18.26 ± 0.233666	12.04 ± 0.074833	10.84 ± 0.05099	11.18 ± 0.066332

Table 5.Effect of EEPP on organ weight

Values are mean ±SEM (n=6). Statistical analysis by (one-way ANOVA) followed by Dunnet's test: Control group compared with all Treatment groups ***P<0.0001, **P<0.001, Standard – AVT (10 mg/kg), EEPP-(250 mg/kg, 500mg/kg)

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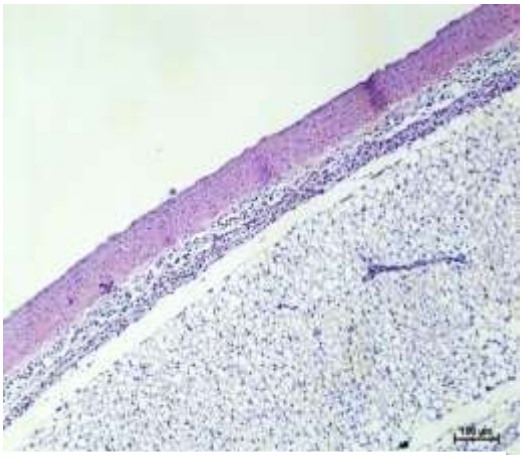
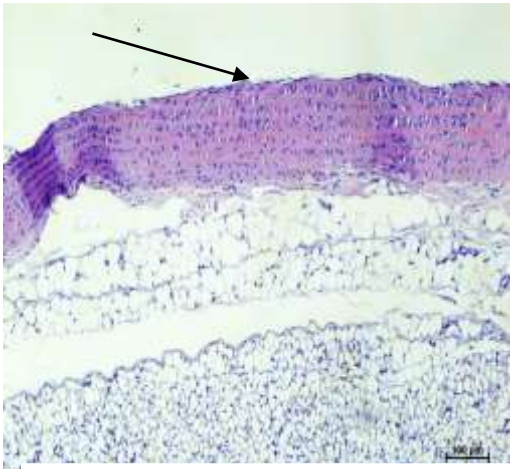
Histopathology of Aorta

NC Aorta



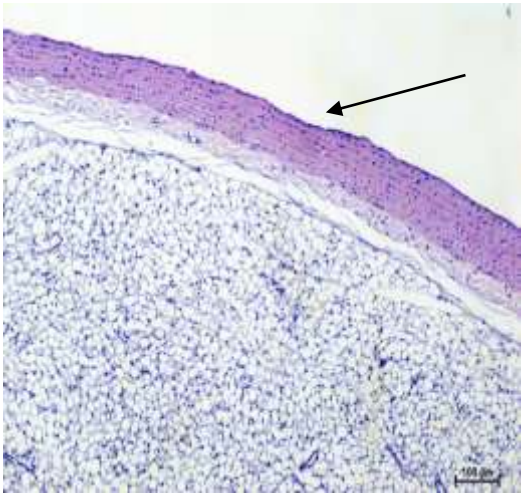
Normal histomorphological features of aorta showing uniform and intact layers of tunica interna and tunica media with intact endothelium. Absence of any cellular pathological changes in the blood vascular tissue

DC Aorta



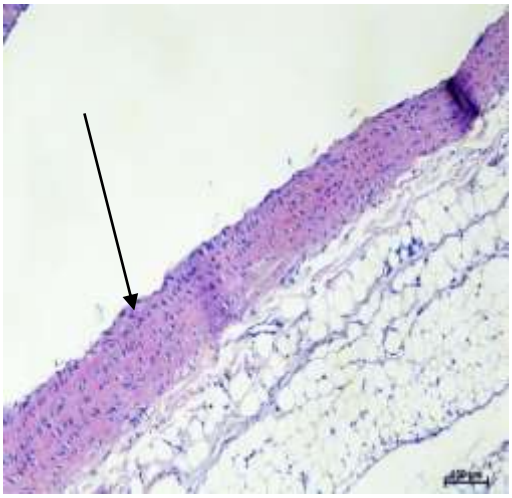
Mild degenerative changes in the endothelial tissue the tunica media and tunica interna layer with focal infiltration of lipid droplets in the medial layer. Focal infiltration of mononuclear inflammatory cells.

STD Aorta



Normal histomorphological features of aorta showing uniform and intact layers of tunica interna and tunica media with intact endothelium. Absence of any cellular pathological changes in the blood vascular tissue.

PP1 Aorta



Endothelial tissue of the tunica interna layer has just a few localised, degenerative alterations.

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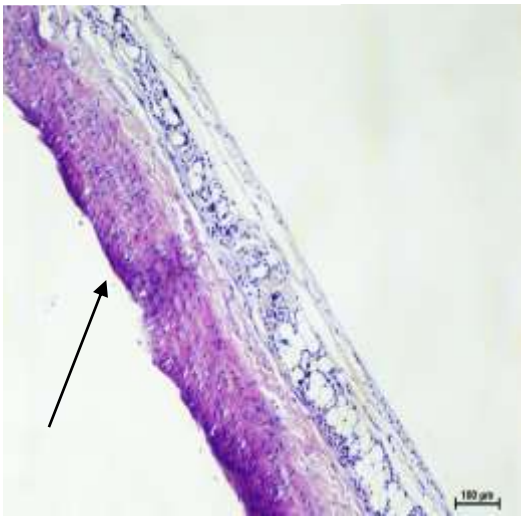
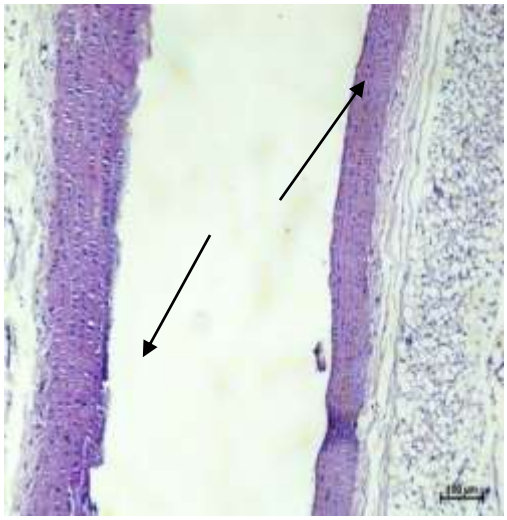
PP2 Aorta



Normal histomorphological features of aorta showing uniform and intact layers of tunica interna and tunica media with intact endothelium. Absence of any cellular pathological changes in the blood vascular tissue.

Antiplatelet Aorta



Endothelial tissue of the tunica interna layer has just a few localised, degenerative alterations.

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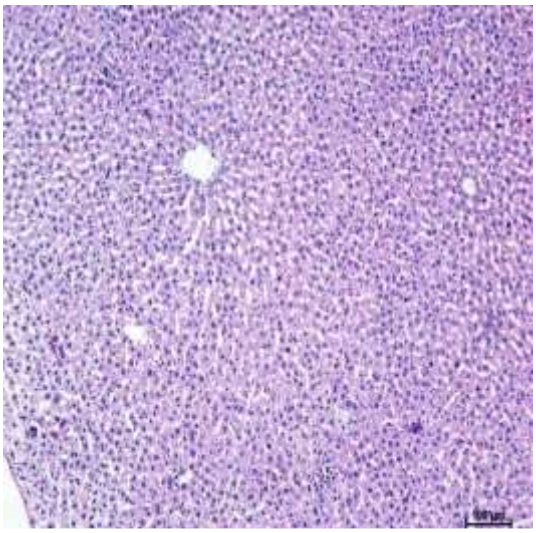
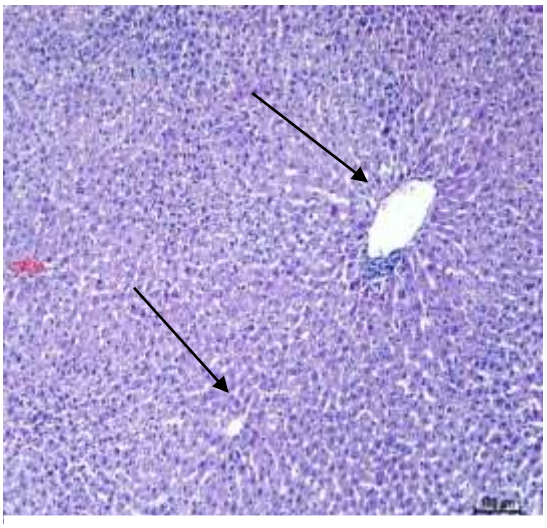
Histopathology of Liver

NC Liver



Hepatocytes have normal histomorphological cellular characteristics, including unbroken cell borders and nuclei. The hepatic parenchyma contained strands of uniformly shaped hepatocytes. The blood vessels including central vein, hepatic artery and portal vein in portal triad showed normal histological features. The bile duct showed normal cellular features with intact epithelium. The hepatic parenchyma shows no signs of inflammation or disease.

DC Liver



Hepatocytes around the major vein showed mild to moderate degenerative alterations. Hepatocytes showed cellular enlargement, an enlarging of the nucleus, and granular cytoplasmic alterations in many foci of hepatic degeneration. A handful of the hepatocytes showed multifocal vacuolar alterations. Congested blood vessels with multifocal areas of hemorrhages in the hepatic parenchyma. Hepatic tissue showed focal mononuclear cell infiltration along with focal vascular tissue congestion.

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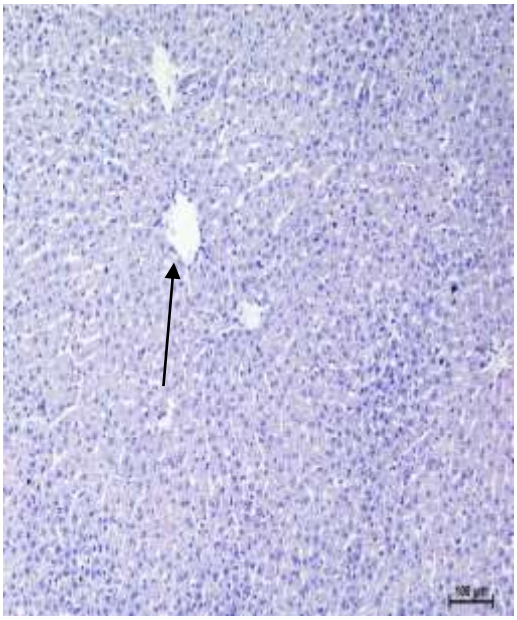
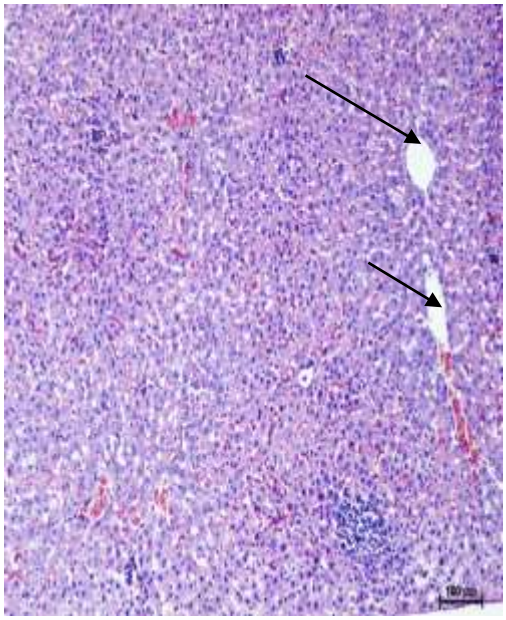
STD Liver



Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Hepatocytes were arranged in strands with uniform morphology in hepatic parenchyma. The blood vessels including central vein, hepatic artery and portal vein in portal triad showed normal histological features. The bile duct showed normal cellular features with intact epithelium. Absence of inflammatory or pathological

PP1 Liver



Focal congested vascular tissue in hepatic parenchyma. Focal areas of fatty infiltration with presence of fatty droplets in the cytoplasm of hepatocytes. Focal degenerative changes in hepatocytes with granular cytoplasm and focal cellular swelling of hepatocytes.

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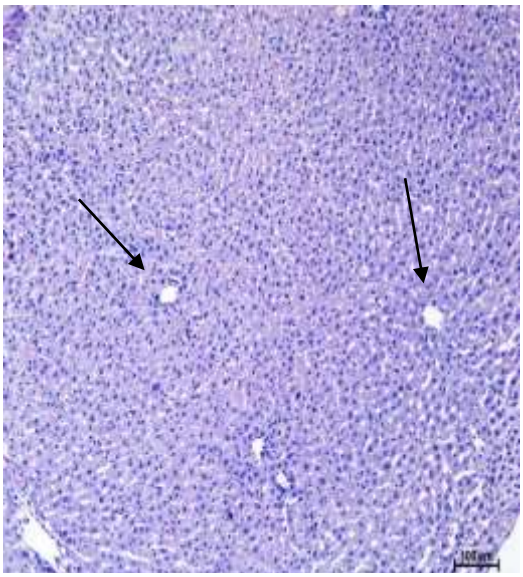
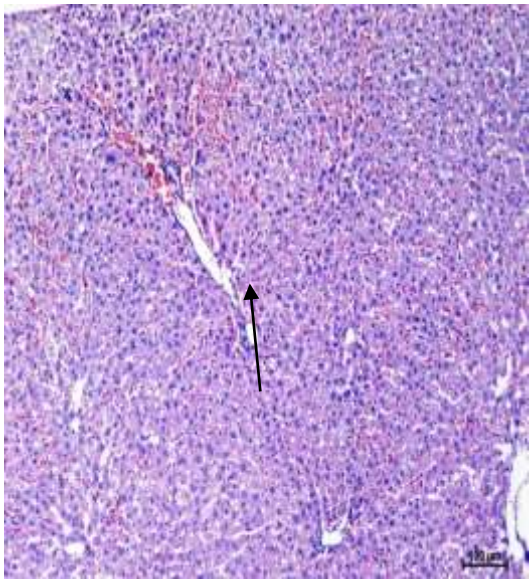
PP2 Liver



Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Hepatocytes were arranged in strands with uniform morphology in hepatic parenchyma. The bile duct showed normal histological features with intact epithelium. Minimal and focal degenerative changes in hepatocytes with granular cytoplasm and focal cellular swelling of hepatocytes.

Anti-platelet Liver



Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Hepatocytes were arranged in strands with uniform morphology in hepatic parenchyma. The blood vessels including central vein, hepatic artery and portal vein in portal triad showed normal histological features. The bile duct showed normal cellular features with intact epithelium. Absence of inflammatory or pathological changes in hepatic parenchyma.

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DISCUSSION

Atherosclerosis is chronic and progressive inflammatory disorder, which involved formation of atherosclerosis lesion .[13] In spite of the discovery of several newer agents, the search for better anti-atherosclerosis drugs continues because of their existing side effects and none of their is suitable for prolonged use.

Recently, various research studies have been conducted for anti-inflammatory and anti-atherosclerotic effects of herbal plant extracts.[14] In this research work we have primarily focused on the investigation of effect of EEPP against antiplatelet conditions associated with atherosclerosis.

As per the research thrombosis, inflammatory responses, and hyperlipidemia all contribute to the emergence of atherosclerosis.[15] Antiplatelet therapy plays a crucial role in the management of atherosclerosis, a condition characterized by the formation of plaques within the arteries. The primary goal of using antiplatelet agents is to prevent the formation of blood clots or thrombi, which can lead to serious complications such as heart attacks and strokes .[16]

The *Peltophorum pterocarpum* stem bark has been proved to effect in anti-platelet activity. In that data it is shown that the EEPP extract possess the fibrinolytic activity as fibrinolysis provoked by a rupture of an atherosclerotic plaque. However, the impact of *Peltophorum pterocarpum* on the development of atherosclerosis has not been previously studied.

According to the research flavonoids present in the *Peltophorum pterocarpum* stem bark inhibits phospolipase A2.[17] Phospholipase A2 may be directly inhibited or indirectly by an extract's effect on the membrane. Inhibiting PLA2 and COX, two essential enzymes necessary for the formation of thromboxane A2, could likely prevent platelet aggregatory response. TXA2 plays a critical role in the induction of platelet aggregation by increasing the intracellular concentration of Ca2+, which encourages the fusion of platelet granules with the membrane and releases its valuable contents. ADP, which is also recognised as a factor in platelet aggregation .[18]

As hyperlipidemia is major responsible conditions to initiate atherosclerosis;[19] High fat diet induced hyperlipidemia is described as an acute atherosclerosis model in experimental animals .[20] Administration of fat rich diet in experimental animals showed increase TG level by dysfunction of liver. It is due to enhanced VLDL production, a delayed hepatic clearance and loss of function of lipoprotein lipase (LPL).

The current research was designed to investigate the potential effects of EEPP (presumably an extract from *Peltophorum pterocarpum*) on atherosclerosis in rats fed a high-fat diet (HFD). In pathological atherosclerosis, the levels of total cholesterol (TC), phospholipids, and serum LDL-C (low-density lipoprotein cholesterol) are substantially increased, while the levels of HDL-C (high-density

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lipoprotein cholesterol) in the blood are significantly decreased. However, the present study demonstrated that treatment with EEPP had a positive impact on lipoprotein profiles. Specifically, rats treated with EEPP showed a significant reduction in TC and LDL-C levels, which are typically associated with the progression of atherosclerosis. Additionally, there was a notable increase in HDL-C levels in the groups that received EEPP treatment. This indicates that EEPP may have the potential to modify lipoprotein profiles favorably and could be beneficial in the context of atherosclerosis management. However, further research is necessary to fully understand the mechanisms and potential therapeutic implications of EEPP in this context. Recent data showed that prevention of the absorption of cholesterol may be due to flavonoids, tannins and saponins present in EEPP that could be a factor causing the rise in plasma lipoprotein levels.

Inflammatory indicators like AST, ALT, and ALP leak from necrotic heart cells into the blood in pathogenic forms of atherosclerosis. The examination of these enzymes collectively can be a sign of myocardial damage even though none of them are specifically related to myocardial injury individually.[21] The current study's findings demonstrated that the diseased control group's serum AST, ALT, and ALP levels were significantly higher than those of the healthy control group due to high fat diet causing damage to hepatocytes or to lipid deposition in the liver .[22] On administration of EEPP, there was a considerable restoration of these enzymes, demonstrating its cardioprotective outcomes.

Blood clotting time lowers in atherosclerosis due to the persistent increase in blood lipid (LDL, cholesterol), which causes lipid to build up in the endothelium of major vascular arteries.[23] This buildup causes endothelial damage, which releases macrophages and pro-inflammatory agents to create blood vessel plaque .[24-25] The present study showed that the clotting time of EEPP (250mg/kg, 500mg/kg) and atorvastatin (10mg/kg) treated group considerably higher than in the disease control group, which prevented the formation of thrombosis in the corresponding groups.

Over the course of the experiment, food intake and body weight grew in all groups. The group with a high-fat diet had the highest body weight at the conclusion of the trial. According to a prior study, this rise in body weight was caused by the animal's bodily tissue having more lipid deposited in it .[26]

In histopathological examinations, the aorta of the disease-control group displayed a sizable foam cell buildup in the endothelial layer. Thus, it is evident that rats fed the HFD exhibited a considerable rise in the level of lipid content in the aorta. The EEPP (250 mg/kg, 500 mg/kg) and Atorvastatin (10 mg/kg) group significantly reduced foam cell deposition and fibrous deposition in the endothelium layer with improvement in the histological structure of the aorta, confirming the efficacy of EEPP stem bark against atherosclerosis.

When compared to livers from the normal group, the histopathology of control group livers provided a high-fat diet revealed increased fat deposition in the liver along with sinusoids (circles) and changed shape of liver cells. EEPP (250 mg/kg, 500 mg/kg) and Atorvastatin (standard) treatment groups, in

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contrast to the disease control group, demonstrated the existence of tiny sinusoids (circles) on the liver. This demonstrates that EEPP works to prevent atherosclerosis.

Conclusion

According to the study's findings, there has been a noticeable increase in the atherogenic index of blood TC, TG, LDL, and VLDL; rats given a high-fat diet displayed HMG-CoA reductase activity and a considerable drop in HDL. The results of the current investigation demonstrated that EEPP is capable of maintaining cholesterol and lipoprotein levels, and the data indicated that the extract exhibits a sizable fibrinolytic activity that is crucial to the progression of atherosclerosis.

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Conflict of Interest: - Author declares there is no conflict of interest.

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