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Evaluation of Anti-Hyperlipidemic and Antioxidant Activity of Ethanolic Extract of *Delonix elata* on High-Fat Diet Induced Rats

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ABSTRACT

Objective: The present study was designed to investigate the antihyperlipidemic activity of aerial part of ethanolic extract *Delonix elata* (EEDE) in high-fat diet induced hyperlipidemic rats. **Methods:** In the present study, chronic administration of High fat diet (HFD) in rats produced significant increase in the body weight, total cholesterol (TC), high density lipoprotein (HDL), very low density lipoproteins (VLDL), low density lipoproteins (LDL), triglycerides (TG) and blood glucose levels. EEDE was administered at a dose of 250 and 500 mg/kg, (p.o) to high fat-induced Hyperlipidemic rats. Atorvastatin (10 mg/kg) is used as reference standard. **Results:** EEDE showed a significant decrease ($p<0.01$) in the levels of serum cholesterol, triglycerides, LDL, VLDL and significant increase ($p<0.01$) in the level of serum HDL. The extract also showed a significant antioxidant activity. The levels of superoxide dismutase (SOD), reduced glutathione (GSH) and catalase were increased significantly indicating the extract is having potent antioxidant activity. Histopathological studies were also observed. **Conclusion:** From the above results it can be concluded that the antihyperlipidemic and antioxidant activity of ethanolic leaf extract of *Delonix elata* may be due to the presence of Flavonoids and Saponins.

INTRODUCTION

Hypercholesterolemia is a metabolic condition that determines the onset of chronic degenerative diseases such as atherosclerosis[1,2]. Hyperlipidemia results from abnormalities in lipid metabolism or plasma lipid transport. Obesity has been found to be associated with various disorders like atherosclerosis, osteoarthritis, diabetes, and hypertension[3,4]. Hyperlipidemia means abnormally high levels of fats in the blood. These fats include cholesterol and triglycerides. Hyperlipidemia is an abnormally high level of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these fatty substances travel in the blood by attaching to proteins forming large molecules called lipoproteins. Allopathic hypolipidemic drugs are available at large in the market, but side effects and contraindications of these drugs have marred their popularity[1,5]. Recently, herbal hypolipidemics have gained importance in overcoming these disadvantages.

Delonix elata belongs to Fabaceae family and is well known for its medicinal uses. *Delonix elata* is a deciduous tree about 2.5-15 m tall, with a spreading, rather rounded crown, poor stem form and drooping branches. The bark is smooth and shining. The plant bark is posessess beta-sitosterol, saponins, alkaloids, carotene. *Delonix elata* is used by folklore for joint pains and in flatulence. It has Anti-inflammatory, Anti-rheumatic and antimicrobial and antioxidant activity. The leaf part was scientifically evidenced to have cytotoxic, Hepatoprotective and free radical scavenging activity. Seven flavonoids glycosides were isolated and identified from the leaves of *Delonix elata*. A decoction of the boiled roots is used as an antidote for a variety of ingested poisons. The bark is soaked in warm water and the resulting liquid is drunk for several days to treat bilharzia. A psychosomatic medicinal use relating to scorpion bite treatment is reported from India. Leaf and seed extracts have antimalarial and anti-ovicidal activity; hence these extracts are used by traditional practitioners to treat malaria.The present study aims to evaluate the antihyperlipidemic activity of ethanolic extract of *Delonix elata* (EEDE) in high-fat diet induced hyperlipidemic rats[6,7].

MATERIALS AND METHODS

Collection and Preparation of Plant extract:

The aerial part of *Delonix elata* was collected from Tirumala Hills, Tirupati, India. The plant was authenticated by Dr. Madhava Chetty, Professor of Botany, Sri Venkateshwara University, Tirupati, and voucher specimen of the plant were preserved at institute herbarium library. The fresh leaves are washed, shade dried and powdered. The powder was subjected to Soxhlet extractor using ethanol. The extract was filtered and the solvent was evaporated under reduced pressure to a solvent-free concentrated mass, which was then stored in air-tight container in a cool and dry condition.

Preliminary phytochemical screening

Phytochemical analysis of the extract was performed for the identification of phytochemicals such as alkaloids, carbohydrates, proteins and amino acids, tannins, flavonoids, steroids, resins[8,9].

Animals

Wistar albino male rats (200-250gm) were used for this study. All the animals were maintained under controlled conditions of temperature (23 ± 2 C), humidity (50 ± 5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free assessed to standard pellets as basal diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Malla Reddy Institute of Pharmaceutical Sciences(Reg. No: 1662/PO/Re/S/12/CPCSEA).

Composition of High Fat Diet

HFD consist of Protein Milk powder (10%) Carbohydrates Wheat flour (61%) Sugar (05%), Fat Butter (16%), salts (04%), vitamins (02%) , fibers (01%), cholesterol (01%) as percentage of total kcal ad libitum, respectively was administered every day[10]. Food intake was calculated every day and body weights were measured once in every two days.

Experimental Methodology

High-fat diet (HFD) induced obesity in rats is considered to be a reliable tool for the evaluation of anti-obesity activity. The animals were divided into five groups. Each group contains six animals. The study was carried out for 28 days[10,11]. Group 1 represented normal control in which the animals were feed on normal diet and free access to watered libitum. Group 2 represented negative control in which the rats feed on high-fat diet. Group 3 represented standard control in which the rats were treated with Atorvastatin (10 mg/kg, p.o). Group 4 represented test treatment in which rats were treated with the dose of ethanolic extract of *Delonix elata* (250 mg/kg, p.o.) along with high-fat diet. Group 5 represented test treatment in which rats were treated with the dose of ethanolic extract of *Delonix elata* (500 mg/kg, p.o.) along with high-fat diet.

Biochemical Estimations

On the 28th day of experiment blood was withdrawn from the retro-orbital plexus and the serum was separated and used for biochemical estimations of TG, TC, HDL, VLDL, LDL, Urea, Creatinine, Uric acid and antioxidant studies SOD, GSH and Catalase.

Histopathological Studies

The cardiac tissues were washed immediately with saline and then fixed in 10% formalin solution. After fixation, the heart tissues were processed in alcohol-xylene series and then embedded in paraffin. The serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under microscope and photographs were taken.

Statistical Analysis

The results were expressed as mean + S.E.M. Statistical analysis was carried out by using one way ANOVA followed Dunnett's multiple comparison test. A P-Value < 0.05 was considered as statistically significant.

RESULTS

Preliminary Phytochemical Screening:

The preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, glycosides, flavonoids, tannins, steroids, saponins, proteins, gums and phenolic compounds in ethanolic extract of aerial parts of *Delonix elata* and absence of fixed oils and amino acids.

Effect of EEDE on body weight of control and high-fat diet rats

At the end of the study, the animals fed with high-fat diet for 28 days produced a significant increase ($p<0.05$) in the body weight compared to the animals fed with normal diet. The group of animals treated with EEDE(250 mg/kg and 500 mg/kg) showed a significant at the decrease in the body weight at the end of the study as represented in table-1.

Table 1: Effect of ethanolic extract of *Delonix elata* on high-fat diet-induced weight gain in rats.

Groups	Weight gain		
	Day 0	Day 14	Day 28
Normal control	122.7±3.10	125± 3.36	128.5± 3.12
High fat diet control	215.5±7.00[a]	224.8±8.21[a]	235.8±7.22[a]
Atorvastatin (10 mg/kg)	200.3±4.79[b]	171.3± 5.03[b]	156±4.55[b]
EEDE (250 mg/kg)	204.5±5.36[c]	190±7.05[c]	174.7±5.01[c]
EEDE (500 mg/kg)	193.3±3.78[c]	175.2±4.68[c]	163±4.87[c]

Data were represented as mean ± S.E.M (n = 6). a=P<0.05 Significant as compared with control rats; b=P<0.05 Significant as compared with HFD rats; c=P<0.05 Significant as compared with HFD rats; EEDE=Ethanolic extract of *Delonix elata.*

Effect of EEDE on serum lipid profile levels in rats

As shown in table-2, the animals fed with high-fat diet showed a significant increase ($p< 0.05$) in the serum TC, LDL-C, VLDL-C, triglycerides as compared to the normal group. However, the animals treated with atorvastatin and EEDE showed a significant decrease ($p<0.05$) in the biochemical parameters compared to hyperlipidemic control group. Further,

atorvastatin-treated group significantly increased the serum HDL-C level in high fat-induced rats.

Table 2: Effect of EEDE on serum lipid profile in control and high fat fed rats

Groups	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal control	57.17± 2.40	38.67± 4.8	18.00± 2.60	11.33± 2.16
High-fat diet control	144.8± 8.18[a]	22.67±2.80[a]	119.3± 7.16[a]	29.67± 3.733[a]
Atorvastatin (10 mg/kg)	94.83± 3.31[b]	34.83± 3.18[b]	48.90± 3.55[b]	19.83± 2.51[b]
EEDE (250 mg/kg)	120.00± 4.74[c]	26.67± 2.60[c]	88.00± 3.23[c]	22.50± 2.739[c]
EEDE (500 mg/kg)	105.8± 3.76[c]	30.5± 2.73[c]	69.33± 2.58[c]	20.83± 3.18[c]

Data were represented as mean ± S.E.M (n = 6). a=P<0.05 Significant as compared with control rats; b=P<0.05 Significant as compared with HFD rats; c=P<0.05 Significant as compared with HFD rats; EEDE=Ethanolic extract of *Delonix elata.;* TG = triglycerides; LDL-C = LDL-cholesterol; HDL-C = HDL cholesterol; VLDL-C=VLDL cholesterol.

Effect of EEDE on serum kidney biomarkers

Urea, uric acid, and creatinine levels were increased due to the high-fat diet. The treatment with EEDE (250 mg/kg and 500 mg/kg) and atorvastatin significantly decrease (p<0.05) the levels of urea, uric acid, and creatinine when compare with high-fat diet group of rats. The results were shown in table-3.

Table 3: Effect of EEDE on serum kidney biomarkers in normal and high-fat diet rats

GROUPS	UREA (mg/dL)	CREATININE (mg/dL)	URIC ACID (mg/dL)
Normal control	16.2±2.30	0.44±0.101	2.2±0.50
High fat diet control	17.5±3.2[a]	0.85±0.12[a]	4.80±1.4[a]
Atorvastatin (10 mg/kg)	10.6±1.70[b]	0.45±0.108[b]	1.30±0.10[b]
EEDE-250 mg/kg	13.2±0.80[c]	0.65±0.09[c]	1.70±0.51[c]
EEDE-500 mg/kg	12.8±0.60[c]	0.62±0.07[c]	1.40±0.23[c]

Data were represented as mean ± S.E.M (n = 10). a=P<0.05 Significant as compared with control rats; b=P<0.05 Significant as compared with HFD rats; c=P<0.05 Significant as compared with HFD rats; EEDE: Ethanolic extract of Delonix *elata.*

Effect of EEDE on Antioxidant parameters

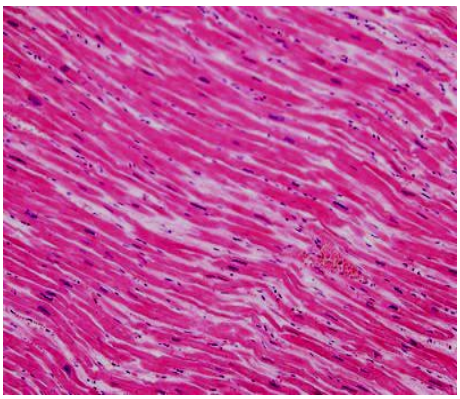
From the results presented in table-4, HFD decreased the levels of SOD, GSH and catalase in plasma significantly (p< 0.05) when compared with that of the normal. The levels of reduced SOH, GSH and catalase were raised significantly (p< 0.05) after treatment with EEDE (250 mg/kg and 500 mg/kg) when compared to HFD treated rats.

Table 4: Effect of EEDE on antioxidant enzymes in control and high-fat diet groups

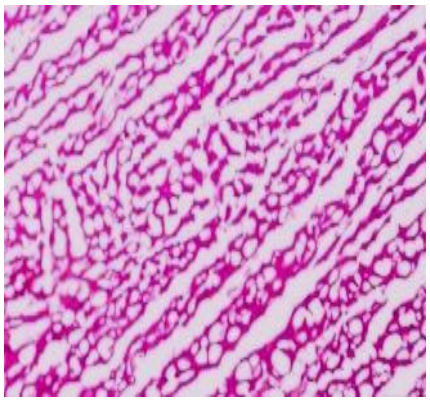
GROUPS	SOD	CATALASE	GSH
Normal control	6.90± 0.60	11.38± 0.81	34.76±1.16
High fat diet control	4.00± 0.61[a]	6.31± 0.69[a]	13.39±0.56[a]
Atorvastatin (10 mg/kg)	5.37± 0.72[b]	7.36± 0.64[ns]	22.12±0.98[b]
EEDE-250 mg/kg	6.08± 0.87[c]	8.41± 0.75[c]	24.19±1.16[c]
EEDE-500 mg/kg	6.61± 0.63[c]	8.83± 0.60[c]	28.57±1.87[c]

Data were represented as mean ± S.E.M (n = 10). a=P<0.05 Significant as compared with control rats; b=P<0.05 Significant as compared with HFD rats; c=P<0.05 Significant as compared with HFD rats; EEDE: Ethanolic extract of Delonix *elata.* GSH: Glutathione; SOD=Superoxide dismutase

HISTOPATHOLOGICAL STUDIES



(a)



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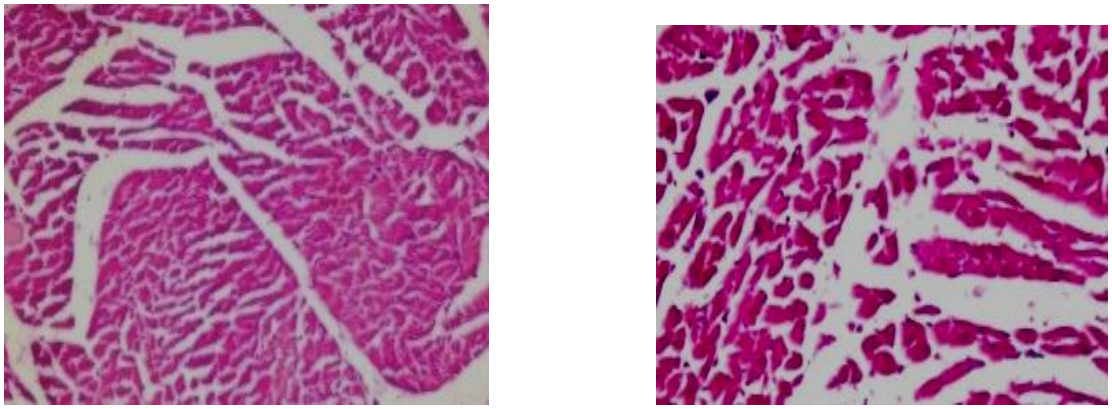
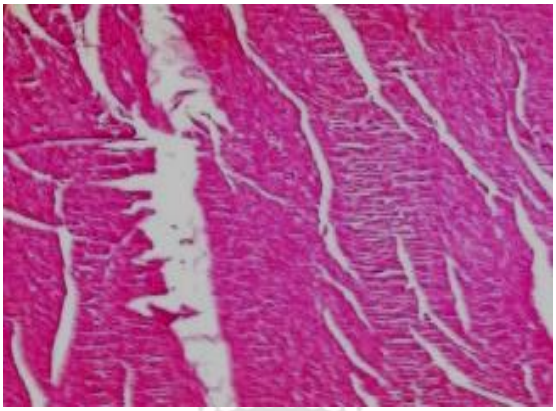


(c) **(d)**



(e)

Figure 1: (a) Cardiac tissues of rats from the negative control group showing normal cardiac tissues. (b) Cardiac tissues of hypercholesterolemic rat from the positive control group showing congestion and marked degeneration of myocardial muscles with ballooning and degeneration. (c) Cardiac tissues of hypercholesterolemic rat treated with standard drug Atorvastatin(10mg/kg). parsley methanol extract restored their normal cardiac structure. (d) Cardiac tissues of hypercholesterolemic rat treated with EEDE(250mg/kg), nearly restored their normal structure. (e) Cardiac tissues of hypercholesterolemic rat treated with EEDE(500mg/kg), nearly restored their normal structure. (H&E ×200)

DISCUSSIONS

Hyperlipidemia is a well-known risk factor for cardiovascular diseases, especially atherosclerotic coronary artery disease (CAD), which is one of the major causes of premature death globally[12]. Several studies revealed that an increase in HDL cholesterol and a decrease

in TC, LDL cholesterol and TG are associated with a decreased risk of ischemic heart diseases [13].

In the present work, as expected, a high-fat diet significantly increased the levels of total lipids TC, TG, LDL-C, and VLDL-C in the serum, compared to animals on a normal diet. When the *Delonix elata* extract (250 mg/kg and 500 mg/kg) was co-administered with the high-fat diet, the levels of these lipids (Triglycerides, LDL-C, VLDL-C) were significantly reduced, whereas plasma HDL-C was increased thereby confirming the anti-hyperlipidemic efficacy of the extract.Urea, uric acid, and creatinine levels were increased due to the high cholesterol diet. There is an association between hypercholesterolemia and kidney damage in which the oxidative stress and inflammatory responses are involved in renal injury was up-regulated by the hypercholesterolemic condition. The levels of Urea, uric acid and creatinine were reduced with the treatment with EEDE (250 mg/kg and 500 mg/kg).

The phytochemical analysis of the *Delonix elata* extract revealed the presence of alkaloids, flavonoids, saponins, tannins, and carbohydrates. Some of these phytoconstituents are known to elicit a wide range of biological activities including hypoglycemic, hypolipidemic and hypoazotemic, among others [14]. As flavonoids proved of having good antioxidant activity the decrease in the lipid levels may be due to the flavonoids. Specifically, according to Oakenfull and Sidhu[15], saponins are known to lower serum cholesterol by a resin-like action. Some saponins with particularly defined structural characteristics form insoluble complexes with cholesterol (e.g. the well-known precipitation of cholesterol by digitonin). When this complexation process occurs in the gut, it inhibits the intestinal absorption of both endogenous and exogenous cholesterol. Conversely, saponins can interfere with the enterohepatic circulation of bile acids by forming mixed micelles[1].

It is already reported that flavonoids are potent natural antioxidants and also having significant increased SOD and catalase activities[16]. High-fat diet brings remarkable changes in the antioxidant defense mechanism against the process of lipid peroxidation. A number of studies have investigated the ability of flavonoid-rich fraction to acts as antioxidants and antihyperlipidemics[10]. The elevated levels of SOD, catalase, GSH with the treatment of *Delonix elata* could be due to the influence of flavonoids. Thus it can be concluded that the antihyperlipidemic and antioxidant activity of ethanolic leaf extract of *Delonix elata* may be due to the presence of these phytoconstituents.

CONCLUSION

Ethanolic extract of *Delonix elata* has the ability to reduced triglycerides levels.By stimulating the lipolytic activity of plasma lipoprotein lipase and reduction in oxidative stress may be responsible for the antihyperlipidemic activity.However, from the literature review hypothesized those medicinal plants containing flavonoids may responsible for the antihyperlipidemic activity. From the phytochemical screening of *Delonix elata* plant extract shows the presence of flavonoids, glycosides, saponins and alkaloids and carbohydrate.Further studies are required to identify the active components and their mode of action

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Conflict of interest

We declare that we have no conflict of interest.

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