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STUDY ON IN VITRO CHOLESTEROL ESTERASE INHIBITORY ACTIVITY OF DELONIX ELATA LEAF EXTRACT

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Abstract:

This study was to evaluate the in vitro cholesterol esterase inhibitory activity of Delonix elata leaf extract. Phytochemical screening of powdered leaves of Delonix elata showed the presence of alkaloids, flavonoids, saponins, tannins, sterols, oil and fat, phenolic compound, protein and amino acid, gums and mucilages, carbo-hydrates, and glycosides. For the extract of DELE IC50 value was calculated and was found to be 46.66±11.66µg/ml. Gallic acid is used as a reference standard and IC50 value was found to be 45.66±10.039µg/ml. Saponins are also reported to precipitate cholesterol from micelles and interfere with enterohepatic circulation of bile acids making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol and hence the reduction in serum cholesterol level. Saponins are also reported to lower triglycerides by inhibiting pancreatic lipoprotein lipase. Similarly in our study also, the presence of both flavanoids and saponins in DELE could have contributed in reducing the levels of lipid status (TC, TG,) elevated levels of serum low density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) are often accompanied by premature atherosclerosis and other CVD. A low level of high-density lipoprotein cholesterol (HDL) is also an important risk factor for cardiovascular disease.

Keywords: *In Vitro, Cholesterol Esterase Inhibitory Activity, Delonix Elata, Leaf Extract*

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INTRODUCTION:

Cardiovascular Diseases (CVDs) that comprised of ischemic heart disease, coronary heart disease, myocardial infarction, cerebrovascular disease, hypertensive heart disease, atherosclerosis, rheumatic heart disease, deep vein thrombosis and pulmonary embolism belongs to noncommunicable disease category, and leads to a very high mortality rate, disability and also, create a significant increase in the socio-economic burden amongst human population.[1] According to World Health Organization (WHO), India has recorded one fifth of CVDs related deaths globally, that is equivalent to 272 per 100000 population.[2] The major conventional etiological risk factors of CVDs include tobacco utility, unhealthy diet, physical inactivity, ageing, hypertension, diabetes, obesity, hyperlipidemia and familial predisposition.[3-5] In India, states such as Punjab, Kerala and Tamil Nadu have recorded a greater number of CVDs cases, and also recorded high prevalence of increased blood pressure and blood cholesterol levels which probably could be the rationale for the contract of CVDs. Henceforth, an imbalance in the blood cholesterol level should be very carefully avoided through follow up of appropriate food style and lifestyle.[6] There are several types of hypolipidemic as well as hypocholesterolemic drugs such as statins, bile aid sequestrants, niacin and orlistat with different mechanism of action are available in the market. Statins can lower the serum LDL level,[7] niacin can reduce both serum triglycerides and LDL,[8] bile acid sequestrants can lower serum LDL level and elevate HDL level,[9] and orlistat can effectively prevent the absorption of lipids through inhibition of pancreatic lipase.[10] Even though different strategies are adopted very effectively to prevent an increase in the triglyceride and cholesterol levels, severe adverse effects such as muscle damage, rhabdomyolysis, hepatotoxicity, gastrotoxicity, insulin resistance and hyperglycemia are documented by the above said drugs. Henceforth, isolation and identification of efficacy oriented alternative nontoxic drugs from plant species are considered as significant therapeutical by the scientific community. India is rich in flora and have a widespread of medicinal plants across the country. Traditionally, whole parts or a particular part of a plant was used to control or cure various diseases and the main rationale for their utilization is due to mild or nil toxicity as well as nil adverse effects. Phytochemicals or secondary metabolites that are significantly distributed in the plants are responsible to execute diversified pharmacological activities.[11] Previous studies related to the assessment of in vitro as well as in vivo cardioprotective potential of the medicinal plants have revealed its pivotal role in the drastic reduction of risk of cardiovascular diseases and its associated complications. [12-15]

Delonix elata Linn. (family: Caesalpinaceae) is a deciduous tree and sparsely distributed in the dry forests of India. Traditional medical practitioners residing in the villages of Chitradurga district, Karnataka, India, used the leaves and stem bark extracts for curing jaundice, hepatic disorders, and bronchial and rheumatic problems. The plant is also reported for problems like pain and stiffness of the joints, especially the knees [16]. Leaves are used for the treatment of bronchitis in infants, fever, malaria, flatulence, and paralysis or as carminative [17]. In our previous study, leaves extract of *D. elata* has shown remarkable antinociceptive activity [18] and antibacterial activity [19]. Leaf extract has been screened for anti-inflammatory activity [20].

Many natural compounds were reported as hypolipidemic agents such as polyphenolics, flavonoids, tannins, alkaloids, phytosterol, unsaturated fatty acids and dietary fibers.The main objective of this study was to evaluate the *in vitro* cholesterol esterase inhibitory activity of Delonix elata leaf extract.

MATERIALS AND METHODS:

Drugs and chemicals

Cholesterol Esterase and pNPB (P-nitro phenyl butyrate) were purchased from the Sigma Aldrich, USA. Acetonitrile, taurocholate were purchased from Loba chemicals, Mumbai. Gallic acid were purchased from HiMedia, Mumbai.

Instrument used

Digital balance (Sartorius Ltd, USA), UV (Jasco UV-spectometry), pH meter (Elico) and GC-MS.

Plant material

The plant material consists of dried powdered leaves of *Delonix elata* L. belonging to the family Fabaceae.

Plant collection and authentication

The leaves were collected from Warangal district, Telangana, India. The plant was identified and authenticated

Plant extraction

The leaves were separated and dried in shade under room temperature, powdered mechanically and sieved through No.20 mesh sieve. The finely powdered leaves were kept in an airtight container until the time of use.

In the cold maceration process, about 25 gm of the whole or coarsely powdered crude drug is placed in a stoppered container with the 70% ethanol and 30%

water and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. On the fourth day the mixture is then strained, the marc is pressed, and the combined liquids are clarified by filtration or decantation after standing.

Phytochemical screening
Chemical tests were carried out for the various fractions of *Delonix elata* for the presence of phytochemical constituents.

Test for alkaloids
To a little of plant leaves extract a few drops of Mayer's test reagent was added. Formation of precipitate indicates presence of alkaloids.

Test for flavonoids
1ml of extract was taken and few drops of very dilute solution of ferric chloride were added. The color changed to pale green or red brown color which indicates the presence of flavonoids.

Test for saponins
One ml extract and one ml alcohol diluted with 20 ml distilled water and shaken well for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for carbohydrates
Small amount of extract was dissolved separately in 5 ml distilled water and filtered. The filtrate was subjected to molisch's test. Formation of reddish-brown ring indicates the presence of carbohydrate.

Test for tannins
To 5ml of extract solution, 1ml of lead acetate solution was added.

Flocculent brown precipitate indicates the presence of tannins.

Test for glycosides
A small portion of extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's test
To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extract produced pink to red color, it indicates the presence of glycosides.

Test for fixed oils and fats
Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soaps or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for phenols (ferric chloride test)
A fraction of the extract was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

Test for gums and mucilage
About 10ml of extract was added to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for amino acids and proteins (1% Ninhydrin solution in acetone)
2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in aboiling water bath for 1-2 minutes and observed for the formation of purple color.

***In vitro* Cholesterol esterase inhibitory activity:**

Enzyme inhibition assay is performed in presence of sodium taurocholate with p-nitrophenyl butyrate as chromogenic substrate. Hydrolysis is performed in the presence of high enzyme concentration and products are identified spectrophotometrically. Assay buffer is sodium phosphate buffer (pH-7.0) whichis made by adding 100 mM sodium phosphate with 100mM Nacl in deionised water.

Stock solution of cholesterol esterase (19.5 ng/mL) and taurocholate (12mM) were prepared by using (100mM) sodium phosphate buffer of pH-7.0. Stock solution of pNPB (200µM), flavonoid compounds and standard of different concentrations (10-320µg/mL) are prepared by using acetronitrile (6%). A final volume of 1 ml is taken into a cuvette containing 430µL of assay buffer, 500µL of taurocholate solution, 40µL of acetonitrile 10µL of pNPB solution and 10µL of flavonoid solution are added and thoroughly mixed. Incubate for 2 minutes at 25º C, the reaction is initiated by adding 10µL of enzyme solution. The absorbance is measured at 405 nm against a blank.

Uninhibited enzyme activity is determined by acetonitrile instead of the inhibitor solution. Control absorbance is measured by adding 100mM sodium phosphate pH 7, instead of enzyme. Gallic acid is used as the reference standard.

Statistical analysis

The results would be statistically analyzed by One

way-ANOVA followed by Tukey's test using GraphPad Instat software. The values would be expressed asmean ± SEM. P<0.05 would be considered statistically significant when compared to standard and negative control.

RESULTS:

PHYTOCHEMICAL SCREENING

Phytochemical screening of powdered leaves of *Delonix elata* showed the presence of alkaloids, flavonoids, saponins, tannins, sterols, oil and fat, phenolic compound, protein and amino acid, gums and mucilages, carbo- hydrates, and glycosides. (Table-1)

Table 1: Phytochemical screening

S.No	Phytochemical	Interpretation
1	Alkaloid	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Sterols	+
6	Oil and Fat	-
7	Phenolic compound	+
8	Protein and Aminoacid	+
9	Gums and mucilage	+
10	Carbohydrates	+
11	Glycosides	+

(+) Present; (-) Absent

***IN VITRO* STUDIES**

Cholesterol esterase inhibitory activity

The ethanolic leaf extract of *Delonix elata* was studied for its cholesterol esterase inhibitory activity at various concentrations ranging from 10, 20, 40, 80, 160, and 320 µg/ml. The absorbance of the mixture was measured at 405nm. It was observed that there is a dose dependent increase in the percentage inhibition from the concentration 10µg/ml to 320 µg/ml and the values are shown Table-2 and . IC50 values of the extract was calculated and compared with the standard Gallic acid.

For the extract of DELE IC50 value was calculated and was found to be 46.66±11.66µg/ml. Gallic acid is used as a reference standard and IC50 value was found to be 45.66±10.039µg/ml.

Table 2: Cholesterol esterase inhibitory activity of Gallic acid

Concentration(µg/ml)	% Inhibition			Mean ± SEM	IC_{50}(µg/ml)
	I	II	III		
5	3.72	26.69	51.52	27.31±13.802	45.66±10.039
10	6.07	38.78	57.78	34.21±15.101	
20	43.0	23.83	46.77	37.866±7.102	
40	20.02	38.78	56.92	38.573±10.653	
80	28.6	32.46	69.04	43.366±12.885	
160	26.15	62.23	65.53	51.303±12.613	
320	37.51	52.42	69.24	53.056±9.165	

Results are Mean ± SEM of two parallel measurements values

Table 3: Cholesterol esterase inhibitory activity of DELE

Concentration(µg/ml)	% Inhibition			Mean ± SEM	IC50(µg/ml)
	I	II	III		
5	16.06	40.09	58.14	38.096±12.188	46.66±11.667
10	20.88	50.98	58.76	43.54±11.550	
20	71.29	58.16	24.84	51.43±13.825	
40	34.74	52.21	61.88	49.61±7.942	
80	42.03	49.02	60.34	50.46±5.335	
160	25.9	59.93	64.48	50.10±12.173	
320	37.64	53.56	69.33	53.51±9.148	

Results are Mean ± SEM of two parallel measurements values.

DISCUSSION AND CONCLUSION

Hypercholesterolemia and the resulting atherosclerosis have been implicated in the pathophysiology of coronary heart diseases and myocardial ischemia. Lowering cholesterol level may decrease the risk of CVD, and therefore enormous efforts have been extended to achieve this aim. *Delonix elata* showed the presence of alkaloids, flavonoids, saponins, tannins, sterols, oil and fat, phenolic compound, protein and amino acid, gums and mucilages, carbo- hydrates, and glycosides. The hypocholesterolemic activity of ethanolic leaf extract of *Delonix elata* against hypercholesterolemia was monitored on the Lipid profile status, antioxidant status, activities of serum cardiac marker enzyme, and histological changes of liver and heart. In this study the high cholesterol diet (HCD) is used which consists of maize, soya meal, coconut cake, rice polish, groundnut cake and animal fat such as tallow which has been used in inducing experimental hypercholesterolemia.

For the extract of DELE IC50 value was calculated and was found to be 46.66±11.66µg/ml. Gallic acid is used as a reference standard and IC50 value was

found to be 45.66±10.039µg/ml. Saponins are also reported to precipitate cholesterol from micelles and interfere with enterohepatic circulation of bile acids making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol and hence the reduction in serum cholesterol level. Saponins are also reported to lower triglycerides by inhibiting pancreatic lipoprotein lipase. Similarly in our study also, the presence of both flavanoids and saponins in DELE could have contributed in reducing the levels of lipid status (TC, TG,) elevated levels of serum low density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) are often accompanied by premature atherosclerosis and other CVD. A low level of high-density lipoprotein cholesterol (HDL) is also an important risk factor for cardiovascular disease.

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