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Hepatoprotective and antioxidant effect of Delonix regia leaf extract against D-Galactosamine induced oxidative stress in rats

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Abstract

The objective of the present study was to investigate and establish the hepatoprotective and antioxidant potential of *Delonix regia* leaves. In order to accomplish the objective, the leaf powder of the plant was extracted with 80% ethanol using soxhlet apparatus. The extract was found to contain alkaloids, phenols, tannins, flavonoids and triterpenoids. The total phenolic content of the extract was found to be 63.27±0.59 GAE mg/g. Hepatoprotective activity of the extract was determined in D-Galactosamine induced hepatotoxicity model at two doses (200 mg/kg and 400 mg/kg). Treatment with both doses of the extract of *Delonix regia* leaves decreased the D-Galactosamine induced alteration in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood. It was also found that the *Delonix regia* leaf extract counteracted the D-Galactosamine-induced free radical activity that is equivalent to that of Vitamin E. SOD, GPx and CAT enzyme levels are statistically significant increased, whereas lipid peroxidation is decreased, when compared to D-galactosamine treated animals ($P < 0.05$).

Keywords: *Delonix regia*, D-Galactosamine, oxidative stress, hepatoprotective, antioxidant, extract

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Introduction

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. Herbal-based therapeutics for liver disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed by Pharmacopeia Foundation to possess liver protecting activity.

Delonix regia was selected as the plant for pursuing our investigation owing to its wide range of pharmacological activities[1-8]

The antioxidant activity in various models for *Delonix regia* has been reported in literature, but no hepatoprotective and action against oxidative stress has been reported till date. The prime objective of the present investigation was therefore to evaluate the effect of *Delonix regia* leaf extract against D-galactosamine induced oxidative stress in rats. The investigation would also assess the effect of the leaf extract on liver function tests.

Material and Methods

All the chemicals and reagents used in the study were of analytical grade and used as obtained without any further purification.

Collection and identification of plant material

The leaves of *Delonix regia* were collected from the local surrounding of Gwalior, Madhya Pradesh in the month of January and authenticated at RB Science; Bhopal by voucher specimen (RB/Herbarium/06). The authenticated plant leaves were washed with distilled water and were dried under shade. The dried leaves were powdered using a blender at low speed. The powdered leaves were stored in closed container till use.

Preparation of Extract

Powdered leaves (250 g) were evenly packed in the extractor of the soxhlet apparatus and extracted using 80% ethanol solution by hot continuous extraction process for about 13 h. The extract was filtered while hot through Whatman filter paper to remove any impurity and concentration by evaporating the solvent using rotary vacuum evaporator. The oleo-resinous extract was collected and placed in desiccator to get rid of the excessive moisture. The dried extracts were stored in desiccator for further experimental procedures.

Qualitative Phytochemical Screening

All the four extracts were evaluated by phytochemical qualitative reactions for identifying the presence or absence of usual plant secondary metabolites. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses

to these tests. Mayer, Wagner, Hager and Dragnedroff's test were performed for detection of alkaloids. Froth test, Kedde's test, Keller Killiani test and Bontrager's test were applied for glycoside detection. Gelatin test, Ferric chloride test, vanillin test and alkaline reagent test were performed for tannins and phenolics. Shinoda test and zinc chloride reduction test were done to detect flavonoids. Proteins and amino acids were detected using Millons test and Ninhydrin test. Detection of sterols and terpenoids was done by Libermann Burchard and Salkowski test.

Total Phenolic Content

The extraction of phenolic compounds was based on a modification of the method reported by Hsu et al[9]. The extract (1 g) was diluted with methanol (20 mL). The solution was stored at 4°C in amber colored bottle and served as the stock solution (50 mg/mL) for subsequent analyses.

For determining the total phenolic content 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent was added to it. After 2 min, 300 µL of 20% Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were treated similarly for constructing the calibration curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Pharmacological Activity

Selection of animals

Male Wistar rats (150-200 gm), were used, and kept in quarantine for 10 days under standard husbandry conditions (27.3°C, Relative humidity 65 ±10%) for 12 h in dark and light cycle respectively and were given standard food and water *ad libitum*. All experiments were approved by the institutional ethical committee and were carried out according to the animal ethical committee guidelines.

Treatment protocol

The extract of *Delonix regia* was dissolved in 20 mL of sterile water and was administered orally at a dose of 200 mg/kg and 400 mg/kg. D-Galactosamine was diluted in sterile water and administered intraperitoneally (i.p) at a dose of 25 mg/kg. Vitamin E was diluted in sterile water and administered orally at a dose of 25 mg/kg.

The acclimatized animals were divided into 5 groups of each 6 animals as per the approved protocol. Each group served individual purpose.

- Group 1: Served as normal control and received normal diet and water.
- Group 2: Toxic control received 25 mg/kg of D-galactosamine i.p for 21 days.

- Group 3: Standard control received 25 mg/kg of vitamin E per oral for 21 days + 25 mg/kg of D-galactosamine i.p for 21 days
- Group 4: Received 200 mg/kg of *Delonix regia* extract (DRE) for 21 days + 25 mg/kg of D-galactosamine i.p for 21 days.
- Group 5: Received 400 mg/kg of *Delonix regia* extract (DRE) for 21 days + 25 mg/kg of D-galactosamine i.p for 21 days.

Blood sample collection

On day 22nd day (24 h after administration of the last dose) the animals from all the groups were sacrificed by cervical dislocation and the blood was collected from the carotid artery and allowed to clot for 45 min at room temperature; serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of various biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP) and serum bilirubin[10-12].

Liver homogenate preparation and evaluation

The livers were dissected out immediately, washed with ice-cold saline and homogenized in phosphate buffer solution (PH 7.4) to prepare 10% homogenates. The homogenate was centrifuged at 1700 rpm for 10 min at 4°C using a refrigerated centrifuge and the level of lipid peroxidation was evaluated. The supernatant was used for the assay of Superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx).

Statistical Analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunett's test. A $P < 0.05$ value was considered as statistically significant.

Results and Discussion

Extraction and phytochemical screening

The yield of the extract obtained using 80% ethanol as solvent was found to be 27.1 % indicating the presence of significant amount of polar and semi-polar secondary metabolites. The extract was dark brown in color and the qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, triterpenoids, phenols and tannins in the extract (Table 1).

Table 1 Qualitative phytochemical screening of *D. regia* extract

Chemical Tests	Observation checked for	Inference
Alkaloids		
Mayer's reagent	cream colour precipitate	+
Hager's reagent	yellow colour precipitate	+
Wagner's reagent	reddish brown precipitate	+
Dragendorff's reagent	reddish brown precipitate	+

Glycosides		
Froth test	Frothing is seen	**-**
Kedde's Test	No color	-
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-
Keller-Kiliani	No color in acetic acid layer	-
Phenols/Tannins		
Ferric chloride	Blue green color	+
Gelatin Solution	White precipitate	+
Alkaline reagent test	Yellow to red precipitate	+
Vanillin HCl test	Purplish red color	+
Flavonoids		
Shinoda test	red color	+
Alkaline reagent test	Yellow color that turns red on acidification	+
Zinc HCl reductino test	red color	+
Proteins		
Millon's Test	white precipitate, turns red on heating	-
Ninhydrin Test	Voilet color	-
Sterols/triterpenoids		
Liberman-Burchard Test	Brown ring at junction Upper layer turns green	+
Salkowski Test	Yellow color in lower layer	-

+ indicates positive observation; - indicates negative observation

Total Phenolic content

The total phenolic content of the extract of *Delonix regia* was calculated from standard calibration curve constructed for gallic acid and was found to be 63.27±0.59 GAE mg/g.

The plant flavonoids and polyphenols are greatly effective free radical scavenging and antioxidants activity[13]. The diseases associated with free radicals are healed by polyphenol and flavonoids. The phenolic compounds have been recognized as antioxidant and have been known to show medicinal activity as well as for exhibiting physiological functions[14-18]. The hydroxyl group present in flavonoids is responsible for radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants.

Hepatoprotective activity

Hepatoprotective activity of the extract was determined in D-Galactosamine induced hepatotoxicity model. In the present study, rats treated with D-Galactosamine, developed considerable liver damage as observed from the elevated serum levels of liver-specific enzymes as well as severe alteration in other biochemical parameters. Values of the biochemical parameters were significantly increased in the D-Galactosamine treated rats (Table 2).

Treatment with both doses of the extract of *Delonix regia* leaves decreased the D-Galactosamine induced alteration in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood. It was found that the test samples offer protection against toxin as evidenced by remarkable reduction in all biochemical parameters ($P<0.05$). It also revealed that decreased biochemical parameter levels of both isolated compound are less compared to standard drug.

Table 2 Effect of Delonix regia extract on SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in D-Galactosamine induced liver toxicity in rats

Group	Treatment	SGOT	SGPT	ALT	ACP	Bilirubin (mg/100 mL of blood)	
						Direct (mg/dl)	Total (mg/dl)
I	Normal Control	73.1 ± 8.6	125.3 ± 8.9	163.4 ± 6.1	136.8 ± 4.6	0.29 ± 0.3	0.65 ± 0.6
II	D-Galactosamine	204.8 ± 6.4[a]	331.4 ± 4.2 [a]	324.0 ± 5.6 [a]	387.3 ± 7.5 [a]	2.36 ± 0.4 [a]	3.41 ± 0.3 [a]
III	Vitamin E	72.4 ± 6.3*	132.7 ± 3.4*	161.9 ± 8.2*	138.2 ± 6.1*	0.31 ± 0.2*	0.69 ± 0.3*
IV	DRE (200 mg/kg)	114.6 ± 4.8*	177.2 ± 6.3*	233.5 ± 6.3*	213.7 ± 3.8*	1.32 ± 0.3*	2.28 ± 0.4*
V	DRE (400 mg/kg)	83.4 ± 6.9*	141.8 ± 7.2*	174.1 ± 4.7*	163.3 ± 8.4*	0.37 ± 0.3*	0.86 ± 0.4*

Values are expressed as mean ± SEM, n = 6 in each group. [a]$P<0.05$ when compared with normal control group, *$P<0.05$ when compared with D-galactosamine treated group.

Antioxidant Activity

The administration of DRE counteracted the D-Galactosamine-induced free radical activity, which resembles that of Vitamin E. SOD, GPx and CAT enzyme levels are statistically significant increased, whereas lipid peroxidation is decreased, when compared to D-galactosamine treated animals ($P < 0.05$). These results provide evidence for strong antioxidant action the *Delonix regia* leaf extract.

Liver participates in various metabolic activities, while releasing a wide variety of enzymes. Liver can be injured by many toxicants, as well as by chemicals or drugs. In our model, D-Galactosamine serves as a toxicant. D-Galactosamine related hepatotoxicity is associated with elevation in enzyme levels, which may be attributed to the generation of trichloromethyl free radical during metabolism by the hepatic microsomes, which in turn begin lipid peroxidation. Hepatocellular necrosis decreases SOD, CAT and GPx activities, and the increase of such activities into basal values, is a clear indication of plasma membrane stabilization and tissue repair as well. Such an effect is in agreement with the view that enzyme activities are restored into normal conditions and healing of the hepatic parenchyma, as well as hepatocyte regeneration, are observed. SOD, CAT, and GPx constitute an enzyme defense mechanism against oxidative damage. Under D-Galactosamine conditions such enzyme activities are decreased, but under the extract-treated conditions, a significant increase in their activities is observed, which may serve as a biochemical strategy to reduce lipid peroxidation[19].

Table 3 Antioxidant activity of *Delonix regia* extract in D-Galactosamine induced liver damage in rats

Group	Treatment	Catalase (mg liver protein)$^{-1}$	Superoxide Dismutase ((mg liver protein)$^{-1}$	Glutathione Peroxidase (mg liver protein)$^{-1}$	TBA (mg liver protein)$^{-1}$
I	Normal Control	249.4 ± 4.9	83.8 ± 2.7	1.33 ± 0.4	1.61 ± 0.4
II	D-Galactosamine	108.9 ± 6.3[a]	31.4 ± 2.3[a]	0.36 ± 0.2[a]	4.23 ± 0.6[a]
III	Vitamin E	258.1 ± 8.6	85.6 ± 6.4	1.38 ± 0.8	1.53 ± 0.5
IV	DRE (200 mg/kg)	194.7 ± 4.9*	58.5 ± 5.4*	0.89 ± 0.7*	2.69 ± 0.4*
V	DRE (400 mg/kg)	233.8 ± 3.8*	76.1 ± 2.8*	1.17 ± 0.9*	1.93 ± 0.4*

Values are expressed as mean ± SEM, n = 6 in each group. [a]P<0.05 when compared with normal control group, *P<0.05 when compared with D-galactosamine treated group.

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