

Hepatoprotective activity of methanol extract of aerial parts of *Delonix regia*

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Abstract

The study was designed to evaluate the possible beneficial effect of methanol extract of aerial parts of *Delonix regia* against CCl₄ induced liver damage in rats. The methanol extract of aerial parts of *D. regia* (400 mg/kg) was administered orally to the Wistar albino rats with hepatotoxicity induced by CCl₄ (2 ml/kg, p.o.). Silymarin (50 mg/kg, p.o.) was given as reference standard. The plant extract was effective in protecting the liver against the injury induced by carbon tetrachloride in rats. This was evident from significant reduction in serum enzymes AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), ALB (albumin), TLP (total protein), DBIL (direct bilirubin) and TBIL (total bilirubin). Histopathological observation showed hugely dilated central vein, disrupted cords of hepatocytes and few hepatocytes shows feathery change, mild inflammation and moderate degree of macro and micro vesicular steatosis. It can be concluded that the methanolic extract of aerial parts of *D. regia* possesses hepatoprotective activity against CCl₄ induced hepatotoxicity in rats.

Key words: *Delonix regia*, Hepatoprotective, Aspartate aminotransferase, ALT, ALP, Histopathology, hepatotoxicity

Introduction

Liver disease is worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine, there are number of medicinal preparations in Ayurveda recommended for the treatment of liver

disorders (Chatterjee, 2000). In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. *Delonix regia* (Caesalpinaceae) is a stirringly ornamental medium sized tree planted in gardens in all the warmer and damper parts of India, native to Madagascar. It is commonly known as 'Gulmohar' in Marathi (Khare, 2007). Traditionally plant is used as anthelmintic, antimicrobial, anticancer, emetic, CNS depressant and in the treatment of anemia and fever (Anonymous, 2003; Anonymous, 1992). Adje *et al.* (2008) did anthocyanin characterization of the plant *D. regia*. The plant has been claimed to be useful as antioxidant (Aquil *et al.*, 2006), larvicidal (Chockalingam *et al.*, 1990), antibacterial, antifungal (Ahmed *et al.*, 2003), anti-inflammatory, analgesic (Muruganandam *et al.*, 2000), nutritional (Grant *et al.*, 1991), antimalarial (Ankrah *et al.*, 2003), antiperiodic, febrifuge, emetic, CNS depressant (Rastogi *et al.*, 1993) and antirheumatic (Khare *et al.*, 2007). Its aqueous and alcoholic extracts were active against roundworm. The bark contains leucocyanidin, lupeol, tannin, β -sitosterol and free OH-proline as major amino acid. Flower anthers are a rich source of zeaxanthin. Leaves contain tannins, lupeol and β -sitosterol (Khare *et al.*, 2007). *D. regia* seeds contain lectins (Pando *et al.*, 2002). Aim of the present work is to validate traditional hepatoprotective effect of *D. regia* aerial parts by using suitable screening model.

Material and Method

Plant Material

Aerial parts of *D. regia* were collected from Ahmednagar (M.S.) region and authenticated at Botanical Survey of India, Pune (Voucher specimen number JA-1).

Extraction

Dried and powdered aerial part was subjected to extraction by using methanol in Soxhlet apparatus (Mukharjee, 2002). Vacuum dried yield of extract was 33.2 % w/w.

Animals

Female adult albino rats (Wister strain) weighing between (180-220 g) were used for the study. The animals were housed under standard laboratory condition and fed with rodent diet and water *ad libitum*. Institutional animal ethical committee approved all experiment protocols of the study.

Determinations of Acute Oral Toxicity (LD_{50})

Acute oral toxicity (AOT) of methanol extract was determined as per OECD guidelines. The animals were fasted 3 h prior to the experiment and as per up and down procedure (OECD 2001). Animals were administered with single dose of methanol extract suspended in 2% w/v acacia and observed for its mortality for 48 h study period (short term) toxicity. Based on short-term profile of drug, the dose of next animals was determined as per as OECD guideline 425. All the animals were also observed for long term toxicity (14 Days).

Table 1. Effect of methanol extract of *D. regia* on different biochemical parameters in CCl₄ induced liver damage in rats.

Treatment (Dose: mg/kg, p.o.)	Serum biochemical parameters						
	AST IU/L	ALT IU/L	ALP IU/L	ALB g/dl	TLP g/dl	DBIL mg/dl	TBIL mg/dl
Control	997.70 ±61.3	840.31 ±64.8	590.04 ±90.7	18.47 ±2.3	62.67 ±5.4	12.47 ±0.09	28.81 ±0.13
Silymarin (50)	128.416* ±35.2	160.683* ±39.6	234.53* ±41.3	5.04* ±1.03	14.72* ±3.7	0.7* ±0.16	0.94* ±0.14
Methanol extract (400)	266.26* ±48.2	226.1* ±40.5	311.21* ±61.8	5.93* ±1.2	14.81* ±3.8	1.13* ±0.07	1.25* ±0.08

The LD₅₀ of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA.

Assessment of Hepatoprotective Activity

Animals were divided into 3 groups (n = 6). Control group was treated with vehicle (2 % acacia, 1 ml/kg, p.o.) for 7 days. Standard group received Silymarin (50 mg/kg, p.o.) for 7 days. Third group was treated with methanolic extract of *D. regia* (400 mg/kg, p.o) for 7 days. Animals from these groups were checked for CCl₄ induced liver damage. Food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals. All groups received a single dose of CCl₄ (2 ml/kg, p.o.) diluted with liquid paraffin (1:1) on 7th day after 1 h of extract treatment and sacrificed 24 hrs after administration of CCl₄ (Shenoy et al., 2001, Mustuda et al., 1991).

Assessment of Liver Function

The blood samples were collected by retro orbital method and serum was used for estimation of AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), ALB (albumin), TLP (total protein), DBIL (direct bilirubin) and TBIL (total bilirubin). The animal were sacrificed and removed liver was washed by normal saline, blotted with filter paper and weighed immediately (Vogel et al., 2002). The liver samples were preserved in 10% formalin for histopathological studies.

Statistical Analysis

All data was expressed as mean ± SEM. The statistical analysis was carried out using one-way ANOVA followed by Dunnett's test. P < 0.05 was considered significant.

Results and Discussion

The results of the hepatotoxicity in terms of increased levels of AST, ALT, ALP, ALB, TLP, DBIL and TBIL were as follows. The degree of hepatotoxicity developed by toxicant CCl₄ free radical during metabolism by hepatic microsomes which in turn cause peroxidation of lipids of cellular membrane simultaneously the hepatoprotective activity of aerial parts of *D. regia* methanolic extract is found to be effective by the decreased levels of

Table 2. Effect of methanol extract of *D. regia* on liver weight and volume in CCl₄ induced liver damage in rats.

Treatment (Dose: mg/kg, p.o.)	Mean liver weight (g/100g)	Mean liver volume (ml/100g)
Vehicle	3.6 ± 0.05	3.75 ± 0.09
Control	4.87 ± 0.14	4.77 ± 0.13
Silymarin (50)	3.74 ± 0.095*	3.95 ± 0.11*
Methanol extract (400)	3.77 ± 0.081*	4.13 ± 0.07*

Value are expressed as mean±S.E.M. (n = 6). *P<0.01 Compared with control group (one way ANOVA followed by Dunnett's test.).

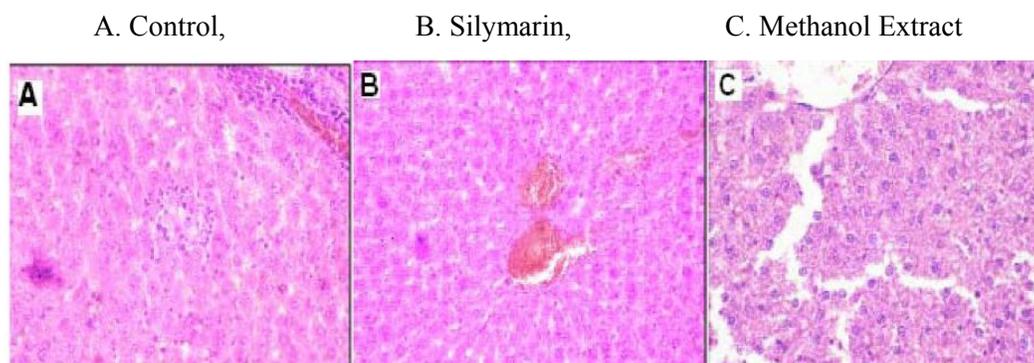


Figure 1. Sections stained with hematoxylin and eosin (H&E 400 X) displaying the liver sections of Wistar albino rats treated with control, Silymarin and methanol extract in acute CCl₄ model.

AST, ALT, ALP, ALB, TLP, DBIL and total bilirubin (Table 1) and reduction in increased liver weight and liver volume (Table 2).

In control animals, liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleolus and central vein. Sinusoids are normal, no inflammatory infiltrate is seen (Fig. 1A). In Silymarin treated animals, the liver sections showed milder degree of liver damage, mild inflammation and milder degree of macro and micro vesicular steatosis. Focal areas of neutrophilic aggregate and focal area of perivascular lympho, plasmacytoid are seen in aggregates (Fig. 1B). In methanol extract treated animals, the liver section showed hugely dilated central vein, disrupted cords of hepatocytes and few hepatocytes shows feathery change, mild inflammation and moderate degree of macro and microvesicular steatosis (Fig. 1C). It can be concluded that methanol extract of aerial parts has shown significant hepatoprotective activity as compared to control group hence it may be used as Hepatoprotective agent.

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