Asian Journal of Chemistry; Vol. 23, No. 1 (2011), 145-148

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Toxicity of the *Delonix elata* (L.) *Gamble* (Caesalpiniaceae) in Leaf

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(*Received*: 9 January 2010; *Accepted*: 23 August 2010) AJC-9008

Delonix elata (family: Caesalpiniaceae) is an indigenous plant for treatment of convulsions, arthritis, measles, piles, chronic bronchitis, rheumatisam and arthritis. *Delonix elata* was evaluated for its toxicity by the oral route in mice and rat. The effect of acute toxicity was carried out in (72 h) and sub-acute (30 days) treatment of the drug with different dosage on liver and kidney functions and hematological and biochemical parameters were studied. The acute toxicity studies with this drug did not produce mortality at any dose level given (5-2000 mg kg^{-1} body weight) and up to the dose level of 5000 mg kg^{-1} did not produce any mortality. No marked adverse alterations were observed in hematological and biochemical parameters during the sub-acute toxicity studies (50, 100, 250 and 500 mg kg^{-1} body weight). In the sub-acute treatment, the highest dose (500 mg kg^{-1} body weight) alone showed a moderate increase in the level of plasma urea, uric acid and creatinine. Decreased level of urinary urea, uric acid and creatinine levels were also observed. Histopathological examination of vital organs showed normal architecture suggesting no morphological disturbances.

Key Words: Toxicity, *Delonix elata*.

INTRODUCTION

Delonix elata (L.) *Gamble* (Caesalpiniaceae) is found in some parts of south India and widely distributed in waste lands. It is a large bush (or) small tree, reaching 9 m height with more or less pubescent leaves and branches and the leaves are no specific taste, soft and feathy to touch and pale green in colour. They are growing in mesic habitats with moderate rainfall and mild temperature[1-3]. The leaves of the plant are used in inflammation rheumatism. The decoction of the root and leaves of the herb is used in rheumatism, antimicrobial[4] nervous diseases, convalescence of measles, piles, chronic bronchitis[5] *etc*. The bark is considered as a good febrifuge and is much appreciated as an antiperiodic[6].

The ethanolic extract of leaves of *Delonix elata* due to the fact that any botanical traditionally used for wound healing, fever, infection, relieving pain, edema or rheumatic disorders is taken as an indicator that the plant should be tested for its antiinflammatory properties[7]. Preliminary phytochemical screening of the ethanolic extracts showed the presence of carbohydrates, proteins, phenolic compounds, tannins, alkaloids, flavonoids and gums. (unpublished data). The present study is therefore, to evaluate the toxicity of *Delonix elata* in acute and sub acute levels in rats.

EXPERIMENTAL

Taxonomic identification of the plant was made from Rapinat Herbarium, St. Joseph's College of Arts and Sciences, Trichy, Tamilnadu, India. Whole fresh plant leaves of *Delonix elata* were collected from Jeyankondam, Perambalur (Dist.), Tamilnadu, India. The leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through 40 mesh sieves.

Preparation of extracts: The powdered leaves (500 g) were successively extracted with ethanol for 24 h by continuous hot percolation method using soxhlet apparatus. The fraction was separated from the solvent by distillation under reduced pressure to yield (5.6 % w/w) solid mass. It was stored in refrigerator and used for further studies.

Animals: The animals for the present study were procured after animal ethical clearance from the Institutional Animal Ethical Committee (IAEC) of Annamalai University, Annamalai Nagar, India. The animal experiments were carried out according to committee for the purpose of control and supervision of experiments on animals (CPCSEA) rules. The albino mice (20-25 g) were used for acute toxicity studies. The Wister rats (150-200 g) were used for subacute toxicity studies. The animals were housed at central animal house (Rajah Muthiah

Medical College and Hospital, Annamalai University, Tamilnadu, India) under standard conditions of temperature (23 ± 1 ºC), relative humidity (55 ± 1 %), 12 h light and dark cycles and fed with standard pellet diet and tap water *ad libtum*.

Acute toxicity study: Acute toxicity studies were performed according to OECD guidelines (acute toxic class method)[8]. Albino mice (n = 3) on either sex selected by using random sampling technique. The animals were on fast for 4 h with free access to water only, after which the extracts were administered orally at the dose level of 5 mg kg^{-1} body weight by gastric intubation and observed for 2 days. If mortality was observed in two out of three animals, then the dose administered was assigned as a toxic dose. If the mortality was observed only in one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose such as 50, 300 and 2000 there is no death then repeated with higher dose 5000 mg kg^{-1} body weight.

Sub acute experiment in rats: The animals were divided into five groups of 6 animals' each. The drug was administered daily at the dose of 50, 100, 250 and 500 mg kg^{-1} body weight for 30 days. The control group received the vehicle under the same experimental condition. The body weight changes were recorded weekly with simultaneous observation of toxic manifestation and mortality. Urine was collected on 28th day for urine analysis. At the end of the 30 days period, the animals were sacrificed by decapitation. The vital organs like heart, liver, lung, kidney, spleen and adrenals were carefully dissected out and weighed.

Hematological and biochemical analysis: The relative blood indices (total red cell and leukocyte counts and haemoglobin) were determined using routine method[9,10]. The differential leukocyte counting was performed with an optical microscopy after staining and, in each case, 100 cells were counted. For biochemical analysis, blood was centrifuged at 1480 × g for 10 min to obtain serum, which was stored at -20 ºC until determination of the following parameters: albumin, plasma urea[11] uric acid[12], creatinine[13], protein[14] and glucose[15] were estimated. Glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (ALP)[16], alkaline phosphatase[17] assays were carried out in serum. Plasma lipid profiles are phospho-lipid[18], triglyceride[19] and free fatty acid[20] were also determined.

Urine analysis: The urine collected on 28th day was analyzed for urea, uric acid, creatinine and protein levels also measured.

RESULTS AND DISCUSSION

During the acute toxicity study, the drug did not produce any external symptoms or mortality up to the dose level of 2000 mg kg^{-1} body weight orally in rats and were observed no mortality level upto the higher dose 5000 mg kg^{-1}. Fig. 1 shows the effect of drug on relative weight of vital organs with respect to the body weight and mortality in percentage. There was no significant change in weight of any of the vital organs (liver, heart, kidney, spleen, lungs and adrenals). No mortality was observed up to the dose of 500 mg kg^{-1} body weight.

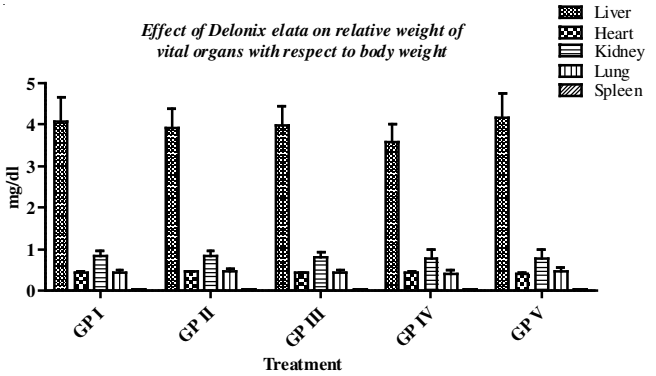

Fig. 1. Effect of ethanolic extract of *Delonix elata* on the relative weight of vital organs with respect to body weight. The experiment was carried out in 5 groups of animals and the effect of leaf extract was compared with control (saline mg kg^{-1}). Values are expressed as mean ± SD for 6 rats. Comparisons were made between group I with II, III, IV and group V

No toxicity signs or deaths were recorded during the 30 consecutive days of treatment by oral route with *Delonix elata* ethanolic extract at doses of 50, 100, 250 and 500 mg kg^{-1}. Neither absolute body weight nor body weight gain was affected by *Delonix elata* administration at all doses throughout the study. The hematological profile of control and treated groups are presented in Table-1. There were no statistically significant differences in all of the hematological parameters analyzed.

Fig. 2. depicts the drug effect on blood parameters like plasma urea, uric acid, creatinine and free fatty acid levels

TABLE-1
EFFECT OF THE *Delonix elata* (50-500 mg kg^{-1}) BY ORAL ROUTE ON HEMATOLOGICAL PARAMETERS IN MALE WISTAR RATS TREATED FOR 30 CONSECUTIVE DAYS

Parameters	Group I	Group II	Group III	Group IV	Group V
Hemoglobin (g/dL)	12.07 3.16	11.18 2.94	12.87 1.79	11.90 1.79	11.08 1.90
RBC (× 10^6 (_L)–1)	8.0 ± 0.1	8.4 ± 0.2	8.1 ± 0.6	8.1 ± 0.1	8.1 ± 0.1
Hematocrit (%)	42.65 ± 0.6	44.18 ± 0.8	42.11 ± 1.4	44.12 ± 0.4	44.0 ± 3 0.4
Platelets (× 10^3 (_L)–1)	1024 ± 16.7	1170± 47.3	1089 ± 67.2	1149 ± 49.3	1143 ± 49.1
WBC (× 10^3 (_L)–1	10.81 ± 1.0	12.18 ± 1.0	11.01 ± 1.0	11.65 ± 0.9	11.01 ± 0.9
PCV	20.46 ± 0.22	14.29 ± 0.43	15.86 ± 0.84	17.29 ± 0.46d	16.14 ± 0.46
Neutrophils (%)	23.01 ± 3.5	21.6 ± 2.7	25.8 ± 2.8	22.15 ± 2.3	22.01 ± 2.3
Eosinophils (%)	1.8 ± 0.43	1.7 ± 0.4	1.7 ± 0.4	1.7 ± 0.4	1.5 ± 0.3
Lymphocytes (%)	68.23 ± 3.3	73.3 ± 2.7	72.13 ± 2.87	64.32 ± 3.4	71.45 ± 1.6
Monocytes (%)	6.1, 0.9	6.01, 0.5	7.02, 0.9	6.5, 0.7	6.2, 0.08
Basophils (%)	0	0	0	0	0

were significantly ($p < 0.05$). No different in all groups and it shows the activity of marker enzymes (ALT, AST, ALP) in serum. No significant changes were observed in the enzymes activity in all groups tested (data now shown).

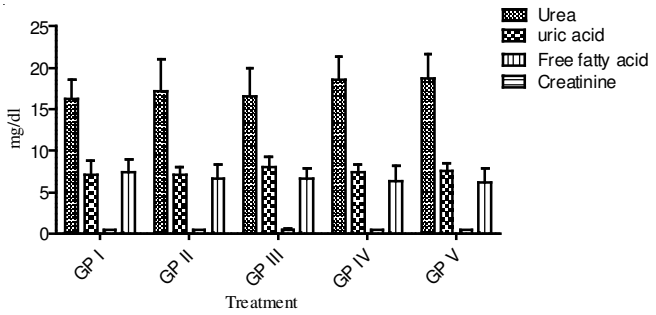

Fig. 2. Effect of ethanolic extract of *Delonix elata* on the urea, uric acid, creatinine and free fatty acid. The experiment was carried out in 5 groups of animals and the effect of leaf extract was compared with control. Values are expressed as mean ± SD for 6 rats. Comparisons were made between group I with II, III, IV, and group V

Fig. 3. represents the level of lipid profiles in experimental and control group animals. Total cholesterol and phospholipids were significantly increased in group III, IV and V when compared with control group. Triglyceride level was significantly decreased in group IV and group V animals. No significant changes were observed in glucose levels in all experimental groups when compared with control animals.

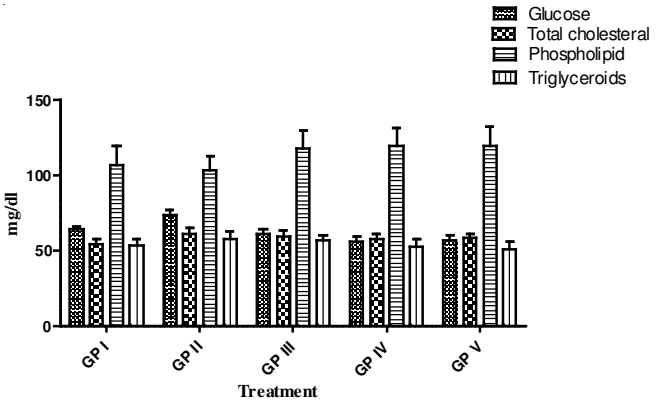

Fig. 3. Effect of ethanolic extract of *Delonix elata* on the glucose and lipids. The experiment was carried out in 5 groups of animals and the effect of leaf extract was compared with control. Values are expressed as mean ± SD for 6 rats. Comparisons were made between group I with II, III, IV and group V

Fig. 4 shows the effect of blood parameters like total protein and albumin. No significant changes were observed in experimental groups.

Fig. 5 shows that the urinary urea, uric acid, creatinine levels were significantly ($p < 0.5$) decreased in group V animals. Urinary protein activity was not altered to a significant extent.

In the acute toxicity study as per OECD guidelines, no mortality was observed in both control and in groups of all selected dose levels. Animals in all groups did not exhibit any signs of adverse effect and thus the no observed adverse effect level (NOAEL) of the extract is greater than 2000 mg kg^{-1}. We were observed that there was no mortality at 5000 mg kg^{-1}

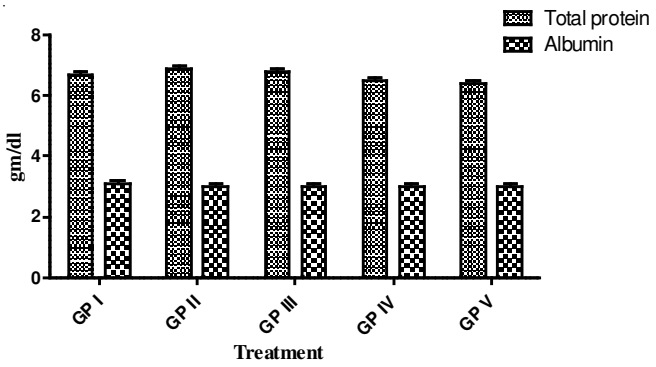

Fig. 4. Effect of ethanolic extract of *Delonix elata* on the protein and albumin. The experiment was carried out in 5 groups of animals and the effect of leaf extract was compared with control (saline mg kg^{-1}). Values are expressed as mean ± SD for 6 rats. Comparisons were made between group I with II, III, IV and group V

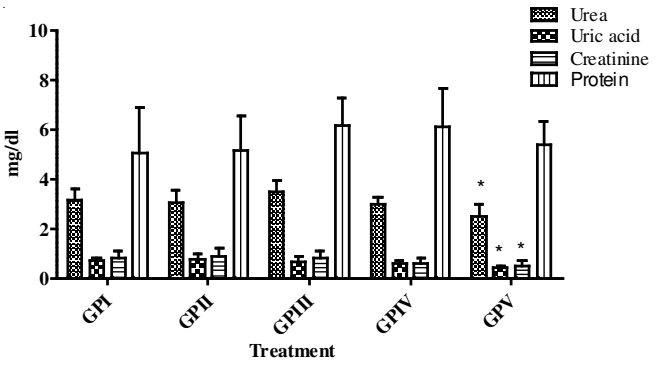

Fig. 5. Effect of ethanolic extract of *Delonix elata* on the urea, uric acid, creatinine and protein. The experiment was carried out in 5 groups of animals and the effect of leaf extract was compared with control (saline mg kg^{-1}). Values are expressed as mean ± SD for 6 rats. Comparisons were made between group I with II, III, IV, and group V. *$p < 0.5$

hence, 1/10th (500 mg kg^{-1}) of this dose was selected for the maximum dose sub-acute toxicity study[20]. The crude extracts of *Delonix elata* have been reported, antibacterial activity[21], antiinflammatory activity (unpublished data). From the present investigation, it is interesting to note that the non-toxic nature and did not induce any toxic effect of mortality upto the dose level 2000 mg, during acute toxicity studies.

Animal behaviour, food and water intake were normal during subacute toxicity study. Changes of body weight have been used to asses the course of the disease response to therapy of drugs[22]. No significant changes were observed in hematological screening indicating the non toxic nature of the drug shows the drugs effects on blood parameters. Plasma urea, uric aid and creatinine levels were increased in group V animals when compared with group I animals. Renal dysfunction may be the cause of raised plasma urea, uric acid and creatinine level accompanied by lowered urine urea, uric acid and creatinine level in high dose of drug treated rats. Raised urea in blood has been observed with impaired renal function or in acute renal failure[23]. In the present study, the alteration is not considerable, the observed small decrease in the urinary content is not sufficient to regard these changes as due to renal failure. There is no alteration in lipid profile and serum enzyme activities. The present study shows that the *Delonix elata* do not induce any toxic manipulation on the biochemical parameters

investigated. From these one can infer and hypothesize that this drug is non-toxic and can be used as therapeutic agent in treating the reported diseases effectively.

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