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Hepatoprotective Activity of Aqueous Extract of leaves of delonix regia against Paracetamol Induced Hepatotoxicity in rats

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ABSTRACT

To evaluate the hepatoprotective activity of the aqueous extract of delonix regia against paracetamol induced hepatotoxicity in albino rats. Hepatotoxicity was induced in albino rats by p.o of paracetamol (2gm/kg for 3 days). The aqueous extract of delonix regia was administered to the experimental animals at two selected doses for 14 days. The hepatoprotective activity of the extract was evaluated by the liver function marker enzymes in the serum (asparitate transaminases AST, alanine transaminase ALT, alkaline phosphatase ALK.P, total bilirubin TB, and histopathological studies of liver. Both the treatment groups showed hepatoprotective effect against paracetamol induced hepatotoxicity by significantly restoring the levels of serum enzymes to normal which was comparable to that of silymarin group. The oral administration of *delonix regia* significantly ameliorates paracetamol hepatotoxicity in rats.

Introduction

Liver is the key organ regulating homeostasis in the body. Because of its unique metabolism and relationship to gastro intestinal tract, the liver is an important target for toxicity produced by the drugs, xenobiotics and oxidative stress. More than 900 drugs, toxins and herbs have been reported to cause liver injury and drugs account for 20-40% instance of liver failure. In the absence of reliable liver, protection drugs in modern medicine, a large number of medicine preparations are recommended for the treatment of liver disorders. This scenario provides a severe necessity to carryout research in the area of hepatotoxicity. Delonix regia, belongs to the family fabaceae. Its leaves contain sterols, tri terpinoids, phenolic compounds, flavonoids, sitosterol, lupeol. It is a good wound healer, anti-inflammatory, good appetizer, good laxative, and widely used in diabetes as it controls the glucose level in body.

Materials & methods

2.1 Animals

In this method albino Wister rats of either sex weighing between 150-250g was used for the study. The rats were housed under standard conditions of constant temperature and lighting (12 hours light/dark cycle). They had access to standard pellet diet and water *ad* libitum

2.2 Collection of plant material

The leaves of Delonix regia was collected from Vijayawada, India.

2.3 Drugs and chemicals

Silymarin (100mg/kg) used as standard drug obtained from microlabs, Bangalore, India Paracetamol (2gm/kg) used as hepatotoxicant

Acute Toxicity studies

Acute oral toxicity studies was carried out on albino mice of either sex weighing between 25-35g and were divided into four groups containing 6 mice each, according to OECD guideline No. 420. The selected leaves extract was administered at a dose level of 200mg/kg body weight. The mice were continuously observed for their mortality and behavioural response for 48h and thereafter once in for 14 days. The selected functional foods were administered at a dose level of 2000 mg/kg body weight.

Experimental Design

The animals were divided in 5 groups of animals each group containing 6 rats. The treatment protocol was planned to study the effect of Delonix regia in curative aspect of paracetamol induced hepatotoxicity (Shenoy AK et al., 2002). The treatment protocol is summarized and given below.

Group I - normal control, 2% w/v gum acacia suspension orally, 1ml/kg once daily for 10 days

Group II- Paracetamol as toxicant 2g/kg orally once daily for 3 days followed by 1ml/kg 2%W/V gum acacia suspension from 4th day to 10th day

Group III- PCM 2g/kg orally for 3days followed by *Delonix regia* 200mg/kg orally from 4th day to 10th day

Group IV - PCM 2g/kg orally for 3days followed by *Delonix regia* 400mg/kg orally from 4th day to 10th day

Group V- PCM 2g/kg orally for 3days followed by silymarin 100mg/kg (Setty SR et al., 2007) orally from 4th day to 10th day

On the 0th day (one day before the dosing) and 11th day blood was collected from each animal for serum analysis. Then on the 11th day the animals were sacrificed and the livers were isolated and washed with fresh saline. Livers were stored in 10% formalin for histopathological study.

Marker enzymes of liver damage

Serum was separated by centrifugation (3000rpm for 15min.) and estimated for the biochemical parameters such as SGOT, SGPT, ALP, Total bilirubin using standard diagnostic kits on semi autoanalyser.

Statistical Analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis was performed with one way analysis of variance (1 way ANOVA) followed by Tukey test. P value less than 0.05 was considered to be statistically significant.

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Table 1: Base levels of selected biochemical parameters in rats for PCM induced hepatotoxicity on 0 day (Curative)

Group	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
1	2% Gum acacia (1 ml/kg; p.o.)	32.54± 1.85	80.67± 2.84	181.9± 4.35	0.89± 0.01
2	PCM (2 mg/kg; p.o.)	32.12± 1.64	82.46± 1.80	180.3± 3.08	0.88± 0.02
3	PCM + *Delonix regia* (2 mg/kg; p.o. + 200 mg/kg; p.o.)	31.25± 1.14	80.96± 3.15	180.5± 2.45	0.86± 0.02
4	PCM + *Delonix regia* (2 mg/kg; p.o. + 400 mg/kg; p.o.)	33.65± 1.54	81.53± 3.13	181.0± 4.09	0.92± 0.03
5	PCM + Silymarin (2 mg/kg; p.o. + 100 mg/kg; p.o.)	31.70± 1.70	81.78± 2.06	182.5± 2.35	0.88± 0.03

Table 2: Influence of *Delonix regia* aqueous extract on biochemical parameters in rats for PCM induced hepatotoxicity on 11th day (Curative)

Group	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
1	2% Gum acacia (1 ml/kg; p.o.)	34.54± 2.85	81.19± 3.84	183.9± 3.68	0.91± 0.01
2	PCM (2 mg/kg; p.o.)	115.1± 6.76	402.46± 5.08	548.0± 10.08	2.92± 0.05
3	PCM + *Delonix regia* (2 mg/kg; p.o. + 200 mg/kg; p.o.)	102.1± 7.04	345.1± 15.15***	524.3± 16.25***	2.05± 0.12***
4	PCM + *Delonix regia* (2 mg/kg; p.o. + 400 mg/kg; p.o.)	85± 3.41***	226.5±12.76***	315.0± 7.23***	1.40± 0.07***
5	PCM + Silymarin (2 mg/kg; p.o. + 100 mg/kg; p.o.)	36.37±3.72***	95.51± 4.28***	214.2± 13.55***	1.04± 0.05***

a values are the mean ± S.E.M. of six rats/treatment;***Significance P<0.001 compared to PCM treated groups

*=p<0.05, **=P<0.01 and ***=P<0.001, when compared with toxicant group.

Results and discussion

The elevation of SGPT, SGOT, ALP and Total bilirubin in Paracetamol intoxication was significantly high when compared to the normal. The elevated levels of SGPT, SGOT, ALP and Total bilirubin in group III & group IV animals (Post treated with aqueous leaves extract of Delonix regia (200 mg/kg, 400 mg/kg) was significantly reduced as depicted in table no.2. The percentage inhibition of SGPT, SGOT, ALP, and Total bilirubin in the extract treated group was calculated and reported as 31%, 23%, 34%, & 42% for delonix regia (200mg/kg) and 39%, 36%, 57%, 66% for delonix regia (400mg/kg) extract respectively. Silymarin was found significant reduction in the level of SGPT, SGOT, ALP and Total bilirubin when compared to the control 87%, 86%, 85%, 85% respectively.

Group I animals treated with gum acacia shown normal architecture of liver (Fig. A), while PCM treated liver shows marked level of fatty changes or degeneration and necrosis of the liver cells (Fig. B). The aqueous leaves extract Delonix regia (200 mg/kg body weight) treated (Fig. C) showed mild reduced hepatic damage. Aqueous leaves extract Delonix regia (400 mg/kg body weight) and Silymarin treated animals reveals reduced hepatic damage, lesser necrosis, demonstrated a normal architecture of liver and no significant pathological manifestations (Fig. D and Fig. E). Based on the enzymatic levels and histopathological observations of the delonix regia treated group, it can be concluded that the leaves extract possess significant hepatoprotective activity.

Conclusion

From the investigations it is evident that, the aqueous leaves extract of *Delonix regia* possessed significant Hepatoprotective activity against paracetamol intoxication in rats. However, further study is required to evaluate using a long term study and beneficial effects of *Delonix regia* leaves.

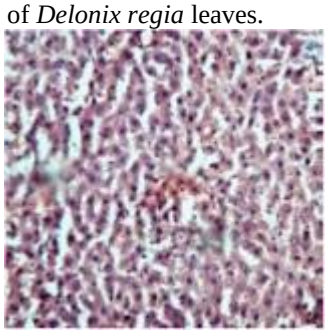

Fig. 1A

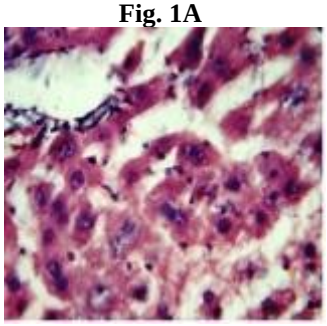

Fig. 1B

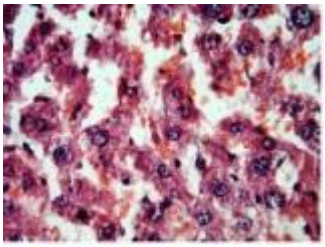

Fig. 1C

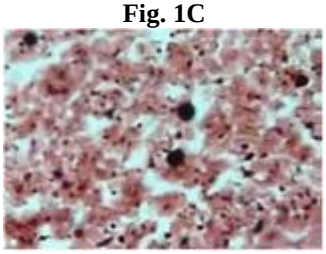

Fig. 1D

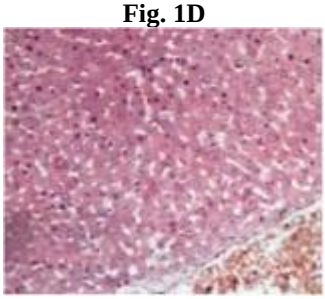

Fig. 1E

Fig.1: Histopathological changes occurred in the liver after paracetamol intoxication and the treatment with Delonix regia aqueous leaves extract

A) Normal B) PCM treated group C) Aqueous D.R leaves extract dose of 200mg/kg D) Aqueous D.R leaves extract dose of 400mg/kg E) Silymarin treated

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