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R E S E A R C H A R T I C L E

Evaluation of Hepatoprotective activity of *Delonix Elata* leaves against Carbon tetrachloride, Paracetamol and Ethanol induced Hepatotoxicity in rats.

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A B S T R A C T

Herbal drugs play a vital role in the management of various liver disorder, most of them speed up the natural healing process of liver. Numerous medicinal plants and their formulations are used in liver disorders in ethno medicinal practices as well as traditional system of medicine in India.The present work deals with hepatoprotective activity of ethanolic extract of Leaves *Delonix Elata (L)*against carbon tetrachloride, ethyl alcohol and paracetamol induced liver damage in rats. *Delonix Elata* produce various pharmacological activities such as aphrodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer and anti-diabetic activities. Liver can participates in a variety of metabolic activity by the presence of no of enzymes and them self-exposed too many toxicants, drugs, chemicals which can damage it. In this Hepatoprotective study, carbon tetra chloride, paracetamol and ethanol are used as hepatotoxic agents to induce liver damage, since it is used by human being for non-medical purposes or by medical purposes. In the present study, the extracts significantly reduced the elevated levels of above mentioned serum marker enzymes and increase in the levels of protein. Hence, at this point it is concluded that the extracts possess hepatoprotectve activity.

Keywords: Medicinal plants, *Delonix Elata*, Ethanolic extract, Hepatoprotective, Carbontetra chloride, Paracetamol

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C O N T E N T S

1. Introduction

Herbal drugs play a vital role in the management of various liver disorders, most of them speed up the natural healing process of liver. Numerous medicinal plants and their formulations are used in liver disorders in ethno medicinal practices as well as traditional system of medicine in India. Various types of treatment modalities are available to treat liver diseases. In allopathic medical practices, herbs play role in the management of various liver disorders. Since however, we do not have satisfactory remedy for disorders of liver, the search for finding out effective hepatoprotective drugs continues. Many unknown and lesser known plants are used is folk and tribal medical practices in India. The medicinal values of these plants are not known to the scientific world. The present work deals with hepatoprotective activity of ethanolic extract of Leaves *Delonix Elata (L)* against carbon tetrachloride, ethyl alcohol and paracetamol induced liver damage in rats.

Plant profile

Taxonomy:

Kingdom : Plantae-plants
Division : Angiosperms
Family : fabaceae
Order : Fabales
Genus : Delonix
Species : D.elata

Common names:

- **Hindi:** Waykaran, Samrsro, Sanesro, Sandeshra
- **Kannada:** kempukenjiga, nirangi, vatanarayana
- **Marathi:** sanchaila, sankasura
- **Sanskrit:** Siddhesvara
- **Tamil:** Perungondrai, Vadanarayanan, Varatti
- **Telugu:** Chinna seribiseri, Chitti keshwaramu

Geographical source:

The tree is harvested from the wild for local use for food, medicine, wood etc. The tree is also planted and protected by local people and is cultivated in India, A distinct, magnificent tree in bloom, it is suitable for cultivation in gardens, avenues and amenity parks.

Uses: The leaf extracts are anti-inflammatory; a root decoction is drunk for abdominal pains. A pychosomatic medicinal use relating to scorpion bite treatment is reported from India.

Ayurvedic uses: The plant holds a species position as a potent adaptogen and aphrodisiac in Ayurvedic system of medicine. It is important ingredient of many Ayurvedic preparations and is considered to have aphorodisiac, immunostimulant and hepatoprotective, and antioxidant, anticancer and anti-diabetic activities.

Figure 1: *Delenux Elata* plant

2. Materials and methods

Chemicals used in this study:

All the chemicals purchased were of analytical grade.

Animals:

Male Wistar albino rats Weight about 150-200 cm. were used for the study. The animals were housed in groups of six and maintained under standard conditions (27±2ºC, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours, respectively) and fed with standard rat diet and purified drinking water and libitum for 1 week before and during the experiments. All experiments and protocols described in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of and with permission from Committee for the purpose of control and supervision of experiments on animals. Ministry of Social Justice and Empowerment, Government of India.

Table 1: Instruments used in this study

S. No	Instruments	Manufactured company
1	UV-Visible Spectrophotometer	UV-1800 Shimadzu, Model, Mfg by Shimadzu Corporation.
2	Centrifuge	Research centrifuge, Mfg by Remi Instruments Ltd, Mumbai
3	Tissue Homogenizer	Type: RO-127A, Mfg by Rajendra Elect, IND. Ltd, Remi Instruments Division, Vasai
4	Sonicator	Pci made in Mumbai.
5	Milli pore water collector	Mfg by TKA smart pure made in made in Germany
6	Soxhlet apparatus	Agarwal
7	Rotary evaporator	Medika instrument Mfg Co.
8	UV chamber	Singhla sciences, Ambala

Collection of plant material:

The plant *Delonix elata* was collected in Tirumala forests, Tirupati, A.P, India in the month of January 2018. The plant sample was further verified and authenticated by a registered botanist Dr. madava chetty, The voucher specimen of the plant was deposited at the college for further reference.

Preparation of *Delonix elata* Leaves extract

Soxhalation process:

The powdered plant material was subjected to the extraction process by separately with water, ethanol and hydro alcohol (50:50) respectively for 18 hrs. Thereafter, they were filtered. The filtrates were dried in rotary evaporator (ROTA VAP) apparatus and suitable extract of was selected based on the percentage of yield and stored in refrigerator at 4˚ C for further studies.

Hepatoprotective studies

Carbon tetrachloride (CCl_4) induced hepatotoxicity:

Rats of either sex were divided into five groups of six animals in each group. (n = 6)

Group I: This group received 0.2% of Carboxy methyl cellulose solution (1ml/kg) once daily for nine days.

Group II: Received 0.2% of Carboxy methyl cellulose solution (1ml/kg) once daily for nine days and carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the 7th day.

Group III: Received standard drug silymarin (25 mg/kg, p.o.) for 9 days once daily and carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the 7th day.

Group IV: This group received *Delonix elata* leaf extract (200mg/kg, BW/PO) for 9 days once daily carbon tetrachloride (1 ml/kg in50% v/v olive oil, s.c.) on the 7th day.

Group V: This group received *Delonix elata* leaf extract (400mg/kg, BW/PO) for 9 days once daily carbon tetrachloride (1 ml/kg in50% v/v olive oil, s.c.) on the 7th day.

On the last day, serum marker enzyme parameters *i.e.*, Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT)and biochemical parameters Alkaline phosphatase (ALP) and biochemical parameters i.e. Total bilirubin and Total protein were analyzed according to the reported methods.

Paracetamol induced hepatotoxicity:

30 Rats were divided into 5 groups (n=6) and the duration of the experiment was 9 days.

Group I: This group received 0.2% of Carboxy methyl cellulose solution (1ml/kg) once daily for nine days.

Group II: This group received Paracetamol (2gm/kg) BW/PO diluted with sucrose solution 40%W/V in 3 divided doses on day nine.

Group III: This group received Silymarin 75mg/kg BW/PO once daily for nine days + Paracetamol (2gm/kg) BW/PO diluted with sucrose solution 40%W/V in 3 divided doses on day nine.

Group IV: This group received *Delonix elata* hydroalcoholic leaf extract (200mg/kg, BW/PO) + Paracetamol (2gm/kg) BW/PO diluted with sucrose solution 40%W/V in 3 divided doses on day nine.

Group V: This group received *Delonix elata* hydroalcoholic leaf extract (400mg/kg, BW/PO) + Paracetamol (2gm/kg) BW/PO diluted with sucrose solution 40%W/V in 3 divided doses on day nine.

Paracetamol (2g/kg) will be administered orally ina volume of 1 ml/day on fourth day to all the animals exceptfor normal and vehicle control. On the last day, serum marker enzyme parameters *i.e.*, Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT)and biochemical parameters Alkaline phosphatase (ALP) and biochemical parameters i.e. Total bilirubin and Total protein were analyzed according to the reported methods.

Ethanol induced hepatotoixicity:

Rats of either sex were divided into nine groups of six animals (n = 6) in each group.

Group I: Received 0.2% of Carboxy methyl cellulose solution (1ml/kg) once daily for 21 days, and served as normal control.

Group II: Received water (5 ml/kg, p.o.) for 21 days once daily and 40%ethanol (v/v, 2 ml/l00 g bw, p.o.) for 21 days.

Group III: Received standard drug silymarin (25 mg/kg, p.o.) for 21 days once daily and 40% ethanol (v/v, 2 ml/l00 g bw, p.o.) for 21 days.

Groups IV & V: Received extract (200&400 mg/kg) 21 days once daily and 40% ethanol (v/v, 2 ml/l00 g bw, p.o.) for 21 days.

On the last day, serum marker enzyme parameters *i.e.*, Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT)and biochemical parameters Alkaline phosphatase (ALP) and biochemical parameters i.e. Total bilirubin and Total protein were analyzed according to the reported methods.

Statistical analysis

All the data were expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dunnett's Multiple Comparison test using a computer based fitness program (Prism, Graph pad.). Statistical significance was determined at $P < 0.001$.

3. Results and discussion

Estimation of Extraction:

Based on the percentage of yield obtained, we selected hydroalcholic extract of *Delonix elata* leaves was selected for further studies and the details of percentage yield obtained are specified in the Table 2.

Phytochemical constituents present in hydroalcoholic extract of *Delonix elata* leaves*:*

Preliminary Phytochemical studies of hydroalcholic extract of *Delonix elata* leaves of *Delonix elata* confirmed the strong presence of desired Phytochemical in hydroalcoholic extracts when compared to Ethanolic, Aqueous extracts. Hence, for the further studies hydroalcoholic extract of *Delonix elata* have been selected.

Hepatoprotective activity

Carbon tetrachloride induced toxicity:

Effect of Hydroalcoholic extract of Delonix elata on biochemical parameters in Carbon Tetrachloride induced hepatotoxic rats. Rats treated with Carbon Tetrachloride developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and Albumin, Total protein and Creatinine when compared to normal control. Pre-treatment with Silymarin, hydro-alcoholic extract had showed good protection against Carbon Tetrachloride induced toxicity to liver. Test group indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which can be shown in the table no 4.

Paracetamol induced hepatotoxicity:

Effect of Hydroalcoholic extract of Delonix elata on biochemical parameters in PCM induced hepatotoxic rats. Rats treated with PCM developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and Albumin, Total protein and Creatinine when compared to normal control. Pre-treatment with Silymarin, hydro-alcoholic extract had showed good protection against paracetamol (PCM) induced toxicity to

liver. Test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which can be shown in the table no 5.

ETHANOL induced hepatotoxicity:

Effect of Hydroalcoholic extract of Delonix elata on biochemical parameters in ethanol induced hepatotoxic rats. Rats treated with ethanol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and Albumin, Total protein and Creatinine when compared to normal control. Pre-treatment with Silymarin, hydro-alcoholic extract had showed good protection against ethanol induced toxicity to liver. Test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which can be shown in the table no 6.

Table 2: Parameters: Estimation of Extraction

S. No	Name of the plant extract	Practical yield	Theoretical yield	Percentage yield
1.	Aqueous extract	24gm	50gm	48%W/W
2.	Ethanolic Extract	20gm	50 gm	36%W/W
3.	Hydroalcoholic Extract	34gm	50gm	65%W/W

Table 3: Qualitative Phytochemical tests

S. No	Name of Phyto constituents	Aqueous Extract	Ethanol Extract	Hydroal-coholic extract
1	Carbohydrtes	Negative	Negative	Negative
2	Alkaloids	Negative	Positive	Positive
3	Flavonoids	Negative	Negative	Positive
4	Cardiac glycosides	Negative	Negative	Negative
5	Anthraquin-one glycosides	Negative	Negative	Negative
6	Saponins	Negative	Negative	Positive
7	Steroids	Negative	Negative	Negative
8	Tri-terpenoids	Negative	Negative	Positive
9	Tannins	Positive	Positive	Positive
10	Phenols	Negative	Negative	Negative
11	Amino acids and proteins	Negative	Negative	Negative
12	Quinones	Negative	Negative	Negative

Table 4: Biochemical Parameters of Effect of Hydroalcoholic extract *of Delonix elata* on Carbon Tetrachloride induced Hepatotoxicity in rats

S.No	Groups	SGOT IU/ml	SGPT IU/ml	Albumin mg/dl	Creatinine mg/dl	Total Protein mg/dl
1	Group I	89.43±3.121	76.14±1.25	5.143±.132	0.7620±0.0438	6.852±0.243
2	Group II	149.3±2.651***	106.4±2.36***	6.573±0.142**	1.543±0.0310***	3.056±1.932*
3	Group III	104.3±3.623***	73.6±3.01***	5.462±0.365**	0.843±0.0274***	6.561±0.316*
4	Group I V	136.9±2.43*	86.4±3.14***	6.052±0.174^{ns}	1.016±0.031***	5.318±0.241^{ns}
5	Group V	129.4±3.219***	79.3±3.62***	5.628±0.126*	0.897±0.030***	6.470±0.184*

All Values are expressed as mean ± SEM, One Way Analysis of Variance, followed by Dunnetts's * P<0.05, ** P<0.01 & *** P<0.001 when compared with G II;G II is compared with G I;

Table 5: Biochemical Parameters of Effect of Hydro alcoholic extract of *Delonix elata* on PCM induced Hepatotoxicity in rats

S. No	Groups	SGOT IU/ml	SGPT IU/ml	Albumin mg/dl	Creatinine mg/dl	Total Protein mg/dl
1	Group I	92.23±2.161	73.0±1.726	4.083±0.1044	0.7571±0.05288	6.883±0.2315
2	Group II	151.7±2.165***	105.6±2.140***	5.567±0.1764***	1.358±0.1576***	4.663±0.1994***
3	Group III	106.3±2.255*	68.52±1.545***	4.417±0.1352**	0.9000±0.02864***	5.980±0.1068**
4	Group I V	132.0±3.543***	76.48±2.543*	4.533±0.19944**	0.8967±0.01498*	5.400±0.1438***

5	Group V	122.3±6.613***	72.70±1.548**	4.483±0.1493***	0.8433±0.03027**	5.667±0.1453***

All Values are expressed as mean ± SEM, One Way Analysis of Variance, followed by Dunnetts's * P<0.05, ** P<0.01 & *** P<0.001 when compared with G II

Table 6: Biochemical Parameters of Effect of Hydro alcoholic extract of Delonix elata on ethanol induced Hepatotoxicity in rats

S. No	Groups	SGOT IU/ml	SGPT IU/ml	Albumin mg/dl	Creatinine mg/dl	Total Protein mg/dl
1	Group I	83.26±2.201	78.0±1.726	4.091±0.128	0.797±0.0498	6.983±0.2415
2	Group II	149.38±2.195***	101.6±2.540***	5.67±0.1764***	1.324±0.1476***	4.633±0.1994***
3	Group III	110.3±2.55*	80.52±3.45***	4.211±0.1256**	0.8900±0.02764***	6.018±1.184**
4	Group I V	128.0±3.415***	90.48±2.543*	4.433±0.184**	0.9067±0.01398*	5.300±0.1438***
5	Group V	120.3±4.213***	79.70±1.548**	4.473±0.1463***	0.8633±0.03127**	5.967±0.453*

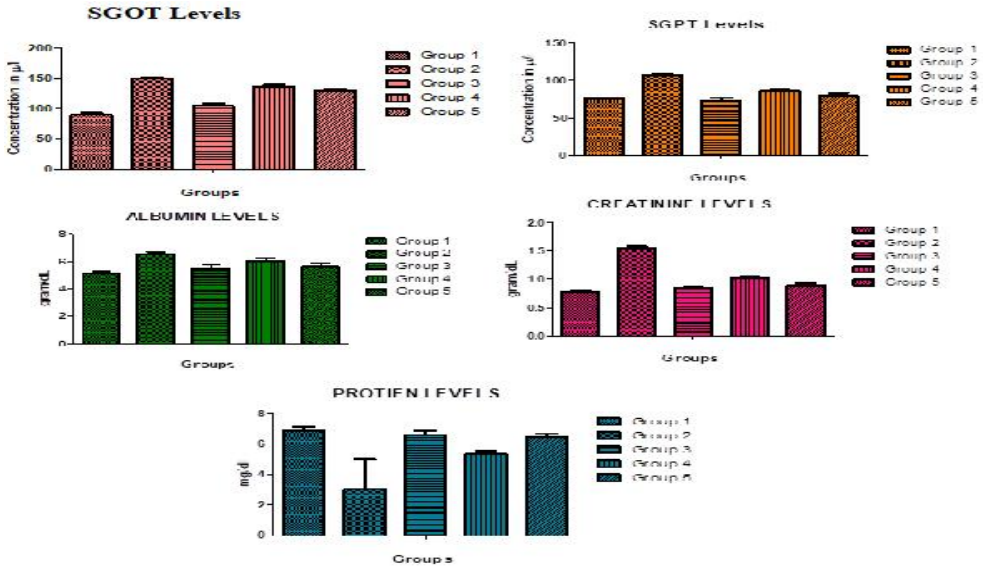

Figure 2: Effect of Hydroalcoholic extract *of Delonix elata* on Carbon Tetrachloride induced Hepatotoxicity in rats

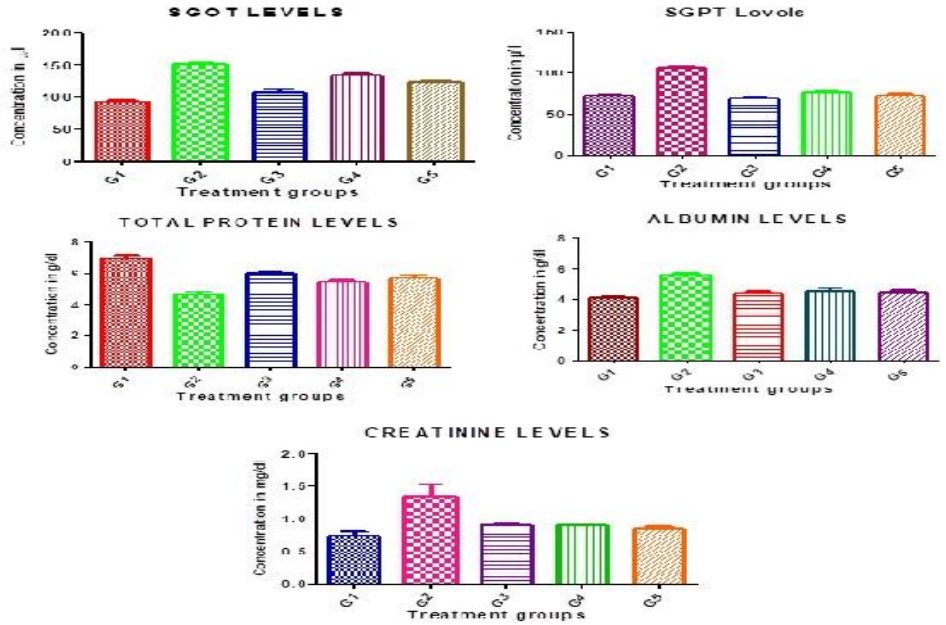

Figure 3: Effect of Hydro alcoholic extract of *Delonix elata* on PCM induced Hepatotoxicity in rats

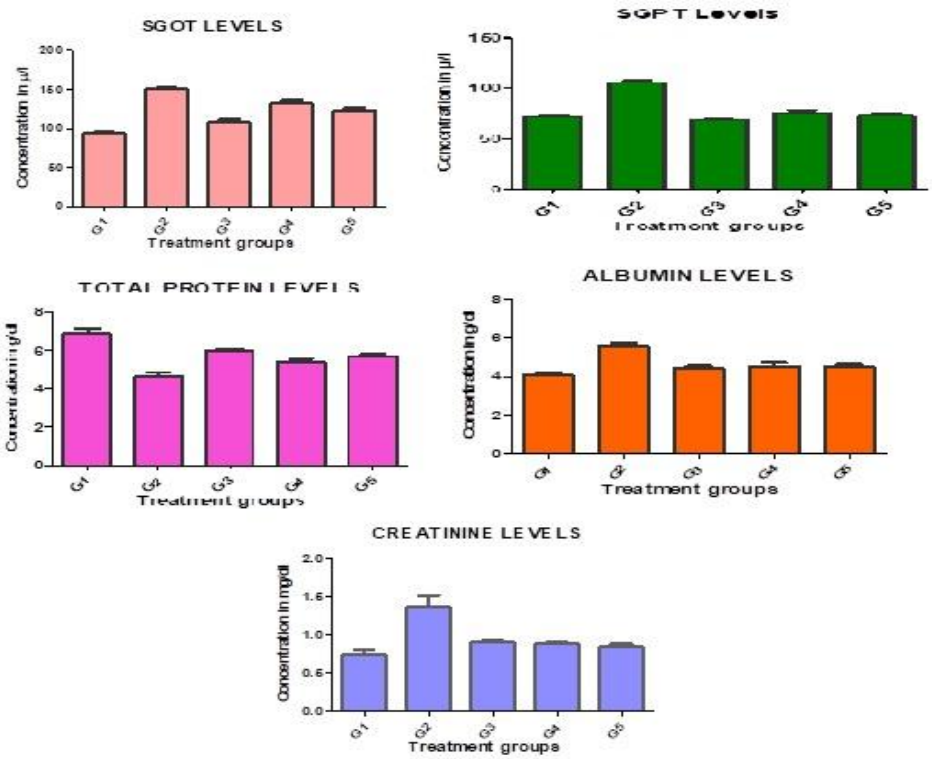

Figure 4: Effect of Hydro alcoholic extract of Delonix elata on ethanol induced Hepatotoxicity in rats

4. Conclusion

The present study was the main objective to assess the hepatoprotective activity of hydro alcoholic extract of Leaves of *Delonix elata*. LD50 studies were conducted in albino rats with hydro alcoholic extract of root of *Delonix elata* according to OECD guideline No.423 and were found safe up to the dose level of 2gm/kg confirming its non-toxic nature. The Hepatoprotective activity was studied in carbon tetra chloride, paracetamol and ethanol induced hepatotoxic animal model. The biochemical parameters like serum SGPT, SGOT, albumin, decreases and total protein increases with hydro alcoholic extract of root of *Delonix elata* Leaves confirmed the hepatoprotective effect of extract under this study. In liver injury models in rats restoration of hepatic cells with minute fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection. Mainly based on the improvement in serum marker enzyme levels it was concluded hydro alcoholic Leaves extract of *Delonix elata* possesses significant hepatoprotective activity in the doses used.

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