

Investigation of Hepatoprotective Effect of Delonix Regia Leaves in Paracetamol Induced Hepatotoxicity in SD Rats

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ABSTRACT

Medicinal plants have been used to treat liver diseases by folk medical practitioners for long time. *Delonix regia* has been used in traditional medicine to alleviate several diseases. Present study investigated the hepatoprotective effect of *Delonix regia* leaves in paracetamol induced hepatotoxicity in rats. SD Rats (140–200g) were used. Acute hepatotoxicity was induced by paracetamol (700mg/kg body weight) administered once daily for one week whereas the test extract was given orally throughout the whole experiment at 300 and 600mg/kg body weight. Silymarin (100mg/kg b.w.) was given orally as standard hepatoprotective drug. The degree of hepatoprotection was determined by the estimation of biochemical parameters like SGPT, SGOT, ALP, bilirubin and total protein. The increased levels of hepatic enzymes including SGPT, SGOT, ALP and bilirubin was found in the paracetamol control group, which may be due to the liver cell damage or changes in the cell membrane permeability indicating severity of hepatocellular damage induced by paracetamol. Treatment with *Delonix regia* and silymarin recovered the increased levels of these hepatic enzymes significantly to reduced levels. The results of the study evidenced that the *Delonix regia* leaves has shown hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: *Delonix regia*, hepatoprotective activity, liver enzymes, paracetamol induced hepatotoxicity

INTRODUCTION

The liver is a vital organ in the body and is primarily responsible for the metabolism of endogenous and exogenous agents. It

plays an important role in drug elimination and detoxification. Hepatotoxicity is the injury to the liver that is associated with impaired liver function caused by exposure

to a drug, or another non-infectious agent [1]. Liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications [2]. Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure [3]. Nevertheless, chemical toxins including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions [4]. A number of plant-based traditional medicines or formulations containing herbal extracts are used against liver disorders. Thus, interest and effort have shifted toward medicinal plants as new sources of hepatoprotective agents [5].

Delonix regia (Family: Fabaceae) is a species of flowering plant, locally known as Krishnachura in Bengali, Gulmohar in Hindi and Urdu, Royal Poinciana or Flamboyant in English). It is a tall tree reaching 05-12m; leaves are even-pinnate, alternate, to 26 inches long, 10-25 pairs of opposite pinnae, 12-40 pairs of small oblong leaflets. It is widely distributed in Bangladesh, South Florida, Egypt, Vietnam, Caribbean, Indian subcontinent, Thailand, Mauritius etc. and found as a wild, also cultivated in most parts of Bangladesh. The plant has traditional uses in pain, inflammation, fever and in several skin disorders [6–8].

The aim of this work was to evaluate the hepatoprotective effect of *D. regia* leaves extract in experimental animals.

MATERIALS AND METHODS

Plant Collection and Extraction

The plant *Delonix regia* (DR) leaves was collected from Savar area and was identified and authenticated by the Dept. of Botany, Jahangirnagar University, Savar, Dhaka. The collected materials were thoroughly washed in water, cut into

smaller parts and shed dried at 35°–40°C and pulverized in electric grinder to get extractable powder. Then powder was extracted in soxhlet apparatus with ethanol (96%).

Experimental Animals

For the experiment, Sprague Dawley rats of either sex, weighing between 140-200g, were collected from the animal research lab in the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: 27.0±1.0°, relative humidity: 55–65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Experimental Design for the Assessment of Liver Functions

Animal study was performed at Pharmacology Laboratory, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. The rats were housed in polypropylene cages at room temperature (27±2°C). The rats were divided into five groups of 6 animals (n = 6) each.

Group I: Received water (10 mL/kg p.o.) once daily for 7 days, and served as normal control

Group II: Received water (10 mL/kg p.o.) and paracetamol 700 mg/kg, p.o. once daily for 7 days and served as positive control.

Group III: Received standard drug silymarin (100mg/ kg p.o.) and paracetamol 700 mg/kg, p.o. once daily for 7 days, serving as STD.

Group IV and V: Received *Delonix regia* (DR) leaves extract (300 and 600 mg/kg respectively) and paracetamol 700 mg/kg p.o. once daily for 7 days.

Rats were anesthetized using ketamine. After sacrifice, blood samples were

collected and the serums were separated by centrifugation. Serum samples were subjected to liver function tests of enzymes such as glutamate pyruvate transaminase (GPT/ALT), glutamate-oxaloacetate transaminase (GOT/AST), alkaline phosphatase (ALP), total bilirubin and total protein.

Statistical Analysis

Statistical analysis for animal experiments was carried out using One way ANOVA following Dunnet's post hoc test using SPSS 16.0 for windows. Data were presented as Mean $\pm$ SEM. The results obtained were compared with the vehicle control group. p values <0.05 , <0.01 and <0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

RESULT AND DISCUSSION

Acetaminophen (AAP) is used as analgesic which causes liver necrosis in high doses by the formation of NAPQI. NAPQI results the depletion of hepatic GSH. At high doses, more NAPQI binds covalently to cellular macromolecules. These macromolecules which leak from the damaged tissues, enzymes are released into the bloodstream, and these enzyme activities in plasma have been found to be important in the assessment of liver damage [9–14].

The increased level of SGPT and SGOT indicates cellular damage and loss of functional integrity of the hepatocyte membrane in the liver [15]. The increase in ALP results from the increased synthesis of this enzyme by cells lining the canaliculi, usually either intra- or extra-hepatic, reflecting the pathological alteration in biliary flow during liver diseases [16].

The present study demonstrated the ability of the DR extract to decrease the SGPT

and SGOT level significantly at 300 mg/kg ($p<0.001$) and at 600mg/kg ($p<0.01$) dose. It also reduced the ALP level significantly ($p<0.01$) at both the doses (Table 2 and Fig. 2).

Reduction of the increased level of serum GPT, GOT, ALP by DR extract indicated the protection and maintenance of the functional integrity of hepatocytes.

An abnormal increase of serum bilirubin indicates hepatobiliary disease and severe disturbance of hepatocellular function [17]. Due to the oral administration of *Delonix regia* (DR) extract showed significant protection against AAP-induced hepatotoxicity. It decreased the levels of bilirubin significantly at 300mg/kg ($p<0.05$) and at 600mg/kg ($p<0.01$) dose which is an indication of protection against hepatic damage caused by AAP (Table 2 and Fig. 3).

Most of the plasma proteins are produced by the liver, except immunoglobulins. Severe liver damage has been associated with decreased production of plasma proteins resulting in reduced serum levels of total protein, albumin, and/ or globulin [18, 19]. Decreased protein production may render other abnormal test values example: depletion of coagulation factors (all are globulins) may result in prolonged prothrombin or activated partial thromboplastin times [20]. And, increased loss of protein via urine or feces due to renal or gastrointestinal disease will reduce serum protein levels. Inflammation anywhere in the body often results in increased production of specific globulin proteins produced by liver [18].

The results indicated that the serum total protein level was significantly ($p<0.05$) increased at both 300mg/kg and 600mg/kg dose (Table 3 and Fig. 4).

Through this study also the consumption of DR for 7 days was found to increase

the body weight insignificantly whereas the extract significantly ($p < 0.01$)

increased the liver weight of rats (Table 1 and Fig. 1).

Table 1: Effect of *Delonix regia* leaves on the body weight and liver weight in paracetamol induced hepatotoxicity in rats.

Group	Body Weight (gm) (Mean±SEM)		Liver Weight (gm)
	Initial Day	Final Day	
Normal Control	158.17±3.50	171.33±3.41	5.73±0.18
Paracetamol Control	158.0±3.0	145.17±2.50	4.45±0.20
STD	159.33±3.74	167.16±3.52	5.03±0.10***
DR 300mg/kg	157.83±4.67	161.83±4.05	4.52±0.25**
DR 600mg/kg	159.0±3.63	164.33±3.85	5.12±0.09**

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean ± SEM, n=6. *($p < 0.05$) = significant, **

($p < 0.01$) = highly significant, *** ($p < 0.001$) = very highly significant as compared to paracetamol control group.

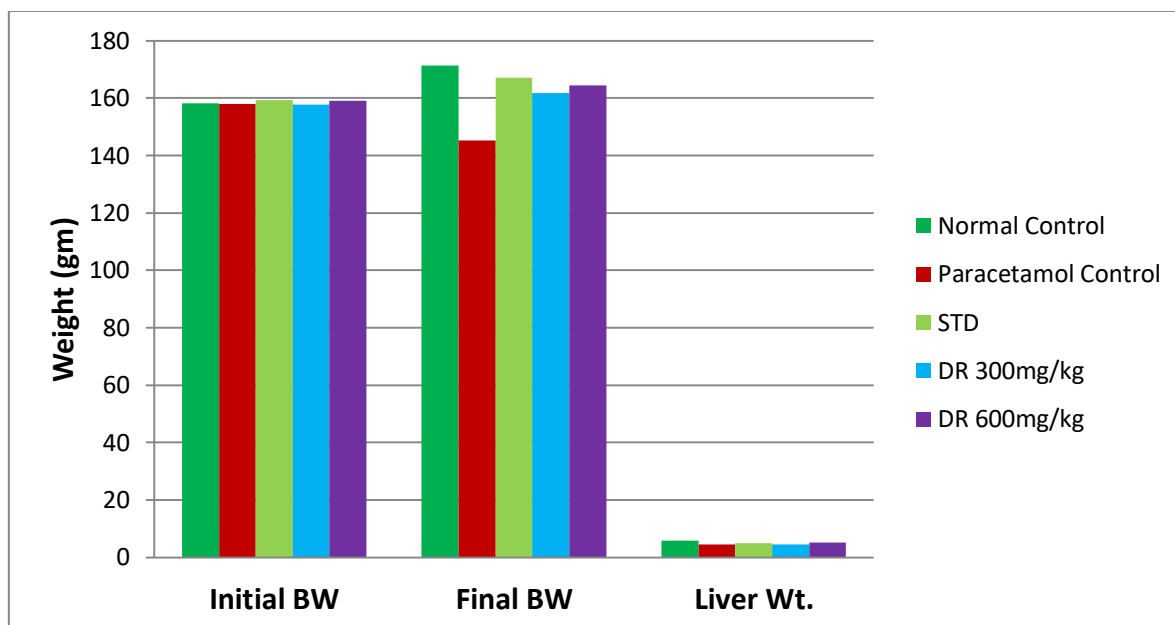


Figure 1: Effect of *Delonix regia* leaves on the body weight and liver weight in paracetamol induced hepatotoxicity in rats.

Table 2: Effect of *Delonix regia* leaves on SGPT, SGOT, ALP and total bilirubin level in paracetamol induced hepatotoxicity in rats.

Group	SGPT (IU/L) (Mean±SEM)	SGOT (IU/L) (Mean±SEM)	ALP (IU/L) (Mean±SEM)	Total Bilirubin (g/dl) (Mean±SEM)
Normal Control	19.50±3.97	24.00±3.31	67.17±3.85	0.73±0.09
Paracetamol Control	58.00±5.84	66.33±4.94	159.67±11.32	2.10±0.15
STD	37.50±3.04**	31.00±3.11***	83.67±5.34**	0.57±0.09**
DR 300mg/kg	49.17±2.63***	46.67±3.85**	122.00±7.19**	1.45±0.15*
DR 600mg/kg	40.33±1.93**	44.83±2.51**	100.83±4.17**	0.92±0.07**

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *(p< 0.05) =

significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant as compared to paracetamol control group.

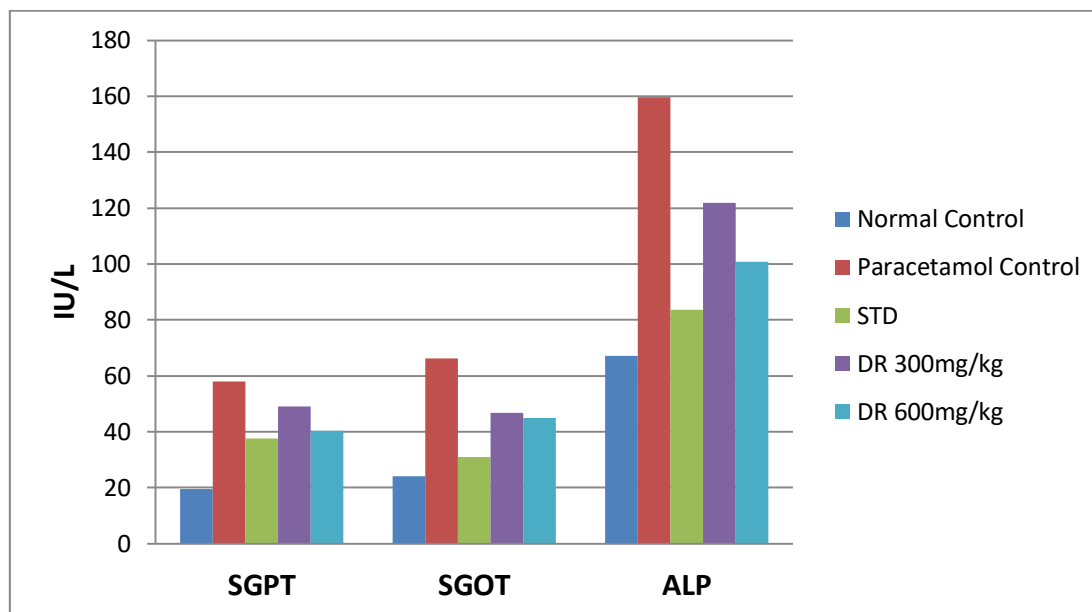


Figure 2: Effect of *Delonix regia* leaves on SGPT, SGOT and ALP level in paracetamol induced hepatotoxicity in rats.

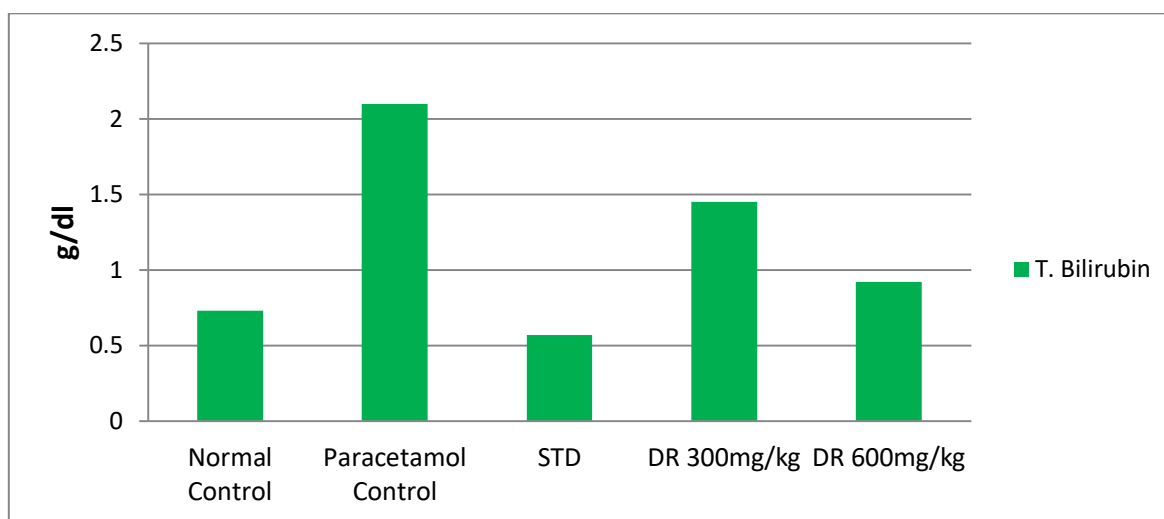


Figure 3: Effect of *Delonix regia* leaves on total bilirubin level in paracetamol induced hepatotoxicity in rats.

Table 3: Effect of *Delonix regia* leaves on total protein level in paracetamol induced hepatotoxicity in rats.

Group	Total Protein (g/dl) (Mean±SEM)
Normal Control	5.72±0.21
Paracetamol Control	3.60±0.22
STD	5.38±0.18**
DR 300mg/kg	3.80±0.28*
DR 600mg/kg	4.62±0.24*

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean $\pm$ SEM, n=6. *(p< 0.05) =

significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant as compared to paracetamol control group.

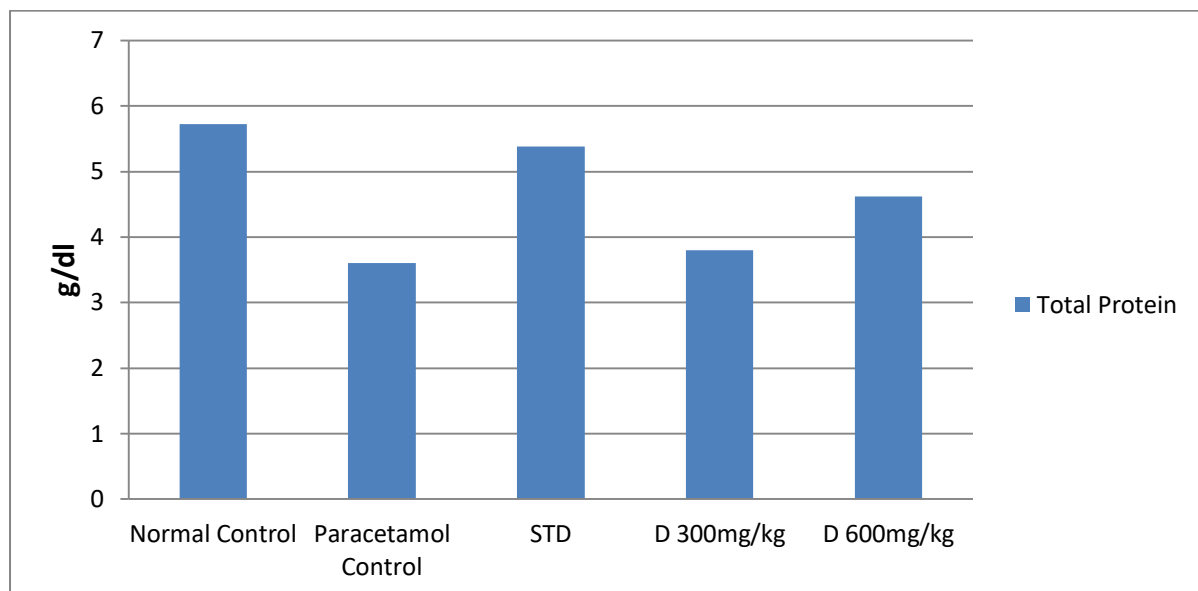


Figure 4: Effect of *Delonix regia* leaves on total protein level in paracetamol induced hepatotoxicity in rats.

CONCLUSION

The overall findings of this study demonstrated that the treatment with *Delonix regia* leaves extract was able to protect the changes in the liver induced by AAP. From the results it can be concluded that *Delonix regia* has significant value as hepatoprotective plant against paracetamol induced liver injury model. Further studies are required to determine the possible hepatoprotective mechanism(s) involved and, to isolate and identify the responsible bioactive compounds.

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