

## HEPATOPROTECTIVE ACTIVITY OF *ABRUS PRECATORIUS* LINN. AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

G.R Battu<sup>a\*</sup>, B.M. Kumar<sup>a</sup>

<sup>a</sup>Pharmacognosy and Phytochemistry Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

Correspondence: [mahimpharm@yahoo.com](mailto:mahimpharm@yahoo.com)

### Summary

The present study was conducted to evaluate the hepatoprotective activity of hydroalcoholic seed extract of *Abrus precatorius* against paracetamol induced liver damage in rats. The hydroalcoholic extract of *Abrus precatorius* (100 and 200 mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5 % Sodium Carboxy methyl cellulose solution. The plant extract 100, 200 mg/kg was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the hydroalcoholic extract of *Abrus precatorius* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

**Keywords:** *Abrus precatorius*, Paracetamol, hepatoprotective and hepatotoxicity.

## **Introduction**

Plants, the first medicines of human being, have played a remarkable role in health care since the ancient times. Traditional plant-based medicines still exert a great deal of importance to the people living in developing countries and also lead to discovery of new drug candidates for a variety of diseases that threaten human health. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (1). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders (2). 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.

The plant *Abrus precatorius* Linn popularly known as Rosary pea, jequirity bean belong to the family leguminosae (Fabaceae) is found throughout India in hedges and bushes in exposed areas. The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form upon long refrigerated storage. Usually seeds are of two types one is scarlet with black spot and the other variety is pure white and traditionally used against leucoderma, wounds, alopecia, asthma, tubercular glands, leprosy, fever, ulcer and tumor (3,4). The seed extract of *abrus precatorius* have also been shown to possess other pharmacologic properties. It was shown to have antifertility effect (5), ureterotonic effect (6), antidiarrhoeal effect (7), antidiabetic (8), arthritis (9), antimicrobial (10), although *abrus precatorius* has been shown to be stable in the gastrointestinal tract, the presence of toxic lectins in its seed limits its pharmacologic utility. Based on the above uses of *A. precatorius*, the present study was undertaken to evaluate hepatoprotective effect of the hydroalcoholic extract against paracetamol induced hepatotoxicity in rats.

## **Materials and Methods**

### ***Plant material***

The plant material (*A. precatarius*) was collected from Kollimallai hills, Salem district in Tamil Nadu during the month of May. The identification of the plant sample was carried out by Dr. M. Venkaiah, Associate Professor, Department of Botany, A.U., Visakhapatnam. The specimen was kept in the Herbarium of the Phytochemistry and Pharmacognosy specialization, Andhra University, Visakhapatnam, Andhra Pradesh, India.

#### ***Chemicals***

Solvent ethanol was procured from Ranbaxy (India).

#### ***Preparation of the extracts***

The seeds of *A. precatarius* were cleaned and ground mechanically to fine powders in a grinder and weighed as 250.5 g. The powdered material was subjected to successive solvent extraction with ethanol: water (1:1) using soxhlet apparatus. The extract was concentrated under vacuum (50 °C), dried completely and weighed (56 g).

#### ***Phytochemical screening***

Standard methods were used for preliminary phytochemical screening of the extract to know the phytoconstituents in the extract (11).

#### ***Animals***

Male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were left for 7 days for acclimatization to animal room conditions were maintained on standard pellet diet and water *ad libitum*. The food was withdrawn on the day before the experiment, but allowed free access of water. Throughout the experiments, animals were processed according to the suggested national ethical guidelines for the care of laboratory animals.

#### ***Acute toxicity studies***

Swiss albino mice of either sex weighing 18 – 22 g were randomly distributed to 6 different groups with 6 animals in each group. The animals were fasted overnight and the drug was administered orally at dose levels of 50, 100, 200, 400, 800 and 1600 mg/kg of body weight. The animals were closely observed at initial and at 2<sup>th</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 12<sup>th</sup> and 24<sup>th</sup> hour following drug administration for behavior pattern, toxic symptoms and for 72 h for mortality rate.

### **Hepatoprotective Activity**

Six groups (A, B, C, D and E) of male wistar rats were taken and each group consists of six animals. Group A served as normal control received 0.5% (CMC) carboxy methyl cellulose solution (1 ml/kg) once daily for 3 days. Group B served as paracetamol control, administered with paracetamol (3 gm/kg) as single dose on day 3. Group C received, *A. precatorius* hydroalcoholic extract (100 mg/kg) once daily for 3 days. Group D received, *A. precatorius* hydroalcoholic extract (200 mg/kg) once daily for 3 days Group E served as reference control, received Silymarin (25 mg/kg) once daily for 3 days. Group C, D and E received paracetamol (3 gm/kg) as single dose on day 3, thirty minutes after the administration of *A. precatorius* hydroalcoholic extract of 100, 200 mg/kg and Silymarin 25 mg/kg respectively. All the test drugs and paracetamol were administered orally by suspending in 0.5 % CMC solution. After 48 h of paracetamol feeding, the blood was collected by retro orbital plexus and was allowed to clot at room temperature and serum was separated by centrifuging at 3000 rpm for 10 min for the estimations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) (12,13) and bilirubin (14).

### **Statistical Analysis**

The values were expressed as mean  $\pm$  SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values  $<0.05$  were considered significant.

### **Results**

In acute toxicity study behavior pattern was unaffected and no toxic effects or mortality was observed up to the dose 1600 mg/kg during the 72 h period for hydroalcoholic extract of *A. precatorius*. Preliminary phytochemical screening of the extract reveals the presence of alkaloid, flavonoids, glycosides, sterols.

The results of hepatoprotective activity of hydroalcoholic extract of *Abrus precatorius* on paracetamol treated rats are shown in Table 1. The concentration of serum enzymes were calculated and plotted a bar graph (Figure 1 and Figure 2). The hepatic enzymes ALT ( $198.83 \pm 0.95$ ), AST ( $248.83 \pm 0.87$ ), ALP ( $297.33 \pm 0.71$ ) and bilirubin ( $2.72 \pm 0.06$ ) in serum was significantly ( $P < 0.001$ ) increased in paracetamol treated animals when compared to control.

The hydroalcoholic extract of *A. precatorius* 200 mg/kg treatment significantly ( $P < 0.001$ ) reversed the levels of ALT ( $102.83 \pm 0.48$ ), AST ( $216.67 \pm 0.49$ ), ALP ( $204.17 \pm 0.48$ ) and bilirubin ( $1.33 \pm 0.03$ ) when compared to paracetamol alone treated rats. The hydroalcoholic extract of *A. precatorius* 100 mg/kg treatment significantly ( $P < 0.001$ ) reversed the levels of ALT ( $85.17 \pm 0.87$ ), AST ( $204.17 \pm 0.48$ ), ALP ( $186.33 \pm 0.71$ ) and bilirubin ( $0.88 \pm 0.05$ ) when compared to paracetamol alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant ( $P < 0.001$ ) decrease in ALT ( $76.67 \pm 0.49$ ), AST ( $188.67 \pm 1.94$ ), ALP ( $175.50 \pm 0.62$ ) and bilirubin ( $0.73 \pm 0.02$ ) levels when compared to paracetamol alone treated rats. From the result it is clear that the drugs show dose dependent activity.

Table 1: Effect of *A. precatorius* on hepatotoxicity induced by paracetamol

Group	Dose	Units	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dL)
Control	---	---	$53.50 \pm 0.76$	$74.83 \pm 0.87$	$56.17 \pm 0.87$	$0.97 \pm 0.05$
Paracetamol	3	gm/kg	$198.83 \pm 0.95$ *	$248.83 \pm 0.87$ *	$297.33 \pm 0.71$ *	$2.72 \pm 0.06$ *
<i>A.Precatorius</i> extract	100	mg/kg	$102.83 \pm 0.48$ **	$216.67 \pm 0.49$ **	$204.17 \pm 0.48$ **	$1.33 \pm 0.03$ **
<i>A.Precatorius</i> extract	200	mg/kg	$85.17 \pm 0.87$ **	$204.17 \pm 0.48$ **	$186.33 \pm 0.71$ **	$0.88 \pm 0.05$ **
Silymarin	25	mg/kg	$76.67 \pm 0.49$ **	$188.67 \pm 1.94$ **	$175.50 \pm 0.62$ **	$0.73 \pm 0.02$ **

\* $p < 0.001$  when compared to control, \*\* $p < 0.001$  when compared to paracetamol treated group.

Figure 1: Effect of *A. precatorius* and Silymarin on serum ALT, AST and ALP levels in paracetamol induced hepatotoxicity.

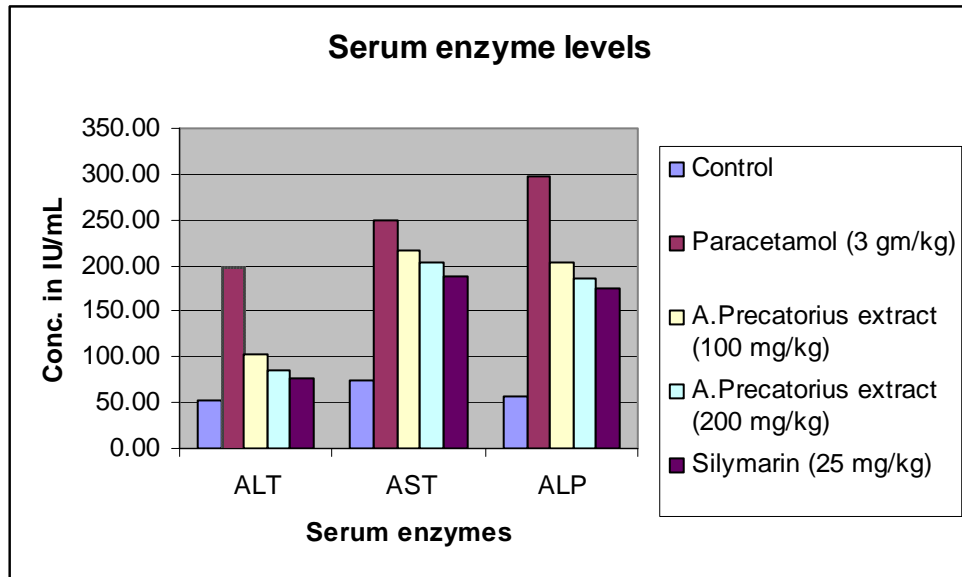
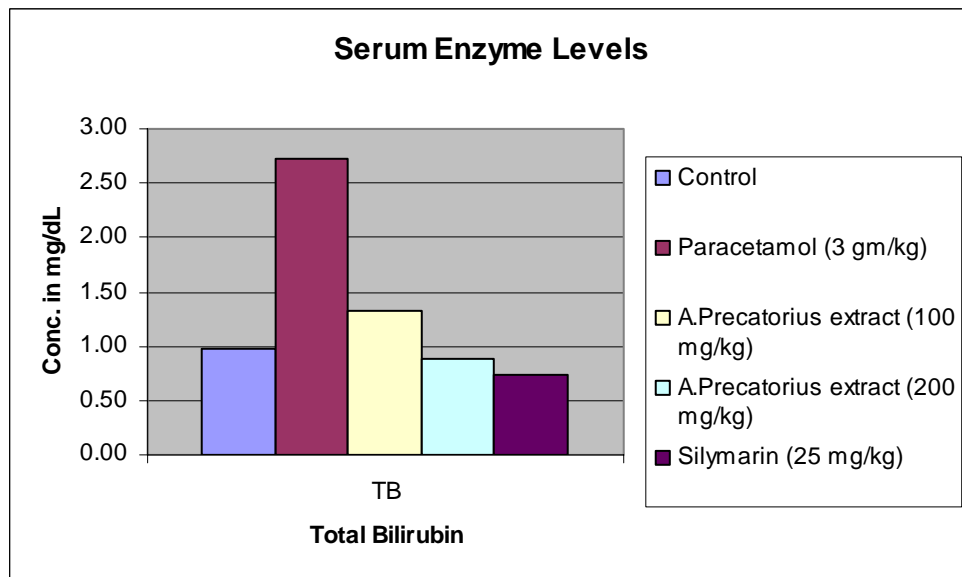


Figure 2: Effect of *A. precatorius* and Silymarin on serum Total Bilirubin levels in paracetamol induced hepatotoxicity.



### Discussion

Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses (15-17). Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations (18-22). Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome (23) or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity (24, 25).

Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present in higher concentration in cytoplasm. When there is hepatopathy/ hepatocyte necrosis or abnormal membrane permeability, these enzymes leak into the blood stream in conformity with the extent of liver damage (26). ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. ALT is more selectively a liver paranchymal enzyme than AST (27).

The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with hydroalcoholic extract of *A. precatorius* reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells. Possible mechanism that may be responsible for the protection of paracetamol induced the following hydroalcoholic extract by it self-act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Its ability is to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1, 1- di phenyl-2-picrylhydrazyl radical (DPPH). Thus, by trapping oxygen related free radicals, hydroalcoholic extract could hinder their interaction with polyester fatty acids and

would abolish the enhancement of lipids peroxidative processes (25, 28).

The active metabolites in the seed of *abrus precatorius* include abrin, abrus agglutinin, glycyrrhizin, gallic acid, trigonelline, precatorine and lipolytic enzymes. Glucine, Coumestrans, resin asparagines and sterols have also been demonstrated (29, 30). Gallic acid, glycyrrhizin and trigonelline are potent antioxidants (31). The hepatoprotective effects of *A. precatorius* noted in this study is probably as a result of the some of its constituents that have antioxidant properties, such as gallic acid, glycyrrhizin, trigonelline.

### **Conclusion**

The hydroalcoholic extract has shown the ability to maintain the normal functional statue of the liver. From the above preliminary study, we conclude that the hydroalcoholic extract of *Abrus precatorius*, is proved to be one of the herbal remedies for liver ailment. We therefore, suggest the isolation and possible characterization of the active constituent(s) from the extracts of this plant species as possible hepatoprotective agents.

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