

Effects of *Rhaphidophora pertusa* (Roxb.) Schott. Methanolic Extract on Acetaminophen-Induced Hepatic Injury in Albino Rats

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ABSTRACT

In the present investigation, we have evaluated the hepatoprotective activity of the methanolic extract of the stem of *Rhaphidophora pertusa* against acetaminophen-induced hepatotoxicity in albino rats. Liver marker enzymes such as serum glutamate oxalo transferase (SGOT), serum glutamate pyruvate transferase (SGPT), alkaline phosphatase (SALP), and total bilirubin (TB), urea and total protein were analyzed. Acetaminophen intoxication (500 mg/kg, p. o.) for 7 days caused significant increase in the levels of bilirubin, liver enzymes, and total protein level ($P < 0.05$). Administration of the methanolic extract (100 and 200 mg/kg) of *R. pertusa* stems resulted in a significant reversal of acetaminophen-induced alterations of all liver function parameters ($P < 0.05$) as evidenced by a decrease in enzyme activities, SGPT, SGOT, SALP and serum bilirubin. The results of the study authenticated the traditional medicinal claim of *R. pertusa*.

Keywords: bilirubin, hepatotoxicity, *Rhaphidophora pertusa*, SGOT, SGPT, SALP, total phenolics

Abbreviations: SALP, alkaline phosphatase; SGOT, serum glutamate oxalo transferase; SGPT, serum glutamate pyruvate transferase

INTRODUCTION

Liver diseases, especially viral hepatitis occurs predominantly in the developing countries (Simonsen *et al.* 1999) with an enormous impact on public health. In the absence of reliable liver protective drugs in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders (Chatterjee 2000). Despite studies for decades as well as advancement of our understanding of the molecular pathogenesis and novel lead drug molecules, the effective therapeutic interventions for liver diseases are still limited. Medicinal plants, especially those with traditional use, have always been considered as a rich repository of new effective drugs. Investigations have indicated that extracts of plant origin and their chemical constituents can protect the liver against the etiologies of chronic hepatic injury (Stickel and Schuppan 2007; Yan *et al.* 2009; Giriwono *et al.* 2010). Recently, several works have been conducted on hepatoprotective effects of medicinal plants (Rathi *et al.* 2009; Srivastava and Shivanandappa 2010; Verma and Khosa 2010).

Rhaphidophora pertusa (Roxb.) Schott., belonging to the Araceae family, is one of the plants used in ethnomedicine in India. Fresh juice of the stem of the plant is administered orally for treating ascites, and enlarged spleen and liver (Pushpangadan and Asha 1998). The stem is bitter, acrid, astringent, digestive and carminative, and good for ulcers, odontalgia and bronchitis (Warrier *et al.* 1995). Stem juice is administered to treat pain in colon and abdominal tumours (Prajapati *et al.* 2003). People of the Nilgiris, India use the stem to treat stomach complaints and tumours (Sasikumar and Doss 2006). The hepatoprotective activity of *R. pertusa* stem on carbon tetrachloride induced hepatitis in rats was evaluated (Hemamalini *et al.* 2000). Stem of the plant was investigated for luteolytic, oestrogenic and follicle-stimulating hormone-like activities (Santhosh *et al.* 2006). The aerial parts of *R. pertusa* were evaluated for anti-inflammatory, analgesic and anti-lipidperoxidative activities (Linnet *et al.* 2010).

The literature reveals that its hepatoprotective effects on acetaminophen-induced liver damage remains to be studied. The present investigation encompasses the effects of methanol extracts of stem *R. pertusa* of against acetaminophen-induced hepatic injury in albino rats *in vivo*.

MATERIALS AND METHODS

Chemicals

The experiments were performed using a scanning mini spectrophotometer (Elico, India). All chemicals used including the solvents were of analytical grade and were procured from SD Fine Chemicals. Acetaminophen and silymarin were purchased from E. Merck (India) Ltd, Mumbai. Gallic acid was purchased from Riedel-de-Hahn, Germany.

Plant material

Stems of *R. pertusa* collected from the Nilgiris, Tamil Nadu, India, was authenticated by the scientists of Botanical Survey of India, Southern Circle, Coimbatore. A voucher specimen was deposited in the department of Biotechnology, Karpagam University, Coimbatore, India. Prior to extraction, the stems were washed in distilled water, shade dried (for 7 days) and ground into powder.

Preparation of extracts

The powder of *R. pertusa* stems (100 g) was exhaustively extracted with methanol in the ratio of 1:7 (w/v) for 24 hrs by a Soxhlet apparatus. The resultant extract was completely evaporated to dryness using rotary flash evaporator (Buchi type) at 45°C. This is followed by standardization of the extract to 200 and 100 mg/kg body weight dosages.

Determination of total phenolics

The phenolic content was determined by UV spectrophotometer using the Folin-Ciocalteu (FC) method (Sadasivam and Manickam

Table 1 Effect of methanol stem extracts of *R. pertusa* (RP) on acetaminophen induced SGOT, SGPT and SAKP elevations in albino rats.

Groups and dosage	SGOT (IU/L)	SGPT (IU/L)	SAKP (IU/L)
Normal control (saline water)	52 ± 1.73	47.05 ± 6.44	160.33 ± 4.51
Acetaminophen (500 mg/kg)	207 ± 25.3*	119.00 ± 3.0*	368.00 ± 5.0*
Acetaminophen (500 mg/kg)+RP (100 mg/kg)	84 ± 2.0**	92.33 ± 5.5**	265.00 ± 5.0**
Acetaminophen (500 mg/kg)+RP (200 mg/kg)	70 ± 1.15**	67.30 ± 0.51**	181.33 ± 7.02**
Acetaminophen (500 mg/kg)+ silymarin (50 mg/kg)	89 ± 5.0**	60.30 ± 4.51**	170.33 ± 4.51**

Values are the mean ± S.D., n = 6 in each group

* P < 0.001 compared with the corresponding value for normal control group

** P < 0.001 compared with the corresponding value for acetaminophen control group

Table 2 Effect of methanol stem extracts of *R. pertusa* on serum urea, total bilirubin and protein in rats.

Groups and dosage	Total bilirubin (g/dl)	Urea (mg/dl)	Total protein (g/dl)
Normal control (saline water)	0.50 ± 0.02	40 ± 3.2	6.2 ± 0.2
Acetaminophen (500 mg/kg)	1.16 ± 0.06*	58 ± 2.4*	4.4 ± 0.3*
Acetaminophen (500 mg/kg)+RP (100 mg/kg)	0.72 ± 0.07**	47 ± 2.7**	5.3 ± 0.2**
Acetaminophen (500 mg/kg)+RP (200 mg/kg)	0.61 ± 0.09**	42 ± 2.6**	5.1 ± 0.02**
Acetaminophen (500 mg/kg)+ Silymarin (50 mg/kg)	0.60 ± 0.09**	41 ± 2.8**	5.8 ± 0.02**

Values are the mean ± S.D., n = 6 in each group.

* P < 0.001 compared with the corresponding value for normal control group

** P < 0.001 compared with the corresponding value for acetaminophen control group

1992). An aliquot of the sample extract (0.1 ml) was mixed with distilled water (3 ml) and 0.5 ml of FC reagent was added. After 3 min. 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for exactly 1 min. It was then cooled and the absorbance was measured at 650 nm using spectrophotometer (Elico Scanning mini spec SL 177, India) against the reagent blank. The results were mean (n=3) standard deviation (±) and expressed as gallic acid equivalent (mg GAE/g).

Animals and diet

Adult Wistar female albino rats (150-180 g) were procured from the Animal House of Karpagam University, Coimbatore. The animals were grouped and housed in polyacrylic cages (cm) with six animals per cage. They were maintained at a constant 25°C with a 12/12h dark/light cycle and were fed with commercial mouse diet (Gold Mohar rat feed, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* during the experiments.

Toxicity profile

The acute toxicity studies of methanolic stem extract of *R. pertusa* was determined in wistar albino rats according to OECD guidelines No. 425 (OECD 2001).

Acetaminophen-induced hepatotoxicity in rats

Animals were randomized and grouped into five groups (I–V) of six animals per group. Animals of group I served as control and received only saline water for seven days. Group II rats were administered with acetaminophen (500 mg/kg body weight) on the seventh day. Groups III and IV received a daily dose of plant extracts for 7 days (200 and 100 mg/kg body weight) and 2 ml of acetaminophen suspension (500 mg/kg body weight) 30 min after extract administration on the seventh day. Group V animals received reference drug (silymarin) at a dose of 100 mg/kg on all the 7 days and 2 ml of acetaminophen suspension (500 mg/kg body weight).

All the animals were anesthetized with chloroform and sacrificed by cervical capitation on the seventh day after 48 hrs of acetaminophen administration. The blood samples were collected into a sterilized centrifuge tube and allowed to coagulate at 37°C. The serum was separated at 2000 rpm at 30°C for 15 min and subjected to various biochemical investigations such as serum glutamate oxalo transferase (SGOT) (E.C. 2.6.1.1), serum glutamate pyruvate transferase (SGPT) (E.C. 2.6.1.2), alkaline phosphatase (SALP) (E.C. 3.1.3.1), total bilirubin, urea and total protein content assays. Hepatotoxicity was indicated by a significant elevation in the activity of the aforesaid liver marker enzymes in acetaminophen-intoxicated rats.

Assessment of liver functions

Activities of serum hepato-specific markers, serum glutamate oxalo transferase (SGOT), serum glutamate pyruvate transferase (SGPT) were estimated by adopting standard procedures (Reitman and Frankel 1957). The SGOT and SGPT activities were expressed as IU/L. Alkaline phosphatase (SALP) was assayed based on the method of King and Armstrong (1980). ALP activity was expressed as IU/L. Total bilirubin, urea and total protein content were determined by the methods of Malloy and Evelyn (1937), Varley *et al.* (1976) and Lowry *et al.* (1951), respectively.

Ethics

All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee.

Statistical analysis

The data shown in **Tables 1 and 2** were expressed as mean ± standard deviation (SD). Statistical differences between the values were analyzed by Student's *t*-test. A value of *P* < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Analysis of total phenolics

The total phenolic content of *R. pertusa* stem was estimated based on the FC method. The amount of phenolic concentration was equal to 860 ± 0.02 mg of GAE of dry material. There is a strong evidence on the preventive effects of phenolics on chronic diseases (Boyer and Liu 2004; Kroon and Williamson 2005).

Acute toxicity studies

There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose of 2000 mg/kg. This result suggests that the extract was safe or non toxic to rats and hence doses of 100 and 200 mg/kg, were selected for the study.

Acetaminophen induced hepatic damage

R. pertusa is known to have wide therapeutic applications in traditional medicine and few studies have authenticated its medicinal claims. The present study on anti-hepatotoxic potential of the plant has further provided the evidence to the claims. In the investigation, the effects of methanol extracts of *R. pertusa* stem on SGOT, SGPT, ALP, urea, bili-

rubin and protein contents on acetaminophen induced liver damage in albino rats were carried out and results are presented in **Table 1**. Administration of acetaminophen (500 mg/kg) after 18 hrs of intoxication resulted in a significant elevation of liver markers such as SGOT (119 ± 3.0 IU/L), SGPT (207 ± 25.38 IU/L) and ALP (368 ± 5.0 IU/L). In a concomitant treatment, plant extract at the concentrations of 200 mg/kg and 100 mg/kg body weight, the levels of marker enzymes were found retrieving towards normal level. Results illustrated in the **Table 2** revealed that the administration of acetaminophen for 7 days caused significant alterations in liver parameters (total bilirubin, urea and total protein) in rats when compared with the control group. An increase in the levels of bilirubin (1.16 ± 0.06 vs. 0.5 ± 0.02 g/dl) and urea (58 ± 2.4 vs. 40 ± 3.2 mg/dl) was observed. Furthermore, a significant decrease in serum total protein level was found in acetaminophen mediated hepatic injury (4.4 ± 0.3 vs. 6.2 ± 0.2). The contents of total bilirubin and urea were significantly reversed on treatment with the methanolic stem extracts in concentration dependent manner. The activity of the plant extract at the concentration of 200 mg/kg was comparable with that of silymarin.

Acetaminophen mediated hepatotoxicity was taken in this study as the experimental model for liver injury. Acetaminophen, a widely used analgesic and antipyretic drug, becomes a hepatotoxin when taken in larger doses (Mitchell *et al.* 1973). Animal hepatic damage by acetaminophen has been widely used to investigate hepatoprotective activities of drugs (Chen *et al.* 2009; Yuan *et al.* 2009; Bairwa *et al.* 2010). Hepatotoxicity induced by acetaminophen resembles various kinds of acute liver diseases with prominent elevations in SGOT and SGPT levels. In the present study, the rats treated with high doses of acetaminophen developed significant hepatic injury, which was observed through a substantial increase in the concentration of serum parameters like SGOT, SGPT, SALP and total bilirubin. These alterations in the liver enzymes will have impact on hepatic structural integrity. The increase in the SGOT level is usually accompanied by an elevation in the levels of SGPT, which plays an important role in the conversion of amino acids to keto acids (Sallie *et al.* 1999). Increase in serum level of SALP is due to increased synthesis in presence of increasing biliary pressure (Moss and Butterworth 1974). Furthermore, a significant reduction in total protein level was found in acetaminophen intoxicated rats, and this authenticates the reported translation inhibition effect of overdose of acetaminophen (Vermeulen *et al.* 1992). However, pre-treatment of the rats with *R. pertusa* extract at 200 and 100 mg/kg body weight for 7 days before acetaminophen administration resulted in a significant protection of acetaminophen induced elevation of liver marker enzymes, urea and in protein level. The crude stem extract of plant appears to be effective in reducing the injurious effect of acetaminophen, observed in the study. Our previous report showed that the methanol stem extracts of *R. pertusa* stem exhibit potent antioxidant activity (Sasikumar and Doss 2006). The crude methanol extract could mitigate the acetaminophen induced oxidative damage in liver since hepatotoxin induced intoxication and cell oxidative damage by reactive oxygen species (ROS) are well relating. The results of this study demonstrate that *R. pertusa* possessed potent antihepatotoxic effects upon acetaminophen induced hepatic injury in albino rats. Thus, the medicinal claims of the plant being used in the treatment of liver disorders may be in part due to the hepatoprotective activity.

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