Prevention of carbon tetrachloride induced hepatotoxicity in rats by *Adhatoda vasica* leaves

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Abstract

The methanolic extract of dried leaf in 1% gum tragacanth was administered orally in CCl_4 induced hepatic damage model. The hepatoprotective activity was assessed using various biochemical parameters like serum bilirubin, protein, alanine transaminase, aspartate transaminase and alkaline phosphatase. There was a significant increase in serum levels of bilirubin, marker enzymes with a decrease in total protein level, in the CCl_4 treated animals, reflecting liver injury. In the ethanol extract treated animals there was a decrease in serum levels of the markers and significant increase in total protein, indicating the recovery of hepatic cells. The ethanol leaf extract of *Adhatoda vasica* afforded significant protection against CCl_4 induced hepatocellular injury.

Key words: Hepatoprotective activity, *A.vasica*, CCl_4 induced hepatic damage

1. Introduction

Adhatoda vasica Nees is a shrub widespread throughout the tropical regions of Southeast Asia. The plant *Adhatoda vasica* Nees (AV) of the Acanthaceae family has been used for thousands of years in India. Extracts of the leaves of A.V. are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergy (Dhuley, 1999; Grange and Snell, 1996; Chakraborty and Brantner, 2001). Several active constituents have also been isolated from different parts of AV (Wagner, 1989). Though the plant is traditionally used in the treatment of jaundice in Bengal, more evidence is needed to substantiate its pharmacological effects. Thus the aim of this study was to determine hepatoprotective activities of ethanolic extract of the leaves of the plant to elaborate and evaluate their potential medicinal use.

2. Material and Method

Fresh leaves of *A. vasica* were air-dried, powdered and soaked in 80% ethanol for 72 h. The extract was then filtered, concentrated under vacuum and lyophilized to obtain a solid mass (6.2%). The chemical constituents of the ethanolic extract were investigated. The extract was subjected to preliminary phytochemical tests. Drug formulations Oral suspensions containing 45 mg/ml and 30 mg/ml of ethanol leaf extracts, were prepared in 1% w/v gum tragacanth.

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Animals Male Wistar albino rats weighing 150-200 g were procured from the IPS College of Pharmacy, Gwalior (M.P.) and maintained under standard housing conditions. The animals were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum. The study was permitted by the Institutional Animal Ethical Committee with Reg. No. 1039/ac/07/ CPCSEA. Acute toxicity studies acute toxicity study was conducted for the extracts by stair case method (Ghosh, 1984). One tenth of the LD_{50} doses were selected for the evaluation of hepatoprotective activity (Jalalpure et al., 2003).

The animals were divided into five groups of six rats each. Group I served as control and received the vehicle (1 ml/kg/ day of 1% w/v gum tragacanth p.o. for 14 days). Group II to IV received 0.1 ml/kg/ day of CCl_4 i. p. (E-Merck, Mumbai, India) for 14 days. Group III animals received the standard drug silymarin (Ranbaxy Lab, Dewas) in the dose of 100 mg/kg/day, p.o. for 14 days, while the ethanol leaf extracts of A. vasica were administered to groups IV in the dose of 45 mg and 30 mg/kg/day, p.o. respectively for 14days.

The CCl_4, silymarin and the extracts were administered concomitantly to the respective groups. All the animals were sacrificed on 14th day under light ether anesthesia. The blood sample from each animal was collected separately in sterilized dry centrifuge tubes by carotid bleeding and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and subjected to biochemical investigations viz., total bilirubin (Mallory and Evelyn, 1937) total protein (Kingsley, 1939) serum alanine transaminase, aspartate transaminase (Reitzman and Frankel, 1957) and alkaline phosphatase

(Bessey, 1964). Results of biochemical estimations are reported as mean ± SEM of six animals in each group.

3. Results and Discussion

The LD_{50} of ethanol leaf extracts were found to be 450 mg/kg, b.w. and 300 mg/kg, b.w, respectively. One tenth of these doses (45 mg/kg, b.w. and 30 mg/kg, b.w.) were selected for the evaluation of hepatoprotective activity. Effect of ethanol leaf extracts of A. vasica on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in Table 1. The CCl_4 treated control group showed a significant increase in serum total bilirubin (2.41±0.01), alanine transaminase (1414.00±1.99), aspartate transaminase (2214.47±32.79) and alkaline phosphatase (442.31±1.56) and a decrease in total protein (4.93 ± 0.01) indicating the liver injury caused by CCl_4 . Whereas animals treated with ethanol leaf extracts exhibited a decrease in total bilirubin (0.60±0.01), alanine transaminase (122.06±1.27), aspartate transaminase (219.66±2.59) and alkaline phosphatase (201.42±1.64) along with a significant increase in total protein (7.41±0.02).

Table 1. Effect of aqueous and ethanol stem bark extract of Pterocarpus santalinus on CCl_4 induced hepatotoxicity in rats

Group N	Total bilirubin	Total protein	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (1% w/v gum tragacanth p.o.)	0.47±0.01	8.44±0.02	151.52±1.43	52.08±1.16	172.99±1.80
CCl_4 (0.1ml/kg/day i.p.)	2.41±0.01*	4.93±0.01*	2214.47±32.79*	1414.00±1.99*	442.31±1.56*
CCl_4+silymarin (0.1ml/kg/day i.p. +100mg/kg/day p.o.)	0.53±0.01†	8.84±0.01†	206.50±2.17†	73.18±1.17†	181.40±1.16†
CCl_4 + ethanol extract (0.1ml/kg/day i.p. +30mg/kg/day p.o.)	0.60±0.01†@	7.41±0.02†@	219.66±2.59†@	122.06±1.27†@	201.42±1.64†@

Values are expressed as mean±SEM. n = 6 in each group. *P<0.01 when compared to control. †P<0.01 when compared to CCl_4. @P<0.01 when compared to silymarin.

Acknowledgement

The author was grateful to Mr. Raju from Laila Impex, Vijayawada, Andhra Pradesh, India, for providing us the gift sample of vasicine and vasicinone.

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