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Hepatoprotective effect of the Aqueous Extract and 5-Hydroxy, 7,8,2′ Trimethoxy Flavone of *Andrographis alata* Nees. in Carbon Tetrachloride Treated Rats

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ABSTRACT

This study was designed to evaluate the hepatoprotective activity of aqueous extract and isolated flavone (5-hydroxy, 7,8, 2'trimethoxy flavone) compound of *Andrographis alata* against CCl_4 induced hepatotoxicity. The hepatotoxicity was induced in albino rats CCl_4 (i.p.). Analysis of serum ALT, AST and alkaline phosphatase activities with the concentrations of albumin, total protein and bilirubin was carried out. The activities of all the marker enzymes reported a significant elevation in CCl_4 treated rats, which were significantly recovered towards an almost normal level in animals simultaneously administered with aqueous extract and flavone compound. This work suggests that aqueous extract and isolated flavone compound of *A. alata* exert significant therapeutic effect on CCl_4 induced hepatotoxicity in rats.
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Introduction

Medicinal plants form the backbone of traditional system of medicine in India. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al.*, 2008). The higher plant products have shown to be effective sources of chemotherapeutic effects without having any side effects (Neetu and Meenakshi, 2003). All aerobic organisms including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended (Duh, 1998).

Andrographis alata Nees. (Acanthaceae) is a perennial branched erect herb is distributed abundantly in Southeast Asian countries namely India, Sri Lanka, Pakistan and Indonesia, but it is cultivated extensively in China and Thailand, the East and West Indies and Mauritius for medicinal purposes (Anonymous, 2001). The genus *Andrographis* posses immense medicinal value. More than 20 diterpenoids and over 10 flavonoids have been reported from this species (Wenkui *et al.*, 2007).

Since it has been demonstrated that flavonoids have potent antioxidant activities by scavenging hydroxyl radicals, superoxide anions and lipid peroxyl radicals (Alan and Miller, 1996), it is reasonable to evaluate the antioxidant property of the aerial parts of this plant. Thus this present investigation was undertaken to evaluate the hepatoprotective activities of aqueous extract and flavone isolated compound of the leaves of *A. alata* against oxidative damage induced by CCl_4 in rats.

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Materials and Methods

Animals

Male Swiss Wistar Albino Rats weighing about 150 to 200 g on the average were selected for clinical studies and were obtained from National College of Pharmacy, Shimoga, Karnataka. The rats were fed with commercial diet (Pranav Agro Industries Ltd., Sangli) and tap water ad-libitum during the experiments. The Institutional Animal Ethical Committee (Reg. No.144/1999/CPCSEA/SMG) permitted this study. Acute toxicity studies were conducted according to 'staircase' method (Ghosh, 1984).

Chemicals

Chemicals used in this experiment are of analytical grade and purchased from various companies like Qualigens, Sisco research laboratories Hi-media and Ranboxy. The reagents used were prepared in all glass-distilled water.

Plant Material and Extraction

The leaves of *A. alata* were obtained from forests of Malebennur Range, Davanagere and Joldhal range, Chitradurga, Karnataka, India. The aerial parts of the plant were collected and shade dried for a week and powdered mechanically (Sieve no. 10/44). Powdered materials were extracted using a Soxhlet apparatus with ethyl acetate for about 48 h. The extract was filtered and concentrated in a vacuum under reduced pressure using a rotary flash evaporator (Buchi, Flawil, Switzerland). The melting point was determined using a melting point apparatus (Jindal, New Delhi). The characterization of the compound was done by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic studies.

100 g of leaf-powdered material of *A. alata* was extracted with 500 ml of distilled water. The aqueous extract was concentrated in a vacuum using a rotavapour and the concentrated extract was dried over the water bath to get an anhydrous powdered material. 500 mg of dried aqueous extract was taken in a conical flask and dissolved in 50 ml of distilled water (1 ml/10 mg). The mouth of the flask was sealed with aluminum foil and the contents are autoclaved at 120 °C for 15 min. 500 mg of flavone compound was taken in a conical flask into which 50 ml of distilled was added (1 ml/10 mg) and the contents are autoclaved at 120 °C for 15 min.

Experimental Design

The rats were randomly divided into four groups. The first group was administered with 0.2 ml/kg body weight of distilled water, intraperitonially (i.p.). This was done biweekly for 4 weeks and was used as control. Liver damage was induced in the groups of second, third and fourth groups by i.p. of CCl_4. Each animal of these groups was administered with 0.2 ml kg body weight of CCl_4. This was done biweekly for a period of four weeks in a similar way. From the third day onwards the third group was administered daily with 1 ml /kg body weight of aqueous extract. Similarly, the fourth group was administered with 1 ml kg body weight of 5-hydroxy, 7,8,2′ trimethoxy flavone. Depending upon the administration of plant part and the compound extract, each group of animals was fed separately.

After the experimental period of seven days, animals were sacrificed by light ether anesthesia and venous blood was collected into sample bottles containing no anticoagulant (Adebayo *et al.,* 2003). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 min (Ogbu and Okechukwu, 2001). The clear serum was removed by pipetting, which was used for the assay of biochemical parameters.

Biochemical Studies

The determination for the assay of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using the method described by Reitman and Frankel, 1957 Further activities and the determination of concentrations of total serum protein, albumin globulin ratio by Biuret method described by Kingsley, 1939, total bilirubin and estimation of serum alkaline phosphatase activity by Bessay et al., 1964. The determination for the assay of aspartate aminotransferase (AST) was done using the method described by Reitman and Frankel, 1957 and alanine aminotransferase (ALT) by Reitman and Frankel, 1957 activities and the determination of concentrations of total serum protein, albumin globulin ratio by Biuret method (Kingsley, 1939), total biliburin. Estimation of serum alkaline phosphatase activity (Bessay *et al.,* 1964).

Antihepatotoxic Studies

The liver samples were excised from the animals of each group washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 h. They are processed for paraffin embedding. The sections were taken at 5 μm thickness, processed in alcohol-xylene series and were stained with alum hematoxylin and eosin. The sections were microscopically examined for the study of histological changes.

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