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Hepatoprotective activity of *Adina cordifolia* against ethanol induce hepatotoxicity in rats

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ABSTRACT

The acetone (AEAC) and aqueous extracts (AQEAC) of *Adina cordifolia*, belonging to the family Rubiaceae, were studied for hepatoprotective activity against Wister rats with liver damage induced by ethanol. It was found that AEAC and AQEAC, at a dose of 500 mg/kg body weight exhibited hepatoprotective effect by lowering the Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphate and total bilirubin to a significant extent and also significantly increased the levels of total protein. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of ethanol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by ethanol and blood samples from the animals treated with AEAC and AQEAC showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells against ethanol induced hepatocellular injury. The effects of AEAC and AQEAC were comparable with standard drug silymarin.

Key Words: Silymarin, SGOT, SGPT, alkaline phosphate, total bilirubin, total protein.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits, and alcohol and prescribed & over-the-counter drug which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease

(Sharma *et al.*, 1991; Subranonium & Pushpangadan, 1999). Thus liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drug available for the treatment of liver disorders (Karan *et al.*, 1999; Chatterjee, 2000). Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model (Rubinstein, 1962; Suja *et al.*, 2002). The medicinal use of many plants (as hepatoprotectives) like *Andrographis paniculata*, *Azadirachta indica*, *Cassia fistula*, *Elephantopus scaber*, *Hibiscus rosasinensis*, *Phyllanthus debilis*, *Picrorrhiza kurroa* has been reported in the literature (Rajesh *et al.*, 2001; Anandan *et al.*, 1999).

Adina cordifolia, (Rubiaceae) (commonly known as Haridru) is found throughout central and south India to Srilanka. The 7-hydroxycoumarin-1 and 7- β -D-glucosylcoumarin-2 were isolated from the root bark

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of *Adina cordifolia*, having antiamoebic activity (Firdoos *et al.*, 2009). According to ayurveda, the bark of this plant is used in liver disorders (Yoganarasimhan 1996). *Adina cordifolia* was also reported for its antiulcer potential activity (Kasinadhuni *et al.*, 1999), antifertility activity (Sabir *et al.*, 1970), anti-inflammatory and anti-nociceptive activity (Jain *et al.*, 2006).

MATERIALS AND METHODS

Procurement and Authentication of the Plant

The leaves of *Adina cordifolia* were collected from local area of village Ingoriya, Ujjain, Madhya Pradesh and it was authenticated by Dr. S. K. Billore, at Vikram University, Ujjain, Madhya Pradesh. The voucher specimen (MIPS/N/012/2010) was deposited at Department of Pharmacognosy, Mahakal Institute of Pharmaceutical Studies, Ujjain, Madhya Pradesh for future reference.

Preparation of extracts of *Adina cordifolia*

The powdered leaves (500g) were extracted by using acetone by sequentially extracted using petroleum ether, chloroform, acetone and ethanol in Soxhlet apparatus. Whereas aqueous extract was obtained by cold maceration processes. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation (Farnsworth, 1966).

Animals

Wistar albino rats (150-200g) used in studies, was procured from Central Drug Research Institute, Lucknow, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavage tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines (CPCSEA, 2003) with regular inspections of rats. The laboratory conditions duly undertaken by registered veterinary practitioner.

Chemicals

All the chemicals and solvents were of analytical grade. Silymarin was obtained as gift sample from Micro Lbs, Goa, India. Standard kits for SGOT,

Table 1: Evaluation of hepatoprotective activity by ethanol induced hepatotoxicity in rats.

Group	Treatment
I	Received water (5ml/kg. p.o) for 21 days once daily, and served as normal control.
I & II	Received water (5 ml/kg. p.o) for 21 days once daily and 40% ethanol (v/v, 2.0ml/100g body wt, p.o.) for 21 days.
I, II & III	Received standard drug silymarin (25 mg/kg. p.o.) for 21 days once daily and 40% ethanol (v/v, 2.0ml/100g body wt, p.o.) for 21 days.
I, II, III & IV	Received AEAC (500 mg/kg) 21 days once daily and 40% ethanol (v/v, 2.0 ml/100 g body wt, p.o.) for 21 days.
V	Received AQEAC (500 mg/kg) 21 days once daily and 40% ethanol (v/v, 2.0ml/100g body wt, p.o.) for 21 days.

Rats were divided into 5 groups of 6 animals (n=6) in each (Kapoor *et al.*, 1994)

SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India.

Acute toxicity studies

Healthy Wistar albino rats of either sex weighing 100-150 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Cooperation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for period of 3 days (OECD, 1996). Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes.

Assessment of hepatoprotective activity

After 24 h of ethanol administration, on 22nd day, blood was obtained from animals by puncturing retro orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT & SGPT (Reitman *et al.*,

Table 2: Effect of AEAC and AQEAC on ethanol induced hepatotoxicity in rats.

Treatment/ Dose	SGPT(μ /min/l)	SGOT(μ /min/l)	ALP(μ /min/l)	Total Bilirubin mg/dl	Total Protein gm/dl
Normal	62.0 \pm 3.71	168.04 \pm 2.80	190.0 \pm 8.01	0.38 \pm 0.06	9.57 \pm 0.24
Induced(ethanol)	98.75 \pm 8.86*	258.42 \pm 4.24*	244.76 \pm 8.82*	6.42 \pm 8.66*	5.40 \pm 8.46*
Standard (silymarin 25mg/kg)	63.76 \pm 4.63**	176.28 \pm 8.47**	194.27 \pm 4.27**	0.45 \pm 2.82**	9.81 \pm 4.26**
AEAC (500mg/kg)	79.88 \pm 8.22**	196.28 \pm 4.24**	210.46 \pm 8.22**	0.68 \pm 4.62**	8.28 \pm 8.12**
AQEAC (500mg/kg)	68.28 \pm 6.76***	186.84 \pm 4.26***	198 \pm 8.44***	0.56.0 \pm 2.20***	6.18 \pm 7.48***

Values are mean \pm SEM, n= 6. (One way ANOVA Followed by Dunnet multiple Comparisons test)
Statistically significance of ** P<0.01, *** P<0.001, when compared with respective control

1957), ALP (Kind *et al.*, 1954), serum bilirubin (Amour *et al.*, 1965) and serum protein (Lowry *et al.*, 1951) After collection of blood samples, the animals were sacrificed under deep ether anesthesia.

Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats (Avadhoot *et al.*, 1991; Bhanwra *et al.*, 2000).

Histopathology studies

A portion of liver tissue of all the animal groups was excised and then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48h and then with bovine solution for 6 h and were then processed for paraffin embedding. By using a microtome, sections of 5 micron thickness were taken and stained with hematoxylin and eosin. These sections were examined under light microscope (Model No: 138, Linco Pvt Ltd, Ambalacantt, Punjab, India) using a magnification of 100X (Mankani *et al.*, 2005).

Statistical Significance (GraphPad Prism)

The results of the study were expressed as mean \pm SEM, n=6. ANOVA (Gennaro *et al.*, 1995) was used to analyze and compare the data, followed by Dunnet's (Dunnet *et al.*, 1964) test for multiple comparisons.

RESULTS

Acute toxicity study

There was no mortality found amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000

mg/kg. This finding suggests that the AEAC and AQEAC were safe or non-toxic to rats and hence doses of 500 mg/kg, p.o. were selected for the study.

Effect of AEAC and AQEAC on serum marker enzyme levels

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT etc, content of ethanol intoxicated animals. In contrast, pretreatment with AEAC and AQEAC (500 mg/kg, po) and silymarin (25 mg/kg, po) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes. The results were showed in table 2.

Effect of AEAC and AQEAC on biochemical parameters

In ethanol treated groups, there was a significant increase in total bilirubin and significant reduction in total protein content. Whereas, pretreatment with AEAC and AQEAC (500 mg/kg, po) caused significant reduction in total bilirubin and significant increase in total protein. The results were showed in table 2.

Effect of AEAC and AQEAC on liver weight

Ethanol intoxicated group of animals, weight of the liver was significantly increased, but it was normalized in AEAC and AQEAC (500 mg/kg, po) treated groups of animals. A significant reduction in liver supports this finding. The results were showed in table 3.

Histopathology

Histopathological studies of liver also provided a supportive evidence for biochemical analysis. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were

Table 3: Effect of AEAC and AQEAC on liver weight variation of ethanol induced hepatotoxicity in rats.

Treatment	Liver weight in gm/100g
Normal	6.84±0.06
Induced (Ethanol)	8.24±0.28*
silymarin 25mg/kg	7.06±0.48***
AEAC (500mg/kg)	8.10±2.48**
AQEAC (500mg/kg)	7.20±0.76***

Values are mean ±SEM, n= 6. (One way ANOVA followed by Dunnette multiple comparisons test)

Statistically significance of ** P<0.01, *** P<0.001, when compared with respective control

observed in ethanol treated (toxic) control group. Both the plant extracts has prevented these histological changes. The results were showed in figure 1a-e.

DISCUSSION

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study ethanol was used to induce hepatotoxicity, since it is clinically relevant. Ethanol produces a constellation of dose related deleterious effects in the liver (Leo *et al.*, 1982). The majority of ethanol is metabolized in the liver and individuals who abuse alcohol by routinely drinking 50-60 g (about 4 to 5 drinks) of ethanol per day are at risk for developing alcoholic liver disease (Zakhari *et al.*, 2007). In addition, both acute and chronic ethanol administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury (Zhou *et al.*, 2003; Thurman *et al.*, 1998; Tsukamoto *et al.*, 2001). Besides the development of fatty liver (steatosis), another early sign of excessive ethanol consumption is liver enlargement and protein accumulation, both of which are common findings in alcoholics and heavy drinkers (Baraona *et al.*, 1975; Baraona *et al.*, 1977).

Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury (Yue *et al.*, 2006). In the present study AEAC and AQEAC at a dose of 500 mg/kg, po caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue

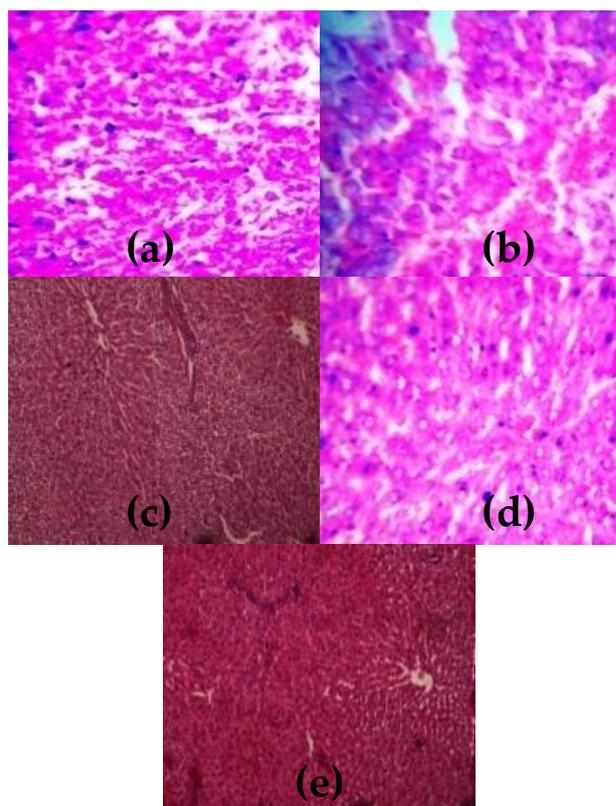


Figure 1: Histopathological Study of liver; (a) Normal control, (b) ethanol induce hepatotoxicity, (c) treated with AEAC, (d) treated with silymarin, (e) treated with AQEAC.

damage caused by ethanol. On the other hand suppression of elevated ALP activities with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants (Mukherjee *et al.*, 2002).

These results indicate that AEAC and AQEAC preserved the structural integrity of the hepatocellular membrane and liver cell architecture damaged by ethanol which was confirmed by histopathological examination.

Phytochemical screening revealed that AEAC and AQEAC contains active pharmacological constituents such as flavonoids, alkaloids, phytosterols and phenolic compounds. However, it has been already reported that such phytoconstituents like phenolic compounds, flavonoids, tannins (Paya *et al.*, 1993) are known to possess hepatoprotective activity in

various experimental models. Therefore it has been suggest that the hepatoprotective activity shown by the *AEAC and AQEAC* can be because of these active phytoconstituents present in the plant which is being also confirmed by the biochemical and histological parameters. The aqueous extract has shown more promising effect as compared to ethanol extracts of leaves of *Adina cordifolia*. The plant selected for the present study has demands for further phytochemical as well as pharmacological research such isolation of principle active phytoconstituents, evaluation of various pharmacological activities. Out of these aspects some respective parameters are already in process at our organization.

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