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Protective effects of *Sida veronicaefolia* against ethanol induced hepatotoxicity in experimental animals

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Abstract

The present study is to evaluate the hepatoprotective effects of the ethanol (*EESV*) and aqueous (*AESV*) extracts of leaves of *Sida veronicaefolia*, against ethanol induced hepatotoxicity in rats. The hepatoprotective effects of *EESV* (500 mg/kg) and *AESV* (500 mg/kg) were estimated by liver function test and serum profile. The results revealed that both the extracts of selected plant produce significant hepatoprotective effect not only by decreasing serum transaminase (SGPT & SGOT), alkaline phosphate and total bilirubin, but also significantly increased the levels of total protein. The effects of *EESV* and *AESV* were comparable with standard drug silymarin.

Keywords: hepatotoxicity; *Sida veronicaefolia; SGPT; SGOT*

Introduction

The liver is an organ of paramount importance, which plays an essential role in the metabolism of foreign compounds entering the body. Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment. In addition, human beings consume a lot of synthetic drugs during diseased conditions which are alien to body organs. All these compounds produce a variety of toxic manifestations (Athar et al., 1997). Conventional drugs used in the treatment of liver diseases are often inadequate. Therefore it is necessary to search an alternative drug for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety. India is well known for a plethora of medicinal plants. The medicinal use of many plants (as hepatoprotectants) 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).

Sida veronicaefolia, (Malvaceae) is a straggling way side herb found very often growing in shady places. It grows mainly in clearing in the forest and as weeds in the over grown grass of public parks and gardens (Lutterodt et al., 1988a). It is also known as Rajbala, Bhumibala, Farid buti, Shaktibala, etc. It has a capability to remove the three *doshas* (vata, pitta, kapha) from the body, and to provide strength and glow to the body (Dash et al., 1991). *Sida veronicaefolia* is very popular with rural womenfolk, especially in the areas where it grows in its natural habitat, and is used extensively in traditional medicine for shortening and reducing the pain of labour in childbirth. It is believed to render parturition almost painless and leads to shorter period of postpartum bleeding. Soup of this plant is taken in the last days of pregnancy (Lutterodt et al., 1988b). Lutterodt reported that alcoholic extract of *Sida veronicaefolia* has abortifacient effect in pregnant rats. An oral dose produces abortifacient effect when administered from day 15 to day 17 day of pregnancy (Lutterodt et al., 1988b). It is also reported that water soluble fraction from an alcoholic extract of *Sida veronicaefolia* has muscarine like active principle (Yua et al., 2008) similarly the leaves of the plant is also reported for its anticancer activity (Saluja et al., 2012). A detailed enthanobotanical survey revealed the presence of various pharmacologically active phytoconstituents in the plant such as alkaloids, flavonoids, triterpenoids, phenolic compounds, saponins, amino acids and protein (Saluja et al., 2012). As there is no such activity has been reported for the above mentioned plant extracts, the present study has been carried to evaluate the hepatoprotective activity of the various plant extract using.

Method And Materials

Plant Material

The leaves of *Sida veronicaefolia* were collected from the local area of village Ingoriya, Ujjain, (M.P.) and were it was authenticated by Dr. S. K. Billore, at Vikram University, Ujjain, (M.P.) The voucher specimen was deposited at the Department of Pharmacognosy, Mahakal Institute of Pharmaceutical Studies, Ujjain,(M.P.) for future reference.

Extraction

The powdered leaves (500g) were extracted by using ethanol 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 et al., 1966).

Animals

Wistar albino rats (150-200 g) used in the present 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. The animals were received the drug by oral gavages tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines (CPCS-

EA, 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. The standard drug silymarin was obtained as gift sample from Micro Lbs, Goa, India. Standard kits for SGOT, 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 Co-operation 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), respireatory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimati-on, perspiration, urinary incontinence and defecation) and central nervous system (drowsi-ness, gait, tremors and convulsion) changes.

Evaluation of hepatoprotective activity by ethanol induced hepatotoxicity in rats

The rats were divided into 5 groups of 6 animals (n=6) in each (Kapoor et al., 1994). Group I: Received water (5 ml/kg. p.o) for 21 days once daily, and served as normal control. Group II: Received water (5 ml/kg. p.o) for 21 days once daily and 40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.) for 21 days. Group III: Received standard drug silymarin (25 mg/kg. p.o.) for 21 days once daily and 40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.) for 21 days. Group IV: Received *EESV* (500 mg/kg) 21 days once daily and 40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.) for 21 days. Group V: Received *AESV* (500 mg/kg) 21 days once daily and 40% ethanol (v/v, 2.0 ml/l00 g body wt, p.o.) for 21 days.

Assessment of hepatoprotective activity

After 24 h of ethanol administration, on 22nd day, the blood was obtained from animals by puncturing retro orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. The 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., 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 the animals, weight of the 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 the 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 mm thickness were taken and stained with hematoxylin and eosin. These sections were examined under light microscope using a magnification of 100X (Mankani et al., 2005).

Statistical Significance

The results of the study were expressed as mean ± 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 *EESV and AESV* were safe or non-toxic to rats and hence doses of 500 mg/kg, p.o. were selected for the study.

Effect of EESV and AESV on serum marker enzyme levels

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP content of ethanol intoxicated animals. In contrast, pretreatment with *EESV* and *AESV* (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 no 1.

Effect of EESV and AESV on biochemical parameters

In ethanol treated group, there was a significant increase in total bilurubin and significant reduction in total protein content. Whereas, pretreatment with *EESV* and *AESV* (500mg

Table 1.Effect of *EESV* and *AESV* 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 ± 3.71	168.04 ± 2.80	190.0 ± 8.01	0.38 ±0.06	9.57 ± 0.24
Induced(ethanol)	98.75 ± 8.86*	258.42 ±4.24*	244.76 ± 8.82*	6.42 ± 8.66*	5.40 ± 8.46*
Standard (silymarin 25mg/kg)	63.76 ±4.63**	176.28 ± 8.47**	194.27 ± 4.27**	0.45 ± 2.82**	9.81 ± 4.26**
EESV (500mg/kg)	70.42± 6.84**	190.86 ±7.22**	202.46± 8.48**	0.58 ±4.86**	8.18±9.66**
AESV (500mg/kg)	65.82 ±5.24***	180.30± 4.24***	196.45±8.24***	0.48 ±2.20***	9.41±8.46***

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.

Table 2. Effect of *EESV* and *AESV* on liver weight variation of ethanol induced hepatotoxicity in rats

Treatment/ Dose	Total Protein gm/dl
Normal	9.57 ± 0.24
Induced(ethanol)	5.40 ± 8.46*
Standard (silymarin 25mg/kg)	9.81 ± 4.26**
EESV (500mg/kg)	8.18±9.66**
AESV (500mg/kg)	9.41±8.46***

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.

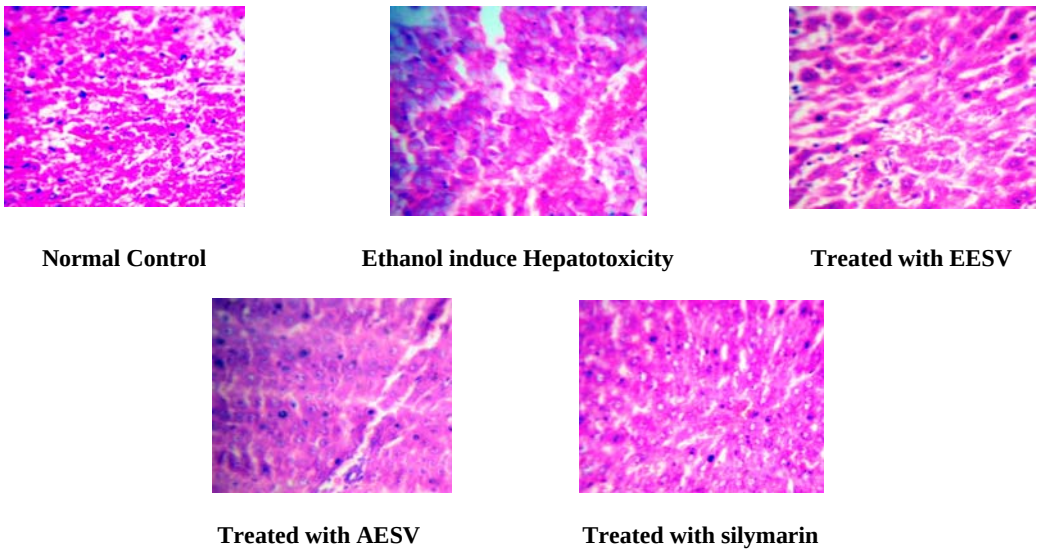

Figure 1. Histopatholocigal profile of liver.

/kg, po) caused significant reduction in total bilirubin and significant increase in total protein. The results were showed in table no.1.

Effect of EESV and AESV on liver weight

Ethanol intoxicated group of animals, the weight of the liver was significantly increased, but it was normalized in *EESV* and *AESV* (500 mg/kg, po) treated groups of animals. A significant reduction in liver supports this finding. The results were showed in table no. 2.

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 observed in ethanol treated (toxic) control group. Both the plant extracts has prevented these histological changes. The results were showed in figure no. 1

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 *EESV* and *AESV* 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 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 *EESV* and *AESV* preserved the structural integrity of the hepatocelluar membrane and liver cell architecture damaged by ethanol which was confirmed by histopathological examination. Phytochemical screening revealed that *EESV* and *AESV* contains active pharmacological constituents such as flavonoids, alkaloids, phytosterols and phenolic compounds (Saluja et al., 2011) 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 *EESV and AESV* 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 *Sida veronicaefolia.* 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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