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Hepatoprotective activity of *Leucas aspera* Spreng against simvastatin induced hepatotoxicity in rats

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Abstract

The present study is to evaluate for hepatoprotective activity of *Leucas aspera* Spreng leaves extract, against simvastatin induced hepatotoxicity for 30days. Leaves of *Leucas aspera* Spreng was successively extracted with ethanol against simvastatin (20mg/kg.p.o) induced hepatotoxicity, using standard drug silymarin (20 mg/kg). There was a marked elevation of biochemical parameters such as increases in serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP), serum bilirubin and decrease the total proteins content in simvastatin treated rats, which were restored towards normalization in *Leucas aspera* Spreng (200 mg/kg and 400 mg/kg) treated animals. The 400 mg/kg dose of *Leucas aspera* Spreng shows the best results than 200mg/kg, similar to silymarin (20 mg/kg). Histopathological observations confirmed the beneficial roles of *Leucas aspera* Spreng and silymarin against ethanol-induced liver injury in rats. Possible mechanism may involve their antioxidant activity.

Key words: *Leucas aspera* Spreng, Antioxidant activity, Ethanol, Simvastatin, Silymarin

Introduction

The liver is the main site of metabolism for drugs and other exogenous compounds. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward *et al.*,1999). The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. Inspite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang *et al.,* 1992). Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders (Ross *et al.*, 1996).

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Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Friedman *et al.*, 2006 and Ostapowicz *et al.*, 2006). Simvastatin hepatotoxicity is hypothesized to occur due to drug-drug interactions (Ricaurte *et al.*, 2006 and Kanathur *et al.*, 2001). Simvastatin (Lipid Lowering Agent) competitively inhibits HMG-CoA(3-hydroxy-3 methylglutaryl coenzyme A) in the formation of mevalonate. Mevalonate is also a precursor of Coenzyme Q10 (CoQ10). Thus, treatment with statins could also lower its levels. CoQ10 acts as an antioxidant, has membrane stabilising effects, and is important for cellular mitochondrial respiration, which is essential for energy production in organs (Frei *et al.*, 1990 and Stocker *et al.*, 1991).Thus, simvastatin causes oxidative stress, mediated hepatotoxicity by depleting antioxidant enzymes (Vaghasiya *et al.*, 2008).

Leucas aspera Spreng (Family: Lamiaceae) commonly known as 'Thumbai', is distributed throughout India from the Himalayas down to Ceylon. Roots are used in the treatment of pneumonia (Pandey *et al*., 2008), leaves in rheumatism and applied locally in snake bites (Kirtikar *et al.*, 2005). The plant is used traditionally as an antipyretic and insecticide (Abdul

Mannan *et al.*, 2010). Flowers used in nasal disorders (Abdul Mannan *et al.*, 2010). Leaves and stems used as oral antihypeglycemic (Parameshwar *et al.*, 2010). The main objective of this study was to assess the hepatoprotective effect of *Leucas aspera* in simvastatin induced hepatotoxicity.

Materials and Methods

Plant collection and identification

The basic plant material of *Leucas aspera* Spreng used in this investigation, was obtained from Sri Venkateshwara University, Tirupati, Andhra Pradesh. The plants were identified and authenticated by the taxonomists, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India.

Preparation of ethanolic extract

The leaves were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder. The coarsely powder leaves of *Leucas aspera* were used for extraction. The shade dry coarsely powder leaves of *Leucas aspera* were used for extraction with ethanol. *Leucas aspera* leaf powder (250 g) was loosely packed in the thimble of Soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 ml Phosphate Buffered Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% Tween 80 was used.

Phytochemical investigation

The ethanolic extracts of *Leucas aspera* were subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents by the following methods (Trease *et al.*, 2002; Kokate *et al.*, 1990 and Khandelwal, 2006).

Experimental animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of Nizam Institute of Pharmacy and Research Centre, Deshmukhi, Pochampally, Ramoji Film City, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional Animal Ethical Committee).

Acute toxicity study

Leucas aspera in the dose of 2000 mg/kg were administered orally to different group of mice comprising of ten mice in each group. Mortality was observed after 72 hours. Acute toxicity was determined according to the method of Litchfield and Wilcoxon (1949).

Experimental design for hepatoprotective activity (Vaghasiya *et al.*, 2009)

Animals are divided into 5 groups, each group comprising of 6 rats.

Group I : Control group

Group II : Simvastatin treated group (20 mg/kg, p.o)

Group III : Simvastatin (20 mg/kg, p.o) +*Leucas aspera* leaf extract (200mg/kg, p.o)

Group IV : Simvastatin (20 mg/kg, p.o) +*Leucas aspera* leaf extract (400mg/kg, p.o)

Group V : Simvastatin (20 mg/kg, p.o) +Silymarin (20mg/kg, p.o)

Group I: rats received a normal standard diet for 24 days; Group II: rats received SMT (20 mg/kg p.o for 30 days), Group III: rats received SMT along with *Leucas aspera* leaf extract (200mg/kg, p.o for 30 days); Group IV: rats received SMT along with *Leucas aspera* leaf extract (400mg/kg, p.o for 30 days); Group V: rats received SMT along with silymarin (20mg/kg/p.o.for 30 days). However, On the 31st day, all the animals were sacrified by mild ether anaesthesia.

Blood biochemistry

Blood samples were collected in glass tube from retro orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT (Reitman and Frankel, 1957), ALP (Walter and Schutt, 1974), Bilirubin (Malloy and Evelyn, 1937) and total protein (Lowry *et al.*, 1951) by a standard method and the samples were sent to AG Labs, Hyderabad for the analysis of various parameters.

Histopathology

Histopathology of liver was carried out by a modified Luna (Luna, 1999). In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days,followed with bovine solution for 6 h. Then the livers were paraffin embedded and 5 μ thickness microtone sections were made (Krajian, 1963). The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

Statistical analysis

The data obtained were analyzed by one-way-analysis of variance (ANOVA), followed by Tukey's multiple comparison test, using Graph pad prism software. p-value <0.05 was taken as the criterion of significance.

Results

Acute toxicity of *Leucas aspera* in the dose of 2000mg/kg were administered orally, shows no signs of toxicity up to 2000mg/kg. The phytochemical screening of *Leucas aspera* shows the presence of alkaloids, carbohydrates, steroids, tannins, flavonoids and glycosides. The effect of ethanol extract of *Leucas aspera* on SGOT, SGPT, alkaline phosphates, bilirubin and total protein level in simvastatin (20mg/kg,p.o) in toxicated rats are summarized in Table 1. There was a significant increase in bilirubin level, SGOT, SGPT and ALP, in simvastatin (20mg/kg,p.o) treated group (Group II), compared to the normal control group. The total protein levels were significantly decreased to 5.4 g/dl in simvastatin (20mg/kg,p.o) treated rats from the level of 7.1 g/dl in normal group. On the other hand, the group which received both *Leucas aspera* extract 200mg/kg and simvastatin (20mg/kg,p.o) (Group III), *Leucas aspera* extract 400mg/kg and simvastatin (20mg/kg,p.o) (Group IV) and simvastatin (20mg/kg,p.o) + silymarin (20mg/kg,p.o) (Group V) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 1)

Table 1: Effect of *Leucas aspera* Spreng on some serum chemical parameters of simvastatin intoxicated rats

GROUPS	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Bilurubin (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
Normal control	24± 1.50	50±1.21	120± 1.50	0.18± 0.42	7.6± 0.30	3.7± 0.23
Simvastatin	155±16.77	84± 10.5	210± 11.5	0.4±0.24	5.6± 0.37	2.8± 0.48
Simvastatin + E. 200mg	94± 2.66*	74±2.21*	180±1.21*	0.32± 0.33*	7.1±0.21*	3.0± 0.36
Simvastatin + E.400mg	68±2.25**	65±1.11**	135±2.21**	0.24± 0.27**	7.9± 0.37***	3.4± 0.52*
Simvastatin +Silymarin	36±2.12***	52±2.32***	130±4.21***	0.20± 0.12***	7.8± 0.28**	3.6± 0.71**

Values are Mean ± $SE_{\bar{X}}$ (n=6), followed by Tukey's multiple comparison test where, * represents significant at <0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001. All values are compared with toxicant.

Figure 1: Section of liver of control group

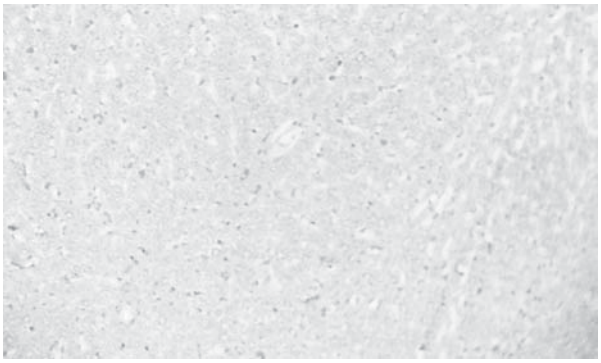


Figure 2: Section of the liver of simvastatin treated group

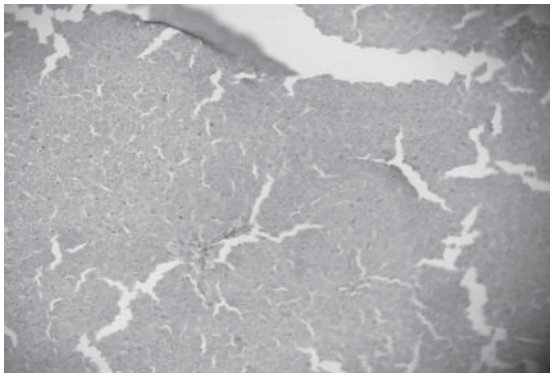


Figure 3: Section of liver of simvastatin and extract(200mg/kg) treated group

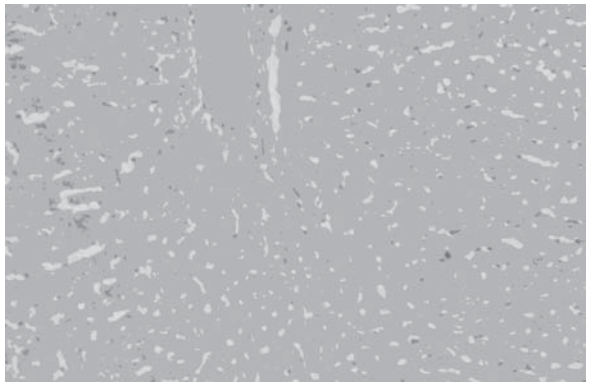


Figure 4: Section of liver of simvastatin and extract(400mg/kg) treated group

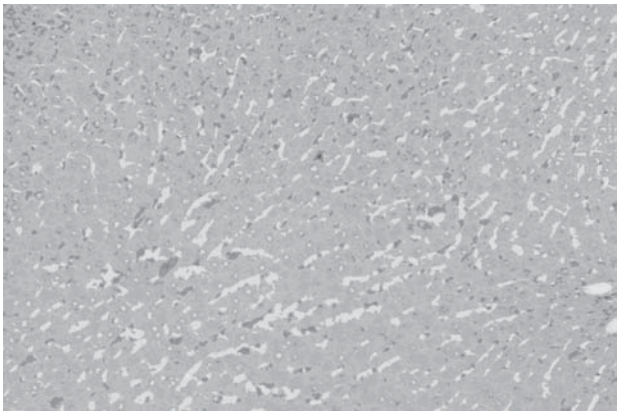
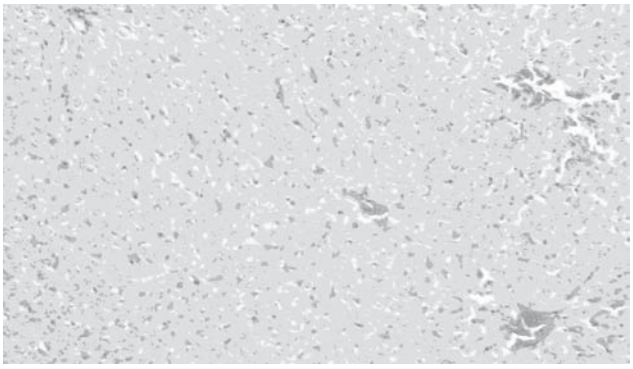


Figure 5: Section of liver of smt and silymarin group



Discussion

The protective effect of *Leucas aspera* Spreng indicates a reduction in enzymes, present in the extra cellular milieu of the liver cell. The medicinal herbs alone or in combination can influence a restoration of the cellular functions and structural integrity of the liver.

The liver can be injured by many chemicals and drugs. In the present study, simvastatin was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. Simvastatin produces a constellation of dose related deleterious effects in the liver (Leo and Arai, 1982). During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration(Deb, 1998). Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in simvastatin control group. All the histological changes observed were in correlation with the biochemical and functional parameters of the liver. These medicinal herbs alone or in combination can influence a restoration of the cellular functions and structural integrity of the liver.

In previous studies, reported that simvastatin caused oxidative stress mediated hepatotoxicity. *Leucas aspera* followed by simvastatin significantly reduced SGPT, ALP and serum bilirubin as compared with simvastatin-treated animals. The protection of liver cells against toxic materials including drugs, lipid peroxidation, and free radical injury may decrease inflammation. Phenols and tannins of *Leucas aspera*, present in high amounts, possess antioxidative which protects liver from free radical injury (Ai Lan Chewa *et al*., 2012). Furthermore, the antioxidative property of *Leucas aspera* crude extracts on free radical mediated DNA damage increases the hepatoprotective effect of *Leucas aspera*(Abdul Mannan *et al.*, 2010 and Parmaeshwar *et al*., 2010). In addition, the anti-inflammatory, (Kirtikar *et al.*, 2005) and antibacterial activity (Ai Lan Chewa *et al.*, 2012) of *Leucas aspera* shows protective effect on simvastatin induced hepatotoxicity.

Conclusion

In conclusion, the results of the present study indicate the hepatoprotective activity of *Leucas aspera* Spreng against simvastatin induced hepatotoxicity. Thus, on the basis of the phytochemical, physical, biochemical and histopathological concluded that the *Leucas aspera* posseses significant hepatoprotective activity. The study suggests a protective role of *Leucas aspera* in drug-induced hepatotoxicity and this effect may be due to its antioxidative effects.

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