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Antiulcer and hepatoprotective effects of *Semicarpus anacardium* Linn. seed extract

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Abstract

The antiulcer effect of *Semicarpus anacardium* Linn. seed extracts was investigated against aspirin plus pylorus ligation induced and ethanol induced gastric ulcers in rats. Both the models revealed ulcer healing property of the seed extracts. The present investigation also revealed the hepatoprotective activity of the seed extracts, against ethanol induced hepatotoxicity. Histopathological studies of the liver showed significant restoration of the normal histomorphological pattern of hepatocytes. The biochemical estimation of serum bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) showed significant reduction in the rats fed with the seed extracts. The study, thus, substantiates the potential anticulcer and hepatoprotective effects of *Semicarpus anacardium* seeds.

Key words: *Semicarpus anacardium*, Antiulcer, Hepatoprotective, Asprin, pylorus ligation

Introduction

Many plant products have been used for the cure of human diseases since antiquity. Even today, crude extracts of medicinal plants are being used successfully by folk physicians to cure human ailments in many underdeveloped countries. *Semicarpus anacardium* Linn. (Anacardiaceae) commonly known as Bhilava, a marking nut is a deciduous tree, distributed in Sub-Himalayan tract and tropical parts of India. It has a high priority and applicability in indigenous system of medicine against various ailments (Premalatha and Sachdanandan, 1999). The crude extracts of *Semicarpus anacardium* nuts have been reported to possess antitumour and antihelmintic activity (Sharma and Chaturvedi, 1965; Chattopadhyay and Khare, 1969). Literature survey reveals that considerable amount of bioflavonoids (Isharatulla *et al*., 1977), phenolic compounds (Rao *et al*., 1973), bhilwanols (Gedam *et al.*, 1974), sterols and glycosides (Indap *et al.*, 1983). It has been reported by several researchers that flavonoids are main responsible for antiulcer (Rajkapoor *et al*., 2002) and hepatoprotective activity (Banskota *et al*., 2001). Based upon the ethanobotanical and literature survey carried out, the seed extracts of *Semicarpus anacardium* was subjected to both antiulcerogenic and hepatoprotective effect using standard rodent models.

Materials and Methods

Plant material

The plant material, *Semicarpus anacardium* Linn. was collected from Konchavaram forest, Gulbarga district, Karnataka (India) in the flowering month of December 2010. The plant material was identified and authenticated by Professor Y. N. Seetaram, Taxonomist, Department of Botany, Gulbarga University, Gulbarga (Voucher no : HGUG -33).

Preparation of extract

The seeds of *Semicarpus anacardium* Linn. were shade dried, after removal of fruit shells. The seeds were crushed in an electric blender and milled into a fine particle meal (2 mm)

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which was sticky in consistency. It was then subjected to successive sequential soxhlet extraction with petroleum ether (40-60 ^{0}C), 95% ethanol and distilled water until the solvents became colourless. The solvents obtained were evaporated to dryness, using rotary flash evaporator and further stored in the refrigerator.

Preliminary phytochemical analysis

All the three extracts of *Semicarpus anacardium* Linn. seeds *i.e*., *Semicarpus anacardium* petroleum ether extract (SAPE), *Semicarpus anacardium* ethanolic extract (SAEE) and *Semicarpus anacardium* aqueous extract (SAAE) were subjected for preliminary phytochemical analysis.

Animals

Albino-Wistar rats of either sex (150-250 g) procured from Mahaveer Enterprises, Hyderabad, (India) were used for the studies. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethics Committee (IAEC Reg. No- 346/CPCSEA). All the animals were housed in polypropylene cages lined with husk, renewed every 24 h under 12/12 h light/dark cycles at 22 ± 2 ^{0}C and at 45%–55% relative humidity. The animals were fed with a standard pellet diet supplied by Lipton India Ltd. and allowed to free access of tap water *ad libitum.* After randomization into various groups, the animals were acclimatized for a period of 7 days. Animals described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water before the experiment was carried out.

Acute toxicity

Healthy adult albino rats were subjected for oral acute toxicity. The animals were overnight fasted and divided into three groups (n = 6) and were orally fed with SAPE, SAEE and SAAE (Ghosh, 1985). The animals were observed continuously for 2h for behavioral, neurological and autonomic profiles and after 24 and 72 h for any lethality (Turner, 1965).

Experimental procedures

Aspirin plus pylorus ligation induced ulcer

The method of Shay *et al*. (1945) was adopted with little modifications. Animals were divided into five groups (n=6). Group 1 received 2 % (w/v) gum acacia (vehicle) and aspirin (200 mg/kg) *p.o* which served as a control. Group II received ranitidine (20 mg/kg) and after 01 h, aspirin (200 mg/kg) *p.o* was administered as positive control. Group III, IV and V were administered with SAAE, SAEE and SAPE (250 mg/kg), respectively. After 01 h of ranitidine administered, aspirin is being given *p.o* for five consecutive days. On fifth day, animals of all the five groups were fasted for 18 h. The animals were anaesthetised with ether prior to pylorus ligation. The animals were sacrificed 4 h later by carbon dioxide anesthesia and the stomach was removed. The gastric content was collected and centrifuged. The volume of gastric juice, free acidity, total acidity, and pH was determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers were counted using a magnifying glass. Mean ulcer score for each animal was expressed as ulcer index. The ulcers were graded using the following scoring system: 0.0: Normal mucosa; 0.5: Red coloration; 1.0: Spot ulcer; 1.5: Hemorrhagic streaks; 2.0: Ulcer e≥3 mm but d 5 mm and 3.0: Ulcer e 5 mm

Ethanol induced ulcer damage in rats

The method of Mizui *et al*. (1987) was adopted in this experimental study. The animals were divided into five groups (n=6). Group I received 2 % (w/v) gum acacia (vehicle) and served as negative control. Group II received omiprazol (20 mg/kg) orally and served as positive control. Group III, IV and V received SAAE, SAAE and SAPE (250 mg/kg), respectively. Day 1, at 8 am, all animals were deprived of food but not water. At 3 pm, the rats were orally fed with SAAE, SAAE and SAPE, respectively. Exactly after one hour, all the animals were orally administered with 0.4 ml/kg of 99.9 % ethanol in all groups. Same procedure was employed on day 2 also. On day 3, 8 am all the animals were sacrificed and the stomach was incised, and then observed for ulcers as mentioned above.

Ethanol induced hepatotoxicity

The method of Gujrathi *et al*. (2007) and Eger (1954) was adopted with some modifications. The animals which were sacrificed in ethanol induced ulcers were used for this study. The liver was removed and subjected for histopathological studies.

Histopathological examination of liver

The liver removed was carefully stored in 10 % formalin. The parietal sides of the liver (left, medium and right lobe and lobus caudotus) were sectioned and stained with hematoxylin and eosin and checked using a stereomicroscope with 25 times magnification. Focal necrosis and peripheral hemorrhage were examined.

Collection of blood serum

Blood samples of the animals were collected from retro-orbitally plexus from the inner canthus of the eye under light ether anesthesia using capillary tubes before the animals were sacrificed. The plasma was separated in a T8 electric centrifuge at 2000 rpm for 2 minutes assessment and then analysed for serum bilirubin and serum markers such as alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) using the standard procedures prescribed in the enzymatic kits (ERBA chemi, Germany).

Data analysis

The results were expressed as Mean ± SEM. Statistical differences between the means were calculated using one-way analysis of variance (ANOVA) by Dunnet's test using Insat (Graphad software Inc., USA) considering $p<0.05$ statistically significant.

Results and Discussion

The antiulcerogenic effect of *Semicarpus anacardium* Linn. by aspirin plus pylorus ligation induced gastric ulcer is shown in table 1. The mean ulcer index of 5.667 ± 0.1667 was observed in the control group which was reduced to 1.250 ± 0.309 in the standard, indicating significant (77.94 %) reduction in ulcer at $p < 0.001$. Ulcer indices with SAAE, SAEE and SAPE were 1.833 ± 0.380, 1.417 ± 0.238, 2.000 ± 0.223, indicating reductions of 67.65 %, 74.99 % and 64.70 %, respectively. Table 1 also represents the effects of *Semicarpus anacardium* on biochemical parameters. All the three extracts neither reduced the volume of gastric juice, free acidity and total acidity nor did they increase pH of the gastric fluid. This clearly indicates that the antiulcer activity may be due to the mucosal damage but the components present in the seed extracts are not antisecretory.

Since decades many indigenous drugs have been shown to possess antiulcer activity. Although in most of the cases, the etiology of ulcer is unknown, it is generally accepted that it results from an imbalance of mucosal integrity through the endogenous defence mechanism (Piper and Steil, 1986). There are many reports on antiulcerogenic effects of different plant extracts on ulcers induced by pylorus ligation and aspirin. Rao *et al*. (2002) have reported that pylorus ligation induced ulcers are due to autodigestion of gastric mucosa and breakdown of gastric mucosal barrier. A few reports have implicated focal mucosal ischemia as a major event in the development of aspirin induced acute erosive gastritis (Rao *et al.*, 2002; O'Brien and Silin, 1986). The antiulcer effect observed with aspirin plus pylorus ligation model in our study may be due to the mucosal damage.

Table 2 represents effect of *Semicarpus anacardium* Linn. on ethanol induced gastric ulcer in rats. Administration of ethanol in control group produced maximum ulcer, the ulcer index being 4.000 ± 0.632, the group fed with standard drug omiprazole indicated the ulcer index being reduced to 1.167 ± 0.307 showing the reduction of 70.82 % at $p < 0.5$. No protection from ulcer was observed in animals which were fed with SAAE. However, significant reduction of 70.82 % and 87.50% were observed in animals fed with SAPE and SAEE, respectively.

Intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals (Ashley and Cheung, 1995; Robert *et al*., 1979). The ulcers observed with this model are caused either by a direct effect of ethanol on the gastric epithelium or are modulated indirectly by the release of vasoactive products from the mast cells resulting in the release of mediators such as histamine (Szabo, 1987). It has been found that ethanol induced ulcers are inhibited by agents that enhance mucosal defence factors such as prostaglandins. Prostaglandins are inhibited by selective action of cyclo-oxygenase (COX). Selvam and Jachak (2004) have reported COX inhibitory biflavonoid from the seeds of *Semicarpus anacardium.* So we can say that antiulcer effect observed is due to inhibition of prostaglandins (Premalatha and Sachdanandan, 1999). It is well documented fact that most of the medicinal plants are enriched with flavonoids. Flavonoids are a major class of phenolic compounds, which are known to possess antioxidant actions and are effective in healing experimentally induced gastric ulcers. Hence, the antiulcer effect observed in this study can be attributed to the presence of flavonoids.

The present study has also investigated hepatoprotective effect. To verify this effect, the extent of liver damage was examined histopathologically and biochemically. Severe central lobular necrosis around central veins and peripheral hemorrhage was observed in the liver of ethanol treated rats. These histological changes were reversed by treatment with seed extracts. This finding led us to investigate serum bilirubin, SGPT, SGOT and ALP (Table 3). In ethanol treated groups, decrease in all the three parameters was observed, but the results were more significant with the parameters SGPT and SGOT, when compared with ALP.

Many chemicals and drugs injure the liver. Ethanol produces constellation of dose-related deleterious effects in liver (Leo and Arai, 1982). Hepatoprotective activity of several herbal extracts using different models has been reported by several researchers (Singanan *et al.*, 2007; Faremi *et al.*, 2008). It seems the extract could protect the liver against ethanol induced oxidative damage by possibly reducing the rate of lipid peroxidation and increasing the antioxidant defence mechanism in rats (Faremi *et al*., 2008; Gupta and Misra, 2006). Gupta and Misra (2006) have also reported that hepatoprotective action combined with antioxidant activity has synergistic effect to prevent hepatic damage. We have already mentioned that flavonoids are responsible for antioxidant nature of the seeds in its antiulcer effect. Similarly hepatoprotective activity also seems to be due to flavonoids present (Oh *et al*., 2004). The hepatoprotective activity observed in the present case may be due to polyphenolic compounds, especially the flavonoids present in the seed extracts.

Conclusion

The present study can be concluded that the *Semicarpus anacardium* Linn. seed extracts not only provides an excellent preventive effect in gastric ulcer models, but also possesses significant hepatoprotective effect. This may be due to the antioxidant nature of flavonoids present in them. Further studies on antioxidant parameters are in progress.

Table 1. Effect of Semicarpus anacardium Linn. seed extract on aspirin plus Pylorus ligation induced gastric ulcers in rats

Group	Treatment	Vol. of Gastric Juice (ml)	Free Acidity (m/Eg/1) 100 g	Total Acidity (m/Eg/1) 100 g	Ulcer Index Mean ± SEM	% Protection Ulcer	pH
I	Control	3.147 ± 0.538	2.667 ± 0.421	4.250 ± 0.559	5.667 ± 0.667	--------	5.68 ± 0.668
II	Ranitidine (20 mg/kg)	1.433 ± 0.519	2.167 ± 0.542	4.667 ± 0.760	1.250 ± 0.309***	78.00	5.46 ± 0.959
III	SAAE (250 mg/kg)	4.383 ± 1.039	4.000 ± 0.365	6.500 ± 0.763	1.833 ± 0.380***	67.65	3.66 ± 0.194
IV	SAAE (250 mg/kg)	4.583 ± 1.121	3.500 ± 0.500	5.833 ± 0.600	1.417 ± 0.238***	74.99	4.00 ± 0.255
V	SAPE (250 mg/kg)	4.583 ± 1.121	3.500 ± 0.500	5.833 ± 0.600	1.417 ± 0.238***	74.99	4.00 ± 0.255

Key: Values are expressed as Mean ±SEM* at p<0.05, ** at p<0.01, *** at P<0.001, ns-indicates non-significant. SAPE= *Semicarpus anacardium* Linn. petroleum ether extract, SAEE= *Semicarpus anacardium* Linn. ethanolic extract and SAAE= *Semicarpus anacardium* Linn. aqueous extract.

Table 2. Effect of *Semicarpus anacardium* Linn. seed extract on ethanol induced gastric ulcer in rats

Group	Treatment	Ulcer Index (Mean ± SEM)	% Protection from Ulcer
I	Control	4.000 ± 0.632	----------
II	Omiprazol (20 mg/kg)	1.167 ± 0.307*	70.82
III	SAAE (250 mg/kg)	4.33 ± 0.881 ns	-8.33
IV	SAEE (250 mg/kg)	0.500 ± 0.223**	87.50
V	SAPE (250 mg/kg)	1.16 ± 0.477*	70.82

Key: Values are expressed as Mean ±SEM* at p<0.05, ** at p<0.01, *** at p<0.001, ns-indicates non-significant. SAPE= *Semicarpus anacardium* Linn. petroleum ether extract, SAEE= *Semicarpus anacardium* Linn. ethanolic extract and SAAE= *Semicarpus anacardium* Linn. aqueous extract.

Table 3. Effect of *Semicarpus anacardium* Linn. seed extract on ethanol induced hepatotoxicity in rats

Group	Treatment	Total bilirubin (mg/dl)	SGPT (Units/ml)	SGOT Units/ml	ALP Units/ml
I	Control	1.933 ± 0.0714	27.86 ± 0.28	37.92 ± 0.205	0.0146 ± 0.0013
II	SAPE (250 mg/kg)	1.616 ± 0.0792**	25.23 ± 0.35**	35.57 ± 0.140**	0.0226 ± 0.0014ns
III	SAEE (250 mg/kg)	1.633 ± 0.0557**	25.65 ± 0.46**	35.69 ± 0.514**	0.0133 ± .0012ns
IV	SAAE (250 mg/kg)	1.700 ± 0.577**	22.40 ± 0.26**	32.40 ± 0.270**	0.0075 ± 0.0015**

Key: Values are expressed as Mean ±SEM* at p<0.05, ** at p<0.01, *** at p<0.001, ns- non-significant. SAPE= *Semicarpus anacardium* Linn petroleum ether extract, SAEE= *Semicarpus anacardium* Linn. ethanolic extract SAAE= *Semicarpus anacardium* Linn aqueous extract. ALP = Alkaline phosphatase, SGOT = Serum glutamate oxaloacetate transaminase and SGPT= Serum glutamate pyruvate transaminase.

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