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Anti ulcer activity of ethanol extract of the stem bark of *Careya arborea* Roxb.

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ABSTRACT

The aim of the study was to determine anti ulcer activity of stem bark of *Careya arborea* Roxb. on the Wistar strain albino rats. Dried stem bark of *Careya arborea* Roxb. was powdered and this coarse powder was extracted with 70% ethanol by soxhlet extraction method to yield a Ethanol extract of stem bark of *Careya arborea* Roxb. (EECA). The extract was subjected for preliminary phytochemical analysis and was evaluated for anti ulcer activity against various models such as Ethanol induced, cold restraint stress induced and Pylorus ligation induced models. In acute toxicity study, EECA was found to be safe till 3000mg/kg. So the doses of EECA at various concentration of 300 and 600mg/kg body weight was administered orally, twice daily for 5 days for prevention of ulcer from Pylorus ligation, Ethanol and cold restraint stress - induced ulcers. Analytical parameters like Percentage of Ulcer protection was calculated based on Ulcer index and Gastric juice volume, pH and acidity of gastric juice. Preliminary phytochemical analysis of EECA showed the presence of carbohydrates, glycosides, phytosterols, phenolic compounds, tannins and saponins. The EECA has shown significant activity at both 300mg/kg and 600mg/kg dose level in a dose dependent manner. Phytoconstituents like tannins and saponins may be responsible for anti ulcer activity of EECA.

Key Words: Phytochemical analysis, ulcer index, ulcer protection, gastric juice, tannins, saponins.

INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice (Akhtar *et al.*, 1992). Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors (Tripathi, 1999). Although a number of antiulcer drugs such as H₂ receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration all these drugs have various undesirable effects such as arrhythmias, impotence and hematopoietic changes and limitations (Ariyoshi *et al.*, 1986; Del *et al.*, 1985). There are many agents in alternative medicine, which have shown promising antiulcer activity without producing above mentioned adverse

reaction. The antiulcerogenic activity of many plant products is reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors (Satoskar *et al.*, 1999; Walker *et al.*, 2003; Tipins *et al.*, 2003).

Careya arborea Roxb. belonging to the family Lecythidaceae is distributed throughout India, Sri Lanka and Malaya peninsula (Gamble, 2005). It is known as "Padmaka" in Ayurveda (Anonymous, 1978). *Careya arborea* Roxb. has ethnopharmacological uses in curing tumour, cough, bronchitis, wounds, dysentery, epilepsy, ulcer and eruptive fever (Kirtikar *et al.*, 1999). It has been reported to possess hepato protective activity, antimicrobial and antioxidant activity, antileishmanial activity (Mandal *et al.*, 2006), anti tumour activity (Natesan *et al.*, 2007), anti-inflammatory and analgesic activity (Kumar *et al.*, 2005) and CNS stimulant activity (Kumar *et al.*, 2008). As review of literature finds no report on anti ulcer activity of the stem bark of *Careya arborea* Roxb, present studies were carried out to prove the folk claim of its anti ulcer activity.

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MATERIALS AND METHODS

Plant material identification

The plant material was collected from vicinity of Tirunelveli, Tamil Nadu state. The plant material collected was identified at the herbarium of M.S.Ramaiah College of Pharmacy, Bangalore and authenticated by Dr. S.N. Yoganarasimhan. The specimen was given a voucher's collection number 27 in the field. A sample of the crude drug material was also preserved in the crude drug museum of the college.

Ethanol extraction

A weighed quantity 100g of the air-dried powdered drug was taken and extracted with Ethanol (70%) in a soxhlet extractor for 72h. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C and was evaporated to dryness. The EECA was subjected for preliminary phytochemical analysis.

Animal

Swiss albino mice weighing 25-30gm and Wister albino rats weighing 150-250 gm of either sex were used in the study. All the pharmacological work was carried out after obtaining the approval from the Institutional Animal Ethics Committee (Ref. No.: MSRCP/M-20/2007) of M. S. Ramaiah College of pharmacy. All animal experiments strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12:12 light:dark cycles. They were acclimatized for seven days. Food was provided in the form of dry pellets and water *ad libitum*.

Acute toxicity studies

Acute toxicity studies were carried out to study acute toxic effects of the drug and to determine minimum lethal dose of the drug extracts. The EECA were administered orally to separate groups of overnight fasted mice at doses of 30, 100, 300, 1000 and 3000 mg/kg. After administration of the extracts, the animals were observed continuously for the first three hours, for any toxic manifestation like increased motor activity, salivation, acute convulsion, coma and death. Thereafter, observations were made at regular intervals for 24 h.

Further, the animals were under observation up to a period of 1 week (Kulkarni *et al.*, 2005)

Ethanol induced gastric ulcer model

Wister strain albino rats were divided into four groups; each group consists of 6 animals. All animals received treatment for 5 days. Group I: Control (2% v/v Tween 80, 5 ml/kg), p.o; Group II: Ranitidine 30 mg/kg body weight, p.o; Group III: EECA of 300 mg/kg body weight, p.o; and Group IV: EECA of 600 mg/kg body weight, p.o. were used. Gastric ulcers were induced in rats by administration of 1ml 90% v/v ethanol p.o. Animals were fasted for 24 h with free access to water prior to the test. EECA, control (2% Tween 80) and the standard drug (ranitidine) were given orally 30 minutes before administration of ethanol (90% v/v, 1 ml/200g) and sacrificed after 15 minutes. The stomach was dissected out, gastric juice was collected and its volume was measured. The stomach was opened along the greater curvature, rinsed under a stream of water and pinned flat on a corkboard. Erosions formed on the glandular portion of stomach were counted and each was given a severity rating on 1-3 scale, based on diameter of the ulcer. The overall total diameter of ulcers in one stomach divided by factor 10 was designated as ulcer index (UI) (Gosh, 2005; Hollard *et al.*, 1985).

Cold Restrain stress induced gastric ulcer model

Wister strain albino rats were divided into four groups, each group consists of 6 animals. All animals received treatment for 5 days. Group I: Control (2% v/v Tween 80, 5 ml/kg), p.o; Group II: Ranitidine 30 mg/kg body weight, p.o; Group III: EECA 300 mg/kg body weight, p.o; and Group IV: EECA 600 mg/kg body weight, p.o. were made. Animals were deprived of food for 12 h with free access to water prior to the tests. The EECA, control (2% Tween 80) and standard drug ranitidine were administered 30 mins prior to immobilizing the animals. For stress induced ulcers, 30 mins after administration of drugs, each animal was individually immobilized in a stress cage and kept for 3 h in a refrigerator, maintained at 4-6°C. The animals were sacrificed by a blow on the head, stomach isolated and gastric juice was collected, volume, pH and total acidity of gastric juice were determined. The stomach was opened along the greater curvature, rinsed under a stream of water and pinned flat

on a corkboard. Erosions formed on the glandular portion of the stomach were counted and each given a severity rating on 1-3 scale based on diameter of ulcers. The overall total diameter of ulcers in one stomach divided by factor 10 was designated as ulcer index (UI) (Khare *et al.*, 2008).

Pyloric ligation model

The ulcer protective effect of EECA was studied as per the method of (Shay *et al.*, 1945). In pyloric ligation induced ulcers, ulcers are caused by accumulation of acidic gastric juice in stomach. Wister strain albino rats were divided into four groups; each group consists of 6 animals. All animals received treatment for 5 days. Group I: Control (2% v/v Tween 80, 5 ml/kg), p.o; Group II: Ranitidine 30 mg/kg body weight, p.o; Group III: EECA 300 mg/kg body weight, p.o; and Group IV: EECA 600 mg/kg body weight, p.o. were formed. Albino rats were fasted for 24 h with free access to water prior to the tests. Under light ether anesthesia, the abdomen was opened by a small midline incision below the xiphoid process; the pyloric portion of the stomach was slightly lifted out and ligated, avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by interrupted sutures. Control vehicle, EECA and standard drug (ranitidine 30 mg/kg) were administered orally immediately after pyloric ligation. After 4h of pyloric ligation, the animals were sacrificed with excess of anesthetic ether and stomach was dissected out; the gastric contents were drained into graduated tubes and its volume, pH, total acidity were determined. Glandular portion of stomach was cut open along the greater curvature and inner surface examined for ulceration. Stomach was rinsed under a stream of water and pinned flat on a corkboard. As in Table 1 Erosions formed on the glandular portion of stomach were counted and each given a severity rating on a 1-3 scale based on diameter of ulcers. The overall total diameter of ulcers in one stomach divided by a factor 10 was designated as ulcer index (UI).

The percentage ulcer protection was calculated using the formula –

$$\text{Percentage of ulcer protection} = 1 - \left[\frac{U_t}{U_c} \right] \times 100$$

where U_t = Ulcer index of treated group and U_c = Ulcer index of the control group.

Table 1: Rating scale for ulcer diameter.

Sl. No.	Ulcer diameter	Rating scale
1	≤ 1 mm	1
2	> 1 mm ≤ 2 mm	2
3	> 2 mm	3

The gastric juice was titrated against 0.01N NaOH using Topfer's reagent as indicator to find out the free acidity and total acidity (Ganguly *et al.*, 1973).

RESULTS

Phytochemical analysis

On phytochemical analysis of EECA, the extract has shown the presence of Carbohydrates and glycosides, Phytosterols, Phenolic compounds and tannins, saponins.

Acute toxicity studies

Acute toxicity studies were carried out on mice for EECA at doses of 30, 100, 300, 1000 and 3000 mg/kg body weight. Each dose was administered to separate groups of mice. No toxic symptoms or death was observed in any of the animals up to the dose of 3000 mg/kg body weight with either extracts, up to one week.

Ethanol induced gastric ulcer model

Table 2 indicates the significant antiulcer activity of EECA at the both doses. Ulcer index reduced at both doses, but the dose 600 mg/kg is significant one. Percentage of ulcer protection of 600 mg/kg is more when compared to 300 mg/kg and the volume of the gastric juice in ml is also reduced in EECA (600 mg/ml). Hence, it can be said that both extracts have anti ulcer activity, but EECA at 600 mg/kg is more potent.

Table 2: Effect of EECA on ethanol induced ulcers.

Sl. No.	Treatment	Ulcer index (mean ± SEM)	% Ulcer protection	Vol. of gastric juice (ml)
1	Control	5.733±0.1626	-	2.966±0.0494
2	Ranitidine (30 mg/kg)	0.683±0.1167***	88.09%	1.716±0.08724***
3	EECA (300 mg/kg)	2.183±0.2257***	61.92%	2.3±0.03651***
4	EECA (600mg/kg)	0.866±0.1116***	84.89%	2.01±0.07491***

Values expressed as mean ± SEM; One way Analysis of Variance (ANOVA) $p < 0.0001$; Tukey Kramer Multiple Comparison Test; *** $P < 0.001$ when compared with control group.

Table 3: Effect of EECA on Cold stress induced gastric ulcer.

Sl. No	Treatment	Ulcer index (mean ± SEM)	% Ulcer protection	Vol. of gastric juice (ml)	pH	Total acidity meq/l/100g
1	Control	3.85±0.2566	-	3.633±0.0557	2.133±0.076	67.5±1.607
2	Ranitidine (30 mg/kg)	0.7167±0.1167***	81.38 %	1.9±0.0730***	3.583±0.065***	43.17±1.014***
3	EECA (300 mg/kg)	1.9167±0.0477***	50.21 %	2.816±0.0601***	3.033±0.049***	50.83±1.167***
4	EECA (600mg/kg)	1.1166±0.2167***	70.98 %	2.016±0.0749***	3.333±0.042***	47.17±1.167***

Values expressed as mean ± SEM; One way Analysis of Variance (ANOVA) $p < 0.0001$; Tukey Kramer Multiple Comparison Test; *** $P < 0.001$ when compared with control group.

Table 4: Effect of EECA on Pylorus ligation ulcer model.

Sl. No	Treatment	Ulcer index (mean ± SEM)	% Ulcer protection	Vol. of gastric juice (ml)	pH	Total acidity meq/l/100g
1	Control	3.917±0.4490	-	6.1167±0.1014	2.116±0.070	78±1.000
2	Ranitidine (30 mg/kg)	0.683±0.1276***	82.56 %	4.033±0.05578***	3.733±0.049***	36.67±0.333***
3	EECA (300 mg/kg)	2.217±0.1621***	43.41 %	4.95±0.07638***	3.216±0.047***	61.33±1.229***
4	EECA (600mg/kg)	0.85±0.1544***	78.29 %	4.683±0.06009***	3.483±0.094***	48.83±0.872***

Values expressed as mean ± SEM; One way Analysis of Variance (ANOVA) $p < 0.0001$; Tukey Kramer Multiple Comparison Test; *** $P < 0.001$ when compared with control group.

Cold restraint stress model

Table 3 shows the dose dependent ulcer reduction and increase in the ulcer protection at higher dose. The volume of gastric juice also reduced at higher dose. The pH also shows significant reduction and acidity of the juice was also reducing significantly at higher dose. Hence EECA shows significant activity at 600 mg/kg.

Pyloric ligation model

Table 4 shows the significant reduction of ulcer index at EECA 600 mg/kg, and increased ulcer protection percentage of EECA is high at 600 mg/kg. And the volume of gastric juice was also reduced at higher dose and acidity was also reduced at higher dose.

DISCUSSION

Antiulcer activities were performed on Wister rats of either sex using ethanol induced, cold stress induced and pyloric ligation models. The 70% Ethanol extracts (300 and 600 mg/kg) showed significant antiulcer activity. The anti ulcer activity was carried out in three different Models, the Model such as Pylorus ligation (PL), Ethanol (EtOH) and

cold restraint stress (CRS) - induced ulcers. The percentage ulcer protection is been observed in all the Models, but the extent of percentage protection is more in ethanol induced ulcer and pyloric ligation ulcer. The percentage of ulcer protection variance with the standard ranitidine 30 mg/kg and EECA 600 mg/kg is very less when compared to cold stress induced ulcer. The ulcer index is also reduced in the higher dose. The volume of gastric juice is reduced in all the Models and pH value is also reduced significantly in cold stress induced and pyloric ligation Models. The acidity of the urine collected in the cold stress induced and pyloric ligation Models are also reduced significantly. The phytoconstituents like flavonoids, tannins, terpenoids, and Saponin have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed (Borelli *et al.*, 2000)

Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating

micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants (Berenguer *et al.*, 2005; Nwafor *et al.*, 1996). The phytoconstituents found in the EECA were carbohydrates and glycosides, phytosterols, phenolic compounds and tannins, saponins. These phytoconstituents present in the EECA could be the possible agents in the prevention of ulcers in the rats.

CONCLUSION

The present study showed that ethanol extract of stem bark of *Careya arborea* Roxb. have shown significant effect on the ulcer induced by three Models such as ethanol induced, cold stress induced and pyloric ligation Model. The maximum ulcer protection of EECA has been shown in the Ethanol induced and cold stress induced models and significant effect was found at both 300 mg/kg and 600 mg/kg dose levels.

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