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RESEARCH ARTICLE

Gastroprotective and Antioxidant effect of Petroleum ether Extract of *Eupatorium triplinerve* Vahl

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ABSTRACT:

The present study was designed to investigate the gastroprotective and antioxidant activity of petroleum ether extract from the leaves of *Eupatorium triplinerve* Vahl in male Wistar rats. The animals orally received 200mg/kg of *E triplinerve* extract for three consecutive days. After 12 hr fasting the animals were treated with aspirin in a dose of 200mg/kg orally. They were sacrificed five hours later and the stomach tissue was subjected to histopathological examination. Extensive damage to gastric mucosal layer with edema and leukocyte infiltration of submucosal layer were evident in aspirin treated animals. However, the intensity of mucosal damage was very much less severe in animals pretreated with *E triplinerve.* The antioxidant activity of *E. triplinerve* extract was estimated in-vitro against DPPH and Nitric oxide radicals. A dose dependent antioxidant activity was recorded for petroleum ether extract of *E. triplinerve.* It is suggested that the protective effect of *E. triplinerve* extract on gastric mucosa may be due to its antioxidant activity.

KEYWORDS: Antiulcer, Antioxidant, Histology, Petroleum ether extract, Aspirin, *E. triplinerve*.

INTRODUCTION:

Eupatorium triplinerve Vahl belonging to the family "Asteraceae" is an ornamental plant native of Brazil and has long been naturalized in India. The infusion of the leaf is used in indigestion, nausea and stomach ulcers in Brazilian herbal medicine[1] while the use as analgesic anti-inflammatory, wound healing and in haemarrhoids are documented in Indian materia medica2. The methanolic extract of *E. triplinerve* is reported to have hepatoprotective activity against carbon tetracholoride induced hepatotoxicity in rats[3], antibacterial, antifungal activity[4] and antiseptic property[5]. The ethanolic extract of the entire plant was active against *Bacillus subtilis.*[4] Scientific evidences are also available for the analgesic and anti-inflammatory activity of *E.triplinerve.* However, the antiulcer property of *E.triplinerve* has not been supported by scientific evidence. Hence in the present study, the gastroprotective property of pet-ether extract of *E.triplinerve* was investigated in aspirin induced ulcers in rats. In addition, the potential antioxidant property which may contribute to ulcer protection was also investigated using *in-vitro* experiments.

MATERIALS AND METHODS:

The leaves of *Eupatorium triplinerve* were collected from Kollam district, Kerala in the month of October and the sample was authenticated by herbarium department of Tropical Botanical Garden and Research Institute, (TBGRI) Trivandrum (Collection No:31691, 31692, Account No: 20391, 20392). Voucher samples have been deposited at TBGRI Trivandrum and Department of Pharmacology, Meenakshi Medical College hospital & Research Institute, Kanchipuram.

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Preparation of extract:
Eupatorium triplinerve Vahl leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was10% w/w.

Animals:
Wistar rats (175-200g) were procured from the institutional animal house. The animals had free access to standard pellet feed (Provomi) and water *ad libitum* under strict hygienic conditions and maintained in room temperature of 25±1°C, relative humidity 45- 55% and a 12:12 h light/dark cycle. All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India) and the study protocol was approved by the institutional animal ethics committee.

Aspirin induced ulcers in rats[6]
The petroleum ether extract of *Eupatorium triplinerve* (200mg/kg) was administered orally in 0.1% tween 80 solution for three consecutive days to a group of Wistar rats. Doses were selected based on the previous study[7]**.** After 12 hr fasting period, aspirin (in 0.1% tween 80 solution) was administered orally in a dose of 200mg/kg to the above treatment groups. A group of control rats was treated with 0.1% tween 80 solution. Five hours after aspirin administration, the rats were sacrificed under excess pentobarbitone sodium i.p and the stomach was excised. It was opened along the greater curvature, then washed in warm water, and examined under a 3-fold magnifier. Gastric tissue samples were fixed in buffered formalin (10%) solution for histological evaluation. 5µm sections were taken and stained with haematoxylin and eosin for microscopical examination.

Antioxidant activity:
DPPH radical scavenging activity was estimated using the method of Yohozowa *et al.*[8] The reaction mixture containing 1ml of DPPH solution (150µM in ethanol) with different concentrations of *Eupatorium triplinerve* extract or ascorbic acid (10, 50, 100, 200, 400µg/ml) was shaken and incubated in dark for 20 min at room temperature. The resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

NO scavenging activity:
The nitric oxide radical scavenging activity was analysed using the method of Alderson *et al*[9]. Three ml of reaction mixture containing sodium nitroprusside (10Mm in phosphate buffered saline) and various concentrations of *Eupatorium triplinerve* extract or curcumin (10, 50, 100, 200, 400µg/ml) were incubated at 37^0 c for 4 hours. To the incubation solution, 0.5ml of Griess reagent was added and the absorbance was read at 546 nm. The percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

RESULTS:

Histological examination of the gastric tissue:
Histological examination revealed extensive damage to gastric mucosal layer with edema and leucocyte infiltration of sub mucosal layer in aspirin treated animals (Fig-1) compared to vehicle control (Fig-2). However, the intensity of mucosal damage was less severe in *E. triplinerve* treated animals. Minimal disruption to the surface epithelial mucosa with minimal edema and leucocyte infiltration of the sub mucosal layer were evident in animals pretreated with 200mg/kg of petroleum ether extract of *Eupatorium triplinerve* (Fig - 3).

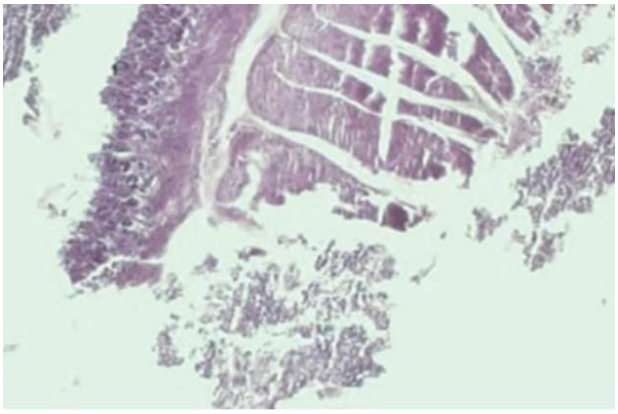

Fig-1: Photomicrograph of Aspirin control group showing the damaged gastric architecture with dead neutrophils and pus formation

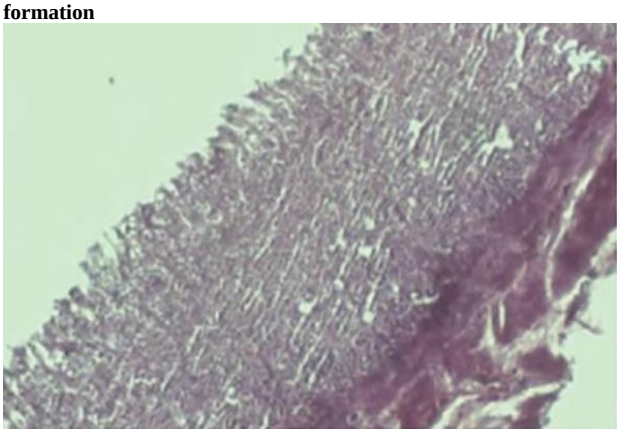

Fig-2: Photomicrograph of vehicle control group showing the normal gastric architecture

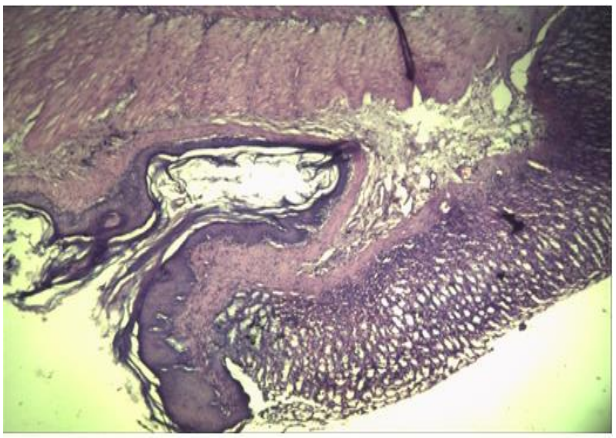

Fig-3: Photograph of petroleum ether extract of *Eupatorium triplinerve* + Aspirin group with minimal edema and leucocyte infiltration of submucosal layer

Antioxidant activity:

A dose dependent inhibition of DPPH (Table-1) and Nitric oxide (Table-2), was evident with pet-ether extract of *E.triplinerve*.

Table 1: DPPH scavenging activity of petroleum ether extract of *Eupatorium triplinerve*

CONCENTRATION (µg/ml)	% INHIBITION OF DPPH ACTIVITY	
	ASCORBIC ACID	*E.triplinerve* EXTRACT
10	79.23±2.46	55.76±3.04
50	81.25±1.68	60.7±2.98
100	83.52±1.96	62.48±2.04
200	86.56±2.34	64.71±1.27
400	88.15±3.14	69.07±0.58

Each value represents mean± SD of three observations

Table 2: Nitric Oxide scavenging activity of petroleum ether extract of *Eupatorium triplinerve*

CONCENTRATION (µg/ml)	% INHIBITION OF NITRIC OXIDE SCAVENGING ACTIVITY	
	CURCUMIN	*E.triplinerve* EXTRACT
10	32.86±2.47	43.20±0.64
50	47.64±2.15	48.08±0.85
100	58.64±1.46	53.85±2.14
200	64.00±2.37	59.50±1.48
400	78.28±2.94	51.60±0.75

Each value represents mean±SD of three observations

DISCUSSION:

Earlier investigations on petroleum ether extract of *E.triplinerve* revealed a potent analgesic effect against various types of nociception in mice and anti-inflammatory activity in Wistar rats[7]**.** Previous studies revealed the anti nociceptive and anti-inflammatory effect of the alcoholic extract of *E.triplinerve* in experimental animals[10]. Both the above reports indicate the presence of active principles with potent antinociceptive and anti-inflammatory activity of *E.triplinerve* extract. Most of the currently employed analgesic and anti-inflammatory drugs like NSAID induced gastric ulceration remains the major adverse effect. Identification of newer analgesic drugs without this adverse effect is highly desirable.

Since petroleum ether extract of *E.triplinerve* demonstrated good antinociceptive and anti-inflammatory effect[7] its propensity to induce gastric mucosal damage was investigated in the present study. The results of the present study revealed that aspirin administration produced extensive gastric mucosal damage accompanied by edema and leukocyte infiltration of the submucosal layer in rats stomach.

However, there was only minimal disruption to surface epithelium of mucosa and minimal edema and leukocyte infiltration of the submucosal layer in animals pretreated with petether extract of *E.triplinerve*. This observation indicates that *E.triplinerve* may not produce severe gastric mucosal damage unlike other NSAID and in fact may offer protection against any ulcers induced by other injurious drugs.

Conventional NSAIDs inhibit cyclo oxygenase enzymes which is responsible for analgesic and anti-inflammatory as well as gastric mucosal damaging effects of these compounds. However, studies on *E.triplinerve* indicates potent anti- nociceptive and anti-inflammatory effect and the present results reveal the gastroprotective property of *E.triplinerve*. This observation suggests a possibility that mechanisms other than COX inhibition may be responsible for the aforementioned beneficial effects of *E.triplinerve*.

Free radical scavenging/antioxidant mechanisms of *E.triplinerve* have been implicated in attenuation of pain and inflammation[11]**.** Furthermore, *E.triplinerve* (200mg/kg) was found to be more effective in acetic acid induced model for Ulcerative colitis by forming a cuticle over the mucosal membrane and also recovered the colonic damage against lipid peroxidaiton and concluded that *E.triplinerve* offers a promising means for the treatment of Ulcerative colitis due to its radical scavenging action.[12] Such a possibility of free radical scavenging activity of *E.triplinerve* has been investigated in the present study. Recent studies showed that ROS are one of the important factors in the pathogenesis of Aspirin-induced mucosal damage[13]. The observations recorded in the present study indicate the pet ether extract of *E.triplinerve* exhibits a potent free radical scavenging activity against DPPH and nitrogen delivered radicals. This free radical scavenging /antioxidant activity may be suggested as one of the possible mechanism for the beneficial effects of *E.triplinerve*. A recent report corroborates this observation, wherein they have reported antinociceptive, sedative and anxiolytic property of *E.triplinerve*.[14]

In conclusion, the present study indicated the gastro protective effect of petroleum ether extract of *E.triplinerve* which may be attributed to antioxidant property.

DISCLOSURE OF INTEREST:

The authors of this article hereby declare that there is no conflict of interest in publishing the article

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