

PHARMACOLOGICAL SCREENING OF *EUPHORBIA NERIIFOLIA* LEAF HYDRO-ALCOHOLIC EXTRACTPapiya Bigoniya¹, A.C. Rana²

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ABSTRACT

Pharmacological screening of *Euphorbia neriifolia* Linn. (Euphorbiaceae) leaf hydro alcoholic extract were performed to explore the analgesic, anti-inflammatory, diuretic, antidiarrhoeal and antiulcer activities. All tests were conducted on rats using 100, 200 and 400 mg/kg dose as LD₅₀ of extract was found to 2779.71 mg/kg. Study revealed strong analgesic effect of *E. neriifolia* against thermal ($P < 0.001$), and in mechanical and chemical ($p < 0.01$) noxious stimuli and anti-inflammatory activity ($P < 0.001$ to 0.01) at the 1000 mg/kg dose. In carrageenan-induced paw edema and cotton pellet induced granuloma model *E. neriifolia* extract showed significant ($P < 0.001$ to 0.01) anti-inflammatory activity. Extract considerably increases urine volume as an effective hypernatraemic and hyperchloraemic diuretic. *E. neriifolia* showed laxative property by increasing wet defecation along with castor oil. Extract showed very prominent protection against ethanol-induced ulceration as well as on pyloric ligated ulceration in dose dependent manner. Extract increases total hexoses ($P < 0.001$), hexosamine ($P < 0.05$), sialic acid and total carbohydrate content ($P < 0.001$) with a decrease in total protein content ($P < 0.001$) of gastric mucosa at 400 mg/kg dose. Presence of phytoconstituents like tannins, flavonoids, alkaloids and triterpenoidal saponins may be responsible for the found pharmacological activities.

Keywords: *Euphorbia neriifolia*, analgesic, anti-inflammatory, diuretic, laxative, antiulcer
Running title: Pharmacological screening of *Euphorbia neriifolia*

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INTRODUCTION

From prehistoric times, various communities and civilizations throughout the world are using herbal medicines. For the past several decades, people are increasingly consuming herbal medicines without prescription. They are traditionally considered as harmless since they belong to natural sources. Herbal formulations have reached widespread acceptability as therapeutic agents like anti-diabetics, anti-arthritics, aphrodisiacs, hepatoprotective, cough remedies, memory enhancers and adaptogens. *Euphorbia neriifolia* Linn. (Euphorbiaceae) was selected for the present study considering its use in traditional medicine thorough literature search to find out scientific basis of the claimed therapeutic potentials.

There are over 1500 species of Euphorbias in the world ranging from annual weeds to trees. *E. neriifolia* grows luxuriously around the dry, rocky, hilly areas of North, Central and South India. It is an herb full of spine, popularly known as 'sehund' or 'thohar' in Hindi. Leaves are thick succulent, 6-12 inch long, ovular in shape. In traditional system, leaves are used as aphrodisiac, diuretic, cough and cold, bleeding piles and in ano-rectal fistula (1). The tribal population of Chhattisgarh region uses the milky latex as an ingredient of aphrodisiac mixture. Latex is used to de-root skin warts, earache and in arthritis. Plant is bitter, laxative, carminative, improves appetite, useful in abdominal troubles, bronchitis, tumors, leucoderma, piles, inflammation, enlargement of spleen, anemia, ulcers, fever, and in chronic respiratory troubles (2). Natives of Chhattisgarh use externally boiled 'thohar' milk in castor oil with salt to cure the deep cracks in soles of legs. The milk of 'thohar' is also used commonly like aloe gel in case of burns. 'thohar' milk can be used successfully for healing of wounds. Application of lukewarm 'thohar' leaves reduces itching pain and swelling in piles (3).

Several triterpenoids like Glut-5-en-3 β -ol, Glut-5(10)-en-1-one, taraxerol and β -amyrin has been isolated from powdered plant, stem and leaves of *E. neriifolia* (4, 5). Antiquorin have been isolated from ethanol extract of fresh root of *E. neriifolia* (6). Neriifolione, a triterpene and a new tetracyclic triterpene named as nerifoliene along with euphol were isolated from the latex of *E. neriifolia* (7, 8).

E. neriifolia is easily available in large quantity in the dry hilly areas of North and Central India. This plant can be used as a cheap source of active therapeutics as Propagation of these plants is easy and cheap which can be grown in large number with very less expenses. *E. neriifolia* latex showed wound healing activity in guinea pig by increasing epithelization, angiogenesis, tensile strength and DNA content in wounds (9). We have already reported mild CNS depressant, wound healing and immunomodulatory activity of leaf hydro-alcoholic extract (10-13). Saponin separated from *E. neriifolia* leaf posses good hemolytic and *in-vitro* antioxidant activity but it is devoid of antibacterial activity upto 10 mg/ml concentration containing euphol as major constituent (14).

E. neriifolia have been widely used to treat a number of ailments in traditional system of medicine in north and central India. Sufficient scientific data is not available to support these above said claims. Based on literature information the present study was undertaken with the aim to confirm its uses in folklore medicine as analgesic, anti-inflammatory, diuretic and ulcer protective.

MATERIAL AND METHODS

Collection and extraction of plant material

E. neriifolia leaves were collected from cultivation field hedge plants of suburban areas of Bhopal (latitude 23.21°, longitude 77.84°, BHOP), Madhya Pradesh, India, in September 2005. The plant was identified with the help of available literature and authenticated by Dr. AP Shrivastava, taxonomist and Principal,

P.K.S Govt. Ayurveda College, Bhopal, India. A voucher specimen was deposited in the herbarium of department (No. 1085).

The leaves were air dried under shade and milled into coarse powder, extracted in Soxhlet extractor successively with different organic solvents such as petroleum ether (60–80°C), chloroform, acetone and 95% ethanol in increasing order of polarity. The marc was dried in hot air oven below 50°C before extracting with next solvent. The extracts obtained with each solvent was distilled to remove 1/4th of solvent then the extracts were dried using a vacuum oven below 30°C and percentage weight calculated in terms of w/w. 95% ethanolic extract was dark brown in color and extractive value was 4.85 % (w/w) of the dry weight of starting material. Presence of triterpenoidal steroids was confirmed by the Salkowski test and Noller's test (15). Presence of saponin was confirmed by Froth test and Hemolysis test (16). Presence of flavonoids was confirmed by Shinoda test and Alkaline reagent test (16).

Test animals

Laboratory bred Wistar albino rats of both sexes (150 – 200 g) maintained under standard laboratory conditions at $22 \pm 2^\circ\text{C}$, relative humidity $50 \pm 15\%$ and photoperiod (12-h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, India) and water were provided *ad libitum*. In order to avoid diurnal variation all the experiments were carried out at same time of the day i.e. between 10 a.m. to 5 p.m. Approval was obtained from Institutional Animal Ethical Committee (approved body of Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India) of Radharaman College of Pharmacy, Bhopal, before carrying out the experiments and care provided to the animal was as per the WHO 'guidelines for the care and use of animals in scientific research.

Determination of LD₅₀

Ld₅₀ was determined according to the guidelines of Organization for Economic Co-operation & Development (OECD) following the Up & Down method (OECD guideline No. 425) and Fixed dose method (OECD guideline No. 420). Based on these agreements a *Limit test* was performed to categorize the toxicity class of the compound and then *Main test* was performed to estimate the exact LD₅₀ (Anonymous, 1992). The limit test was started from 2000 mg/kg dose. LD₅₀ was found greater than the test dose so the test substance could be classified in the hazard classification as class 5, $2000 \text{ mg/kg} < \text{LD}_{50} < 5000 \text{ mg/kg}$ in the Globally Harmonized System (GSH) (17).

The suspension of the extract was prepared freshly with 2% carboxyl methyl cellulose (CMC). Animals were divided into five groups of 6 rats each. Group 1 (vehicle control) was treated only with 2% CMC (0.5 ml/100gm). Group 2 animals were treated with standard drug as per the protocol of study design and group 3-5 with different doses of *E. neriifolia* leaf extract.

Analgesic activity Study

Thermal stimulus (Eddy's hot plate method): Analgesia test was carried out by placing a rat on Eddy's hot plate (Techno, India) at $55\pm 0.5^\circ\text{C}$ for a maximum period of 30 sec. and noting the basal reaction time i.e. licking front paws or making an effort to jump out of the chamber (18). Increase in reaction time after drug administration against basal reaction time was noted in sec. Cut-off time of 30 sec. was selected to avoid tissue damage. The percentage of pain inhibition after 60 minutes of drug treatment was calculated according to the following formula (19).

Pain inhibition percentage (PIP) = $(T_1 - T_0) / T_0 \times 100$. T_1 = Post drug latency (reaction time after drug treatment) and T_0 = Pre drug latency (basal reaction time).

Thermal stimulus (Tail flick method): Analgesia test was carried out by placing the tail tip (last 1-2 cm) of rat on radiant heat of the Tail Flick analgesiometer (Techno, India) heated at $55\pm 0.5^\circ\text{C}$ for a maximum period of 15 sec and noting the basal reaction time (flicking of tail). Increase in tail flick response 45 min after drug treatment was noted and percentage increase in reaction time calculated (18).

Mechanical stimulus: Application of an arterial clip (with jaws covered by a rubber tubing) to the tail base of the rat induces physical pressure due to compression and act as a mechanical stimulus. Attempt to dislodge the clip (biting or kicking etc.) in <15 sec were considered as positive response (20). Failure of such response in treated animals was taken as criteria for analgesia. Basal reaction time and reaction time 45 min after drug administration was noted and percentage increase in reaction time calculated.

Chemical stimulus: Writhing is defined as a stretch, torsion or constriction of abdomen and extension or drawing up of a hind leg etc, given the writhing agent, 3% aqueous acetic acid (2 ml/kg i.p). The writhing episodes produced for 10 minutes after 30 minutes of administration of extracts or standard drug (aspirin, 100 mg/kg, p.o) were counted and percentage protection was calculated as shown below (20).

Percentage protection = $(100 - \text{no. of wriths in test} / \text{no. of wriths in control}) \times 100$

Anti-inflammatory activity study

Carrageenan-induced rat hind paw edema: Inflammatory edema was produced by injecting 0.1 ml of 1% w/v carrageenan solution in normal saline beneath the sub-plantar surface of right hind paw of all the animals. The volume of the paw before and three hr after carrageenan treatment was measured by mercury displacement technique using plethysmometer and percentage inhibition calculated (21).

Cotton pellet induced granuloma: Sterilized pre weighed cotton pellets were implanted in both axillae and groin regions through a single midline incision on the dorsal surface according to the method of

D'Arcy *et al.* (22) on rats anaesthetized with pentobarbitone sodium (30 mg/kg). Drugs were administered 3 h after implantation and continued for seven days. On the 8th day the pellets were dissected out, dried at 60°C the dry weights were determined. The difference between the initial and final weight of cotton pellet was considered the weight of granulomatous tissue produced.

Diuretic activity

Urine output along with electrolyte concentration: Animals were deprived of food and water for 16 hours. All the rats received priming dose of normal saline 25 ml/kg orally. Immediately after administration of vehicle, different doses of extract and standard drug frusemide (5 mg/kg, p.o) all the rats were placed in metabolic cages (group wise) specially designed to separate urine and faeces at room temperature of 25±0.5°C (23). The urine was collected in measuring cylinder up to 5 h after drug administration. During this period no food and water was made available to animals. Concentration of Na⁺ and K⁺ in urine was measured by Flame photometer (Elico, India). Chloride ion concentration was estimated by titration with silver nitrate solution (N/50) using 3 drops of potassium chromate solution as indicator (24).

Experimental Models for antidiarrheal study

Castor oil-induced diarrhea on rats: The method of Awouters *et al.* (25) was followed with minor modification. 24 h fasted animals were treated with vehicle, standard drug (loperamide 0.5 mg/kg) and different doses of extract. After 1 h, each animal received 1 ml of castor oil orally and was then observed for defecation placing them in separate cages up to 6 h. The consistency and frequency of faecal matter, and the number of respondents were noted on filter papers placed beneath the perforated metal cages. Purging index (PI) was calculated as follows.

$$\text{Purging index (PI)} = \% \text{ respondent} \times \text{average no of stools/average latent period}$$

Experimental Models for antiulcer study

Pyloric ligation-induced gastric ulceration: Under light ether anaesthesia, the abdomen was opened by a small midline incision of 1 cm below the xiphoid process. Stomach was exposed and a tight knot was applied around the pyloric sphincter. The stomach was placed carefully and abdomen wall closed by interrupted sutures. Vehicle, ranitidine (20mg/kg) and test extract were administered orally 15 min before pyloric ligation. After 4 hours animals were killed by decapitation, abdomen was opened and the stomach was isolated after suturing the lower esophageal end. The stomach was then cut open along the greater curvature and ulcer index was determined using a hand lens. Gastric contents were collected in a graduated centrifuge tube, volume measured, pH determined, centrifuged at 1000 RPM for 10 min and subjected to biochemical analysis. Ulcer grading was determined following the scoring system suggested by (26).

Ulcer index = $10/X$ where, X = Total area of stomach mucosa / Total ulcerated area. (27).

Gastric contents (1ml diluted with 9 ml of D.W.) titrated against 0.01N NaOH using Topper's reagent till orange color, corresponds to free acidity and further titrated to pink color with phenolphthalein, total volume of NaOH corresponds to total acidity (28).

Acidity expressed as Vol. of NaOH \times Normality \times 100 / 0.1 mEq/L/100g

Total hexose's, fructose of the gastric juice was determined as per the method of Zachariah and Landrum (29), hexosamine following Dische and Borenfreund (30), sialic acid by Warren (31). Total carbohydrate (TC) measured as sum of hexose, fucose, hexosamine and sialic acid. The total protein content (PR) of the gastric juice was also measured following method of Lowry *et al.* (32). The mucin activity measured as total carbohydrate to protein ratio (TC/TP).

Ethanol-induced gastric ulceration: Animals were administered with test extract 45 min before oral dose of absolute alcohol (1ml) (33). One hour after ethanol administration, animals were sacrificed by giving overdose of ether. The stomach was removed, opened along the greater curvature, rinsed with normal saline and scored for the severity of ulceration as mentioned earlier.

Statistical Analysis

Experimental data were analyzed using one way ANOVA followed by Turkey-Kramer multiple comparison test. P value less than 0.05 were considered statistically significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS

E. neriifolia hydroalcoholic extract was found to contain sugar, tannins, flavonoids, alkaloids, triterpenoidal saponin on preliminary phytochemical analysis. LD₅₀ of *E. neriifolia* leaf extract was found to 2779.71 mg/kg from main test. A dose range of 100, 200 and 400 mg/kg was selected for pharmacological screening.

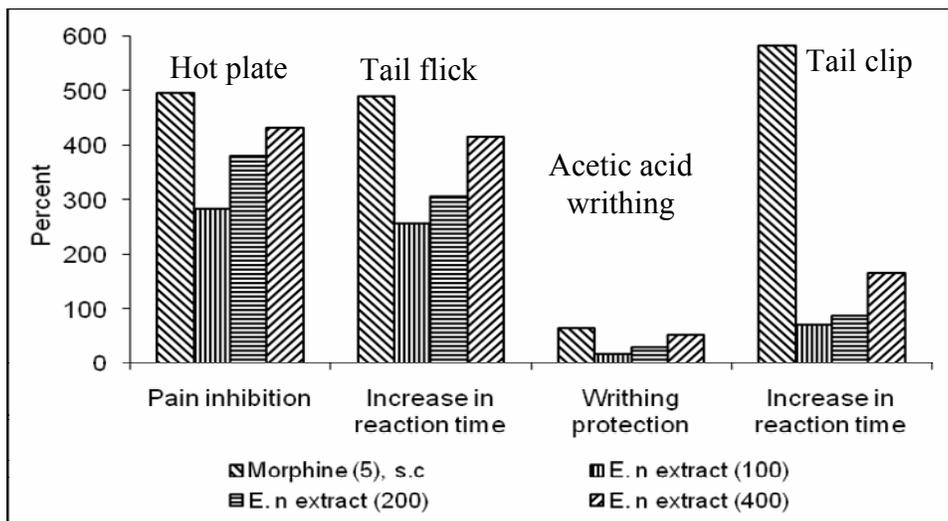


Figure I. Effect of *E. neriifolia* extract treatment on different analgesia models in rats n = 6 per group.

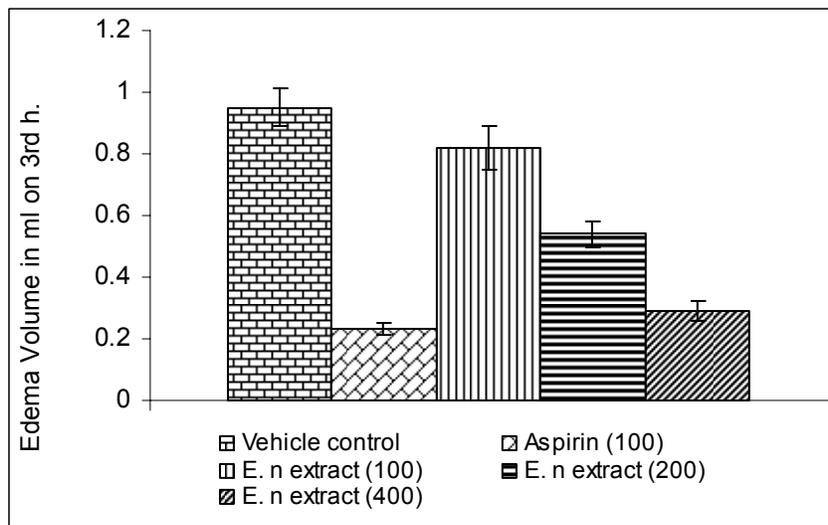


Figure II. Effect of *E. neriifolia* extract treatment on carrageenan-induced paw edema in rats

n = 6 per group. ** $P < 0.01$, *** $P < 0.001$ and ns = not significant as compared to control values. Aspirin and *E. neriifolia* (400 mg/kg) treatment showed 75.78% and 69.47% inhibition of edema volume compared to vehicle control group.

E. neriifolia extract in 400 mg/kg dose showed 432.22% pain inhibition ($P < 0.001$) in Eddy's hot plate method after 60 minutes of drug treatment. Increase in tail flick and tail clip response 45 min after drug treatment was noted to be 416.36% ($P < 0.001$) and 165.94% ($P < 0.01$) respectively in 400 mg/kg dose. In this same dose acetic acid induced writhing episodes protection was found to be 53.83% ($P < 0.01$).

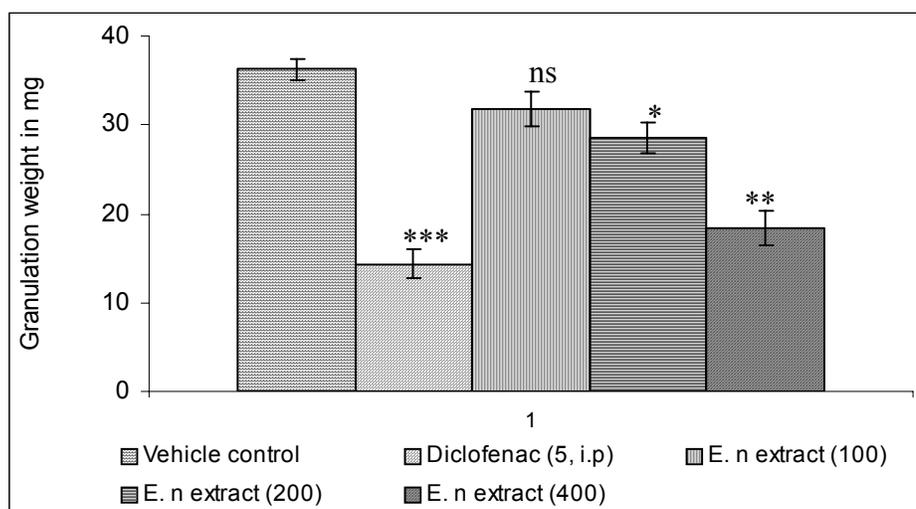


Figure III. Effect of *E. neriifolia* extract treatment on cotton pellet induced granuloma tissue development in rats

n = 6 per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and ns = not significant as compared to control values. Diclofenac and *E. neriifolia* (400 mg/kg) treatment showed respectively 66.14% and 60.61% inhibition in granulomatous tissue mass development compared to vehicle control group.

E. neriifolia extract produced dose dependent diuretic activity, onset of diuresis was extremely significant at 400 mg/kg dose whereas increase in total volume of urine formed was extremely significant at both 200 and 400 mg/kg dose. Fifth hour urine volume for 400 mg/kg dose was 17.45 ml as compared to 6.65 ml of control shown in Table 1. The electrolyte changes induced by the standard drug, vehicle and different doses of extract are shown in Table 2.

Table – 1 Effect of *E. neriifolia* extract treatment on urine output in rats

Treatment (mg/kg, p.o)	Collection time for 1 st drop of urine in min. (M ± SEM)	Total volume of urine on the 5 th hr. (M ± SEM)
Vehicle control	12.5 ± 1.03	6.65 ± 0.96
Frusemide (5)	2.8 ± 0.12***	15.72 ± 1.25***
E. n extract (100)	8.2 ± 1.04*	9.4 ± 0.82 ^{ns}
E. n extract (200)	7.6 ± 1.12*	14.26 ± 1.05***
E. n extract (400)	5.2 ± 1.05***	17.45 ± 1.63***

* $P < 0.05$, *** $P < 0.001$ and ns = not significant when compared to control group.

Table – 2 Effect of *E. neriifolia* extract treatment on urinary electrolyte excretion in rats

Treatment (mg/kg, p.o)	Mean defecation/gr (M ± SEM)	Mean number of wet faeces/gr (M ± SEM)	% Respondent	Mean latent period in min (M±SEM)	Purging index	% Protection
Vehicle control	36.43 ± 4.27	24.49 ± 4.18	67.21	12.14 ± 2.05	201.63	0.00
Loperamide (0.5)	18.99 ± 3.03**	3.57 ± 0.02***	18.80	48.12 ± 5.63***	07.41	85.42
<i>E. n</i> extract (100)	36.13 ± 1.44 ^{ns}	24.94 ± 2.56 ^{ns}	68.56	15.80 ± 1.80 ^{ns}	156.78	- 1.84
<i>E. n</i> extract (200)	38.24 ± 1.03 ^{ns}	26.77 ± 2.60 ^{ns}	69.23	12.73 ± 1.45 ^{ns}	207.96	- 9.31
<i>E. n</i> extract (400)	41.50 ± 2.17 ^{ns}	29.46 ± 3.47 ^{ns}	72.90	10.57 ± 1.44 ^{ns}	286.22	- 20.29

** $P < 0.01$, *** $P < 0.001$ and ns = not significant when compared to control group.

E. neriifolia leaf extracts increased frequency of defecation also the number of wet and deformed faeces. Extract increased purging index to 286.22 as compared to 201.63 of vehicle control and showed increase in wet defecation. Although the tested doses of *E. neriifolia* leaf extract did not produce diarrhoea alone but with castor oil, it produced diarrhoea, which was 20.29 % more than the castor oil alone (Table 3). *E. neriifolia* leaf extract has not been tested for other parameters of antidiarrhoeal activity as it itself shows laxative properties.

Table – 3 Anti-diarrhoeal activity of *E. neriifolia* extract against castor oil induced diarrhea on rats

Treatment (mg/kg, p.o)	Electrolyte excretion				
	Na ⁺ in mEq/L (M ± SEM)	K ⁺ in mEq/L (M ± SEM)	Cl ⁻ in mEq/L (M ± SEM)	Na ⁺ /K ⁺ ratio	% increase in Na ⁺ excretion
Vehicle control	189.25 ± 12.72	99.08 ± 12.33	195.31 ± 6.08	1.91	---
Furosemide (5)	310.53 ± 14.64***	564.85 ± 12.76***	360.05 ± 10.57***	0.55	64.08
<i>E. n</i> extract (100)	236.68 ± 8.63 ^{ns}	102.73 ± 5.98 ^{ns}	202.45 ± 8.46 ^{ns}	2.30	25.06
<i>E. n</i> extract (200)	286.71 ± 7.90***	154.52 ± 6.30 ^{ns}	285.60 ± 10.24**	1.85	51.49
<i>E. n</i> extract (400)	302.78 ± 14.90***	178.24 ± 6.51**	332.68 ± 12.33***	1.69	59.99

Purging index (PI) = % respondents \times average no of stools / average latent period. % protection = $C - T / C \times 100$ (Mean no. of wet faeces). ** $P < 0.01$, *** $P < 0.001$ and ns = not significant when compared to control values.

E. neriifolia showed anti ulcer activity at 200 and 400 mg/kg dose. *E. neriifolia* reduced pH of gastric content significantly both at 200 ($P < 0.05$) and 400 ($P < 0.001$) mg/kg dose. Volume of gastric content was significantly ($P < 0.05$) reduced only at 400 mg/kg dose. Reduction in free acidity, ulcer index and ulcer grading was extremely significant ($P < 0.001$) at 200 and 400 mg/kg dose although reduction in total acidity was significant ($P < 0.01$) only at 400 mg/kg dose. All the results were recorded in Table 4.

Table – 4 Effect of *E. neriifolia* extract treatment on secretory parameters and ulcer index in pyloric ligated rats

Treatment (mg/kg, p.o)	pH of gastric content (M \pm SEM)	Volume of gastric content in ml/100g (M \pm SEM)	Free acidity in mEq/L/ 100g (M \pm SEM)	Total acidity in mEq/L/ 100g (M \pm SEM)	Ulcer index (M \pm SEM)	Ulcer Grading (M \pm SEM)
Vehicle control	2.10 \pm 0.09	2.58 \pm 0.14	17.57 \pm 2.02	41.51 \pm 3.76	5.40 \pm 0.78	2.5 \pm 0.00
Ranitidine (20)	4.80 \pm 0.47***	1.08 \pm 0.05***	5.22 \pm 0.75***	18.25 \pm 2.57***	0.61 \pm 0.03***	0.40 \pm 0.02***
<i>E. n</i> extract (100)	2.93 \pm 0.26 ^{ns}	2.45 \pm 0.32 ^{ns}	10.67 \pm 1.74*	35.80 \pm 2.94 ^{ns}	3.04 \pm 0.57*	1.85 \pm 0.04***
<i>E. n</i> extract (200)	3.50 \pm 0.42*	1.95 \pm 0.25 ^{ns}	7.12 \pm 1.17***	28.92 \pm 2.72 ^{ns}	1.78 \pm 0.07***	1.04 \pm 0.05***
<i>E. n</i> extract (400)	4.2 \pm 0.35***	1.73 \pm 0.15*	6.74 \pm 1.09***	21.51 \pm 2.02**	1.52 \pm 0.02***	0.62 \pm 0.02***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and ns = not significant when compared to control group. All the values are expressed per 100 gm body weight of experimental rats.

Table – 5 Effect of *E. neriifolia* extract treatment on biochemical parameters in pyloric ligated rats

Treatment (mg/kg, p.o)	Total hexoses in mg/ml (M±SEM)	Fucose in mg/ml (M±SEM)	Hexosamine in mg/ml (M±SEM)	Sialic acid in mg/ml (M±SEM)	T C in mg/ml (M±SEM)	T P in mg/ml (M±SEM)	TC /T P ratio
Vehicle control	115.35 ± 7.42	2.79 ± 0.42	75.24 ± 4.82	11.76 ± 1.54	205.14 ± 14.30	174.20 ± 7.80	1.18
Ranitidine (20)	140.19 ± 4.86*	7.32 ± 0.53***	124.53 ± 7.93***	78.70 ± 3.45***	350.74 ± 16.50***	92.47 ± 5.75***	3.79
E. n extract (100)	119.30 ± 4.63 ^{ns}	2.86 ± 0.86 ^{ns}	76.65 ± 2.52 ^{ns}	25.46 ± 2.06**	224.27 ± 10.63 ^{ns}	158.74 ± 4.31 ^{ns}	1.41
E. n extract (200)	137.73 ± 3.22*	3.97 ± 0.78 ^{ns}	80.29 ± 3.49 ^{ns}	46.18 ± 2.53***	268.17 ± 10.45*	122.75 ± 3.58***	2.18
E. n extract (400)	156.41 ± 3.65***	5.09 ± 0.59 ^{ns}	97.11 ± 3.62*	61.64 ± 2.91***	320.25 ± 12.34***	102.21 ± 3.05***	3.13

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and ns = not significant when compared to control group. TC = total carbohydrate content of gastric juice, TP = total protein content of gastric juice. All the values are expressed per ml gastric juice.

Effect of *E. neriifolia* leaf extract on increase of total hexoses ($P < 0.001$) and hexosamine ($P < 0.05$) was significant at 400 mg/kg dose but had no effect on fucose ($P > 0.05$) content. Leaf extract increased sialic acid at all the tested doses which was extremely significant ($P < 0.001$) at 200 and 400 mg/kg dose and increase in total carbohydrate content was extremely significant ($P < 0.001$) at 400 mg/kg dose. Decrease in total protein content was extremely significant ($P < 0.001$) at 400 mg/kg dose as shown in Table 5.

E. neriifolia leaf extract decreased ulcer index, ulcer grading and free acidity on ethanol induced ulceration which was extremely significant ($P < 0.001$) at all the tested doses. Decrease in total acidity and volume of gastric content was significant at 200 ($P < 0.05$) and 400 ($P < 0.01$ & 0.001) mg/kg dose respectively which is reported in Table 6.

Table – 6 Effect of *E. neriifolia* extract treatment on secretory parameters, ulcer index and grading in ethanol induced gastric ulcerated rats

Treatment (mg/kg, p.o)	Volume of gastric content in ml/100g (M±SEM)	Free acidity in mEq/L/ 100g (M±SEM)	Total acidity in mEq/L/100g (M±SEM)	Ulcer index (M±SEM)	Ulcer grading (M±SEM)
Vehicle control	4.26 ± 0.12	4.28 ± 1.06	16.02 ± 1.10	6.33 ± 1.07	2.50 ± 0.00
Ranitidine (20)	1.89 ± 0.04***	0.43 ± 0.10***	5.94 ± 0.42***	3.94 ± 0.65 ^{ns}	2.50 ± 0.00 ^{ns}
Sucralfate (100)	2.03 ± 0.37***	0.97 ± 0.09***	7.65 ± 1.04**	0.31 ± 0.03***	0.15 ± 0.05***
E. n extract (100)	3.09 ± 0.64 ^{ns}	1.46 ± 0.03***	12.60 ± 1.59 ^{ns}	2.54 ± 0.13***	1.42 ± 0.08***
E. n extract (200)	2.72 ± 0.14*	0.94 ± 0.06***	9.81 ± 1.05*	1.09 ± 0.03***	0.91 ± 0.02***
E. n extract (400)	2.25 ± 0.27**	0.47 ± 0.02***	6.55 ± 0.82***	0.63 ± 0.02***	0.09 ± 0.00***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and ns = not significant when compared to control group. All the values are expressed per 100 gm body weight of experimental rats.

DISCUSSION

E. neriifolia showed excellent analgesic activity against thermal stimuli, moderate activity against mechanical stimulus and less against chemical noxious stimuli. The hot plate model is commonly used to assess analgesic activity of narcotic analgesics and other drugs such as sedative, hypnotics or psychomimetic drugs, which act centrally (34). The abdominal writhing elicited by acetic acid has been reported to be a very sensitive but less selective model that enables the detection of antinociceptive activity of compounds in laboratory animals. Collier *et al.* (35) proposed that acetic acid acts indirectly by releasing endogenous mediators, which stimulate neurons that are sensitive to other drugs such as narcotics and centrally acting agents. The abdominal constriction response is thought to involve local peritoneal responses. Several studies demonstrated that steroids produce antinociception when assessed in several chemical models of nociception in animals, which might be true with extract due to presence of steroids and alkaloids (36).

The psychopharmacological profile suggested that *E. neriifolia* leaf extract exhibited an anxiolytic and antipsychotic action without effecting motor coordination and spontaneous activity. This may be due to the generalized central depressant activity of the extract that also correlates with anxiolytic activity observed on elevated plus-maze (10). *E. neriifolia* behaved like other central nervous system depressants by

elevating pain threshold when tested against different noxious stimulus. *E. neriifolia* produced analgesia against thermal as well as on mechanical and chemical noxious stimuli. This indicated that *E. neriifolia* was effective on acute as well as on chronic pain and the antinociceptive effect was mediated centrally.

Carrageenan induced paw edema model is prototype of exudative phase inflammation. The edema development method is biphasic; the initial phase is due to release of histamine, serotonin and kinins in the first few hours after injection of carrageenan (37). The more pronounced second phase occurs in 2-3 hrs due to release of prostaglandin like substances. The significant anti-inflammatory activity of *E. neriifolia* may be due to the presence of steroidal saponins or flavonoids by exerting predominant inhibition of inflammatory mediators from phlogogenic stimuli (38). The association of both analgesic and anti-inflammatory effects is well documented for various non-steroidal anti-inflammatory agents.

E. neriifolia leaf extract produced potent diuresis, increasing the urine volume three times than the control by increasing urine sodium and chloride concentration along with water. Though extract appeared to cause good diuresis, the actual mode of action is not clear from the test. *E. hirta*, another Euphorbiaceae plant is used as diuretic agent by the Swahilis and Sukumas of East Africa. The diuretic effect of extract is mediated by increasing Na^+ , K^+ and HCO_3^- concentration in urine (39). The delay in onset of diuresis induced by the extracts may be attributed to poor absorption of the active principles present in the crude preparations. From the above observations, it can be suggested that *E. neriifolia* extract is an effective hypernatraemic and hyperchloraemic diuretic.

Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in the pyloric ligated rats. Reflex or neurogenic effect in addition to acid secretion has also been suggested to play an important role in the formation of gastric ulcer in this mode (40). *E. neriifolia* leaf extract decreased gastric lesions and pH of gastric content as well as total and free acidity. Effect on volume of gastric content was evident only at high dose. The extract was more effective for reduction of gastric acidity than the volume of gastric content. The effect of extract on soluble mucosubstances showed that increase in total hexose and sialic acid was extremely significant, it also increased hexosamine but had no effect on fucose content. The extract increased total carbohydrate and decreased total protein of gastric content suggesting stimulation of gastric mucosal growth and protection against high acidity.

E. neriifolia leaf extract offered extremely significant protection against ethanol induced ulceration on all tested doses. The extract reduced gastric lesions, volume and acidity of gastric fluid. It is well known that in ethanol induced ulceration leucotrienes cause gastric damage while prostaglandin E_2 protects gastric mucosa against various ulcerogens. Ranitidine does not decrease severity of ulceration in ethanol induced injury to the gastric mucosa as its activity is independent of luminal acid (41). Prostaglandins form a vital component of gastric mucosal defense locally throughout the gut in high concentrations and the major stimulant for their synthesis is cell trauma by acid or alkali, and is known to have an antisecretory effect on

gastric acid production. It has been proposed that non-prostanoids protects gastric mucosa through the mobilization of endogenous prostaglandins. As reported earlier sucralfate inhibit alcohol induced ulceration via stimulation of endogenous prostaglandins release from the gastric mucosa implicating cytoprotection as a possible mechanism (42). The antiulcerogenic effect of the extract may be due to increase in microcirculation, mobilization of prostaglandins in gastric mucosa in addition to its ability to reduce total acidity and to increase TC/TP ratio.

E. neriifolia leaf extract showed very prominent protection against ethanol induced ulceration as well as on pyloric ligated ulceration but the effects were more pronounced on protection of gastric lesions and acidity. The phytochemical analysis of the extract revealed prominent presence of tannins and flavonoids, these substances are known to affect the integrity of mucous membrane. Tannins being astringent may precipitate microproteins in the site of ulcer thus preventing absorption of toxic substances forming a protective layer and resisting the mucous layer against the attack of proteolytic enzymes. Tannins could prevent ulcer development with their protein precipitating and vasoconstrictory effects (43). Flavonoids also protect ulcer development by improving microcirculation and increasing capillary resistance in turn increasing gastric defensive factors (44)

In conclusion, these experimental studies showed potent analgesic, anti-inflammatory, mild diuretic and anti-ulcer activity of *E. neriifolia*, which gives scientific basis to its uses in traditional medicine. The results indicated that *E. neriifolia* extract exerts the cytoprotective effect in addition to their gastric antisecretory activity that could be due to, partly at least, to the presence of flavonoids (41). Presence of tannins may also be responsible for its protective effect by maintaining an efficient gastric mucosal microvascular supply. Further studies are required to establish and elaborate the mechanism of antiulcer activity of *E. neriifolia* leaf extract. Detailed pharmacological studies should be carried out to establish the mode of action and bioactive components responsible for the therapeutic usefulness.

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