Antiulcer effect of *Euphorbia neriifolia* Linn leaf extract

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Introduction: Ulcer is a common gastrointestinal disorder which is seen among many people. It is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance.

Aim & Objective: Present study to evaluate the antiulcer potential of *Euphorbia neriifolia* linn. Leaf extract

Methods: The 70% v/v hydro-alcoholic extract of dried leaves of *Euphorbia neriifolia* was evaluated for its antiulcer activity using two models. Models are pylorus ligation induced gastric ulcers model and ethanol induced gastric ulcer model in mice. It was found that the hydro-alcoholic extract of leaves have significant antiulcer activity in dose dependent manner where 3 different oral doses prepared (100 mg/kg of body weight, 200 mg/kg of body weight and 400 mg/kg of body weight). Evaluation was done on both models comparing with reference standard Omeprazole (20 mg/Kg/ p. o.).

Result: The compounds like sugar, tannins, flavonoids, alkaloids, 24-methylene cycloartenol, triterpennoidal and saponins were detected by usual chemical test in hydro-alcoholic extract.

Summary & Conclusion: The above result shows that *Euphorbia neriifolia* leaves probably contains some active ingredients that could be developed for above mentioned abnormal condition as have been claimed by traditional system of medicine.

Key words: *Euphorbia neriifolia, gastric ulcer, ethanol, pylorus.*

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INTRODUCTION

Many groups and civilizations throughout the world have used herbal remedies from the ancient era. Since a few decades ago, the number of individuals using herbal remedies without a prescription has increased. Since they come from natural sources, they are typically seen to be safe. Herbal preparations, such as anti-diabetics, anti-arthritics, aphrodisiacs, hepatoprotective,

cough cures, memory boosters, and adaptogens, have gained universal acceptance as medicinal agents. Since *Euphorbia neriifolia* Linn. (Euphorbiaceous) is used in traditional medicine, it was chosen for the current investigation. A thorough literature search was conducted to determine the scientific foundation for the stated therapeutic potentials. Over 1500 different species of euphorbias exist worldwide, ranging from weedy annuals to trees. Around the arid, rocky, mountainous regions of North, Central, and South India, *E. neriifolia* flourishes opulently.It is a spine-filled plant commonly referred to as "sehund" or "thohar" in Hindi. The leaves are thick succulent, ovular-shaped, and 6–12 inches long. According to the conventional method, leaves are employed as aphrodisiacs, diuretics, cough and cold remedies, for bleeding piles, and for ano-rectal fistulas (1). The creamy latex is used as an aphrodisiac by the tribal people of the Chhattisgarh area. Arthritis, earaches, and skin warts can all be treated with latex. Plant is bitter, laxative, carminative, increases hunger, and is helpful for chronic respiratory issues, tumours, bronchitis, leucoderma, piles, inflammation, and enlargement of the spleen (2). To treat the severe cracks in the soles of legs, Chhattisgarh natives topically boil 'thohar' milk with castor oil and salt.For burns, 'thohar' milk is frequently used like aloe gel. The milk known as "thohar" can be used to cure wounds. The swelling and irritation associated with piles are reduced when lukewarm 'thohar' leaves are applied (3).

From the powdered plant, stem, and leaves of *E. neriifolia*, many triterpenoids including Glut-5-en-3-ol, Glut-5(10)-en-1-one, taraxerol, and amyrin have been identified (4, 5). From an ethanol extract of the fresh root of E. neriifolia, antiquorin has been extracted (6). From the latex of E. neriifolia, nerifolione, a triterpene, and nerifoliene, a novel tetracyclic triterpene, were extracted (7, 8). In the arid hilly regions of North and Central India, E. neriifolia is readily accessible and can be found in huge quantities.This plant can be produced in huge quantities for very little money, making it an affordable source of therapeutically effective substances. In guinea pigs, E. neriifolia latex increased wound epithelization, angiogenesis, tensile strength, and DNA content to demonstrate wound healing activity (9). We have earlier documented the leaf hydroalcoholic extract's modest CNS depressant, wound-healing, and immunomodulatory activities (10–13). Despite having high hemolytic and in-vitro antioxidant activity and having euphol as a prominent ingredient, saponin isolated from *E. neriifolia* leaves lacks antibacterial action up to 10 mg/ml concentration (14). The traditional medical system in north and central India has traditionally employed *E. neriifolia* to treat a variety of illnesses. Sufficient scientific data is not

available to support these above said claims. Based onliterature information the present study was undertaken with the aim to confirm its uses in folkloremedicine as analgesic, anti-inflammatory, diuretic and ulcer protective.

MATERIAL AND METHODS

Collection and extraction of plant material

In September 2005, *E. neriifolia* leaves were gathered from field hedge plants in suburban Bhopal, Madhya Pradesh, India (latitude 23.21°, longitude 77.84°, BHOP). With the use of accessible literature, the plant was recognised and verified. The leaves were air dried in the shade, ground into a coarse powder, and gradually extracted in a Soxhlet extractor using increasing amounts of polarity of petroleum ether (60-80°C), chloroform, acetone, and 95% ethanol. Before extracting with the following solvent, the marc was dried in a hot air oven below 50°C. The extracts produced with each solvent were then distilled to remove a quarter of the solvent, dried in a vacuum oven below 30°C, and their percentage weight was determined in terms of w/w.95% ethanolic extract was dark brown in color and extractive value was 4.85 % (w/w) of thedry weight of starting material. Presence of triterpenoidal steroids was confirmed by the Salkowski test andNoller's test (15). Presence of saponin was confirmed by Froth test and Hemolysis test (16). Presence offlavonoids was confirmed by Shinoda test and alkaline reagent test (16).

Test animals

cultivated in a lab For the experiment, Wistar albino rats of both sexes (weighing between 150 and 200 g) were kept at a temperature of 22 2 °C, a relative humidity of 50 15%, and a photoperiod of 12 h of darkness and light. Water and commercial pellet diet (Hindustan Lever, India) were freely available. All of the studies were conducted at the same time of day, from 10 a.m. to 5 p.m., to prevent diurnal fluctuation. The Institutional Animal Ethical Committee, a recognizedorganization by the Committee for the Purpose of Control and Supervision of Experiments on Animals in Chennai, India, granted approval.

Determination of LD_{50}

According to the Organization for Economic Co-operation & Development's (OECD's) recommendations, the LD50 was calculated using the Up & Down approach (OECD guideline No. 425) and fixed dosage method (OECD guideline No. 420). Based on these agreements, a Limit test was carried out to classify the compound's degree of toxicity, and a Main test was

carried out to determine the precise LD50 (Anonymous, 1992). The dosage used to begin the limit test was 2000 mg/kg. The test drug might be categorized in the Globally Harmonized System (GSH) as class 5, 2000 mg/kgLD_{50}5000 mg/kg, since the LD_{50} was discovered to be larger than the test dosage (17). Freshly made with 2% carboxyl methyl cellulose (CMC), the extract suspension. Animalswere divided into five groups of 6 rats each. Group 1 (vehicle control) was treated only with 2% CMC (0.5ml/100gm). Group 2 animals were treated with standard drug as per the protocol of study design and group3-5 with different doses of *E. neriifolia* leaf extract.

Experimental Models for antiulcer study

Pyloric ligation-induced gastric ulceration:

The abdomen was opened by making a little midline incision of 1 cm below the xiphoid process while under mild ether anaesthesia. A tight knot was tied around the pyloric sphincter while the stomach was exposed. The abdominal wall was closed by interrupted sutures after the stomach had been appropriately positioned. 15 minutes before pyloric ligation, test extract, Omeprazole (20 mg/kg), and vehicle were all given orally. Animals were decapitated after 4 hours, their abdomens were opened, and the stomach was separated after the lowed esophageal end had been sutured. After that, the stomach was sliced open along its larger curvature so that the ulcer index could be measured using a hand lens. The contents of the stomach were gathered in a graduated centrifuge tube, assessed for volume and pH, spun at 1000 RPM for 10 minutes, and then submitted to biochemical analysis. Ulcer grading was determined following the scoring system suggested by (18).Gastric contents (1ml diluted with 9 ml of D.W.) titrated against 0.01N NaOH using Toper's reagent tillorange color, correspond to free acidity and further titrated to pink color with phenolphthalein, totalvolume of NaOHcorresponds to total acidity (19).

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

Ethanol-induced gastric ulceration:

Animals were administered with test extract 45 min before oral doseof absolute alcohol (1ml) (20). One hour after ethanol administration, animals were sacrificed by givingoverdose of ether. The stomach was removed, opened along the greater curvature, rinsed with normal salineand 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 Version3.02 was used for statistical calculations.

RESULTS

E. neriifolia hydroalcoholic extract was found to contain sugar, tannins, flavonoids, alkaloids, triterpenoidalsaponin on preliminary phytochemical analysis. LD_{50} of *E. neriifolia* leaf extract was found to 2779.71mg/kg from main test. A dose range of 200 and 400 mg/kg was selected for pharmacologicalscreening.

Table – 1 Effect of *E. neriifolia* extract treatment on secretary parameters and ulcer index in pyloricligatedrats

Treatment (mg/kg,p.o)	pH ofgastriccontent (M±SEM)	Volume ofgastriccontent inml/100g (M±SEM)	Free acidityin mEq/L/100g (M±SEM)	Totalacidity inmEq/L/100g (M±SEM)	Ulcer index (M±SEM)	UlcerGrading (M±SEM)
Vehiclecontrol	2.10 ±0.09	2.58 ±0.14	17.57 ±2.02	41.51 ±3.76	5.40 ±0.78	2.5 ±0.00
Omeprazole (20)	4.80± 0.47***	1.08± 0.05***	5.22± 0.75***	18.25 ±2.57***	0.61± 0.03***	0.40 ±0.02***
E. n extract(200)	3.50 ±0.42*	1.95 ±0.25^{ns}	7.12 ±1.17***	28.92 ±2.72^{ns}	1.78± 0.07***	1.04± 0.05***
E. n extract(400)	4.2± 0.35***	1.73±0.15*	6.74± 1.09***	21.51± 2.02**	1.52± 0.02***	0.62± 0.02***

*P<0.05,**P<0.01,***P<0.001andns=notsignificantwhencomparedtocontrolgroup.Allthevaluesareexpressedper100gmbodyweightof experimentalrats.

E. neriifolia leaf extract decreased ulcer index, ulcer grading and free acidity on ethanol induced ulcerationwhich was extremely significant (P<0.001) at all the tested doses. Decrease in total acidity and volume ofgastric content was significant at 200 (P<0.05) and 400 (P<0.01 & 0.001) mg/kg dose respectively which isreported inTable 1.

Table 2: Effect of *E. neriifolia* extract treatment on secretary parameters, ulcer index and grading in ethanol induced gastric ulcerated rats

Treatment (mg/kg,p.o)	Volumeofgastric content inml/100g (M±SEM)	Free acidity inmEq/L/ 100g (M±SEM)	Total acidity inmEq/L/100g (M±SEM)	Ulcerindex (M±SEM)	Ulcergrading (M±SEM)
Vehiclecontrol	4.26±0.12	4.28±1.06	16.02±1.10	6.33±1.07	2.50±0.00
Omeprazole (20)	1.89 ±0.04***	0.43 ±0.10***	5.94 ±0.42***	3.94 $\pm0.65^{ns}$	2.50 $\pm0.00^{ns}$
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 per100 gm body weight of experimental rats.

Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in thepyloric ligated rats. Reflex or neurogenic effect in addition to acid secretion has also been suggested to playan important role in the formation of gastric ulcer in this mode.*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 thanthe volume of gastric content. The effect of extract on soluble muco substances 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 gastricmucosa against various ulcerogens. Omeprazole does not

decrease severity of ulceration in ethanol inducedinjury to the gastric mucosa as its activity is independent of luminal acid (22). Prostaglandins form a vitalcomponent of gastric mucosal defense locally throughout the gut in high concentrations and the majorstimulant for theirsynthesisis cell traumaby acid oralkali, and is known to have anantisecretory effectongastric acid production. It has been proposed that non-prostanoids protects gastric mucosa through themobilization of endogenous prostaglandins. As reported earlier sucralfate inhibit alcohol induced ulcerationvia stimulation of endogenous prostaglandins release from the gastric mucosa implicating cytoprotection asa possible mechanism (23). The anti ulcerogenic effect of the extract may be due to increase in microcirculation, mobilization of prostaglandins in gastric mucosa in addition to its ability to reduce totalacidityand to increaseTC/TPratio.

E. neriifolia leaf extract showed very prominent protection against ethanol induced ulceration as well as onpyloric ligated ulceration but the effects were more pronounced on protection of gastric lesions and acidity.The phytochemical analysis of the extract reveled prominent presence of tannins and flavonoids, thesesubstances are known to affect the integrity of mucous membrane. Tannins being astringent may precipitate micro proteins in the site of ulcer thus preventing absorption of toxic substances forming a protective layerand resisting the mucous layer against the attack of proteolytic enzymes. Tannins could prevent ulcerdevelopment with their protein precipitating and vesoconstrictory effects. Flavonoids also protect ulcerdevelopment by improving microcirculation and increasing capillary resistance in turn increasing gastricdefensivefactors (24)

Conclusion

These experimental researches concluded that *E. neriifolia* had powerful anti-ulcer action, providing a scientific justification for its traditional medical use. In addition to its gastric antisecretory action, the results showed that *E. neriifolia* extract also has cytoprotective properties, which may be caused, at least in part, by the presence of flavonoids. By preserving an effective stomach mucosal microvascular supply, tannins may also be to blame for their protective effect. To clarify and establish the mechanism of *E. neriifolia* leaf extract's antiulcer efficacy, more research is needed. To determine the mode of action and bioactive components responsible for the therapeutic usefulness, thorough pharmacological research should be conducted.

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