"GASTROPROTECTIVE EFFECT OF *TABERNAEMONTANA DIVARICATA* (L). R.BR. FLOWER METHANOLIC EXTRACT IN RATS"

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Summary

Tabernaemontana divaricata (L).R.Br belonging to Apocynaceae family is traditionally used by people in many parts of the world to treat various disorders. The present study was undertaken to investigate anti-ulcer property of *Tabernaemontana divaricata* flower methanolic extract (TDFME 500 mg/kg, p.o) by pyloric ligation induced gastric ulceration model using Omeprazole (8mg/kg, p.o) as a standard drug in wistar rats. Five parameters i.e. volume of gastric juice, pH, free & total acidities and ulcer index were assessed. The test extract significantly ($p< 0.01$) decreased volume of gastric juice, free & total acidities and ulcer index. Like standard, it also raised pH of gastric acid. The observed percentage protection for standard and test were 89.84% and 79.53% respectively. Thus, TDFME 500 mg/kg had a positive effect on all the parameters under study and the results were similar to that of standard. From the above results, it can be concluded that TDFME exhibits remarkable gastroprotective effect.

Keywords: Peptic Ulcer Disease (PUD), *Tabernaemontana divaricata* flower methanolic extract (TDFME), pyloric ligation induced gastric ulceration model and ulcer index.

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Introduction

Peptic Ulcer Disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. An estimated 15,000 deaths occur each year as a consequence of PUD [1]. The patho-physiology of PUD involves imbalance between offensive (acid, pepsin & *H.pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) [2]. PUD is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy [3]. Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric ulcer and second with reinforcing gastric mucosal protection [1,2]. The type of drug differs from being a H_2 receptor antagonist, proton pump inhibitor or cytoprotective agents such as sucralfate. Unfortunately, most of these drugs confer simpler to severe side effects like arrhythmias, gynaecomastia, enterochromaffin like cell hyperplasia and hematopoietic changes [4].

Considering the morbidity caused by PUD and dyspepsia over the world, cheap and easily available treatments will always be in demand especially for the people of non-industrialized countries [5]. Thus, there is an urgent need to search an indigenous drug with fewer side effects to have a better and safer alternative for the treatment of peptic ulcer. In this context, extensive studies and research has been undertaken which mainly focuses on search of anti-ulcer agents of plant & marine origin [6].

Herbal medicines are now used by up to 50% of the western population, in a number of instances (~10%) for the treatment or prevention of digestive disorders [7]. Ginger (rhizome of *Zingiber officinale* Roscoe) is among the 20 top- selling herbal supplements in the USA and its retail sales in mainstream of the USA market in 2001 amounted to $ 1.2 million [8]. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including PUD [9]. *Tabernaemontana* is one of the genera that are used in Ayurveda, Chinese and Thai traditional medicine for the treatment of fever, pain and dysentery [10, 11].

Tabernaemontana divaricata (L.) R.Br. Ex Roem. & Schult. (Synonyms: *Ervatamia coronaria*, *E. microphylla*, *E. divaricata*, *T. coronaria*) belongs to family Apocynaceae. The common vernacular names are Crepe Jasmine, Carnation of India, East Indian Rosebay and Pinwheel Flower (English), Nandivrksha (Sanskrit), Chandni (Hindi), Nandivardhanam (Telugu), Nandiar vattai & Nantiyavattam (Tamil), Sagar (Gujarati), Ananta & Tagar (Marathi), Nantyarvattam (Malayalam), Kottubale & Nandibatlu (Kanada) and Nanta Phool (Konkani) [12].

It is found in Tropical Asia, Australia and Polynesia. In India, it occurs in upper Gangetic plain, Garhwal, East Bengal, Khasia Hills, Assam, Burma, hills of Vishakapatnam. It is cultivated as an ornamental plant, grows wild in hedges and shady forests [13,14]. *T. divaricata* is glabrous, evergreen shrub 1.8-2.4 m in height with silvery grey bark and milky latex; leaves are simple,

opposite, elliptic or elliptic – lanceolate, smooth, glossy green, acuminate and wavy margins; flowers are white, sweetly fragnant in 1-8 flowered cymes at the bifurcations of the branches, lobe of corolla overlapping to right in the bud; fruit follicles are 2.5-7.5 cm long, ribbed and curved, orange or bright red within narrowed into a slender curved beak; seeds are dull brown, minutely pitted, irregular, enclosed in a red pulpy aril [12].

This plant is traditionally used by people in many parts of the world to treat various disorders like abdominal tumours, arthralgia, asthma, diarrhea, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, oedema, paralysis, piles, rabies, rheumatic pain, skin diseases, ulceration and vomiting. It is also used as antihelmintic, antihypertensive, aphrodisiac, diuretic, emmenagogue, hair growth promoter, purgative, remedy against poisons and tonic for brain, liver & spleen [12,13,15-17].

Growing evidences suggests that this plant has medicinal benefits and its extracts could possibly be used as pharmacological intervention in various diseases. Phytochemical studies on various parts reveal that it contains at least 66 indole alkaloids, non-alkaloidal constituents like enzymes, flavonoids, hydrocarbons, phenolic acids, phenyl propanoids, steroids and terpenoids [18]. Alkaloids, flavonoids and terpenoids are the main secondary metabolites that exhibit many physiological and pharmacological properties on living cells [19]. The flowers contain 3, 4, 14, 19 – tetrahydro-Olivacine, 11-methoxy-N-methyl dihydro-Pericyclivine, 19-epivoacangine, Apparicine, Isovoacangine, Isovoacristine, Tabernaemontanine, Tabersonine, Voaphylline, N-1-methyl-Voaphylline and Vobasine [20-27].

There are still many indole alkaloids and their derivatives whose pharmacological activities are yet to be studied. They may contain beneficial pharmacological properties [18]. In the view of its traditional use for healing ulcers and presence of novel & unscreened alkaloids, present study was carried out to evaluate the effect of TDFME on gastric ulcers.

Material and Methods

Plant material: The flowers of Crepe Jasmine (*Tabernaemontana divaricata*) were collected in the month of November 2009 from the local areas of Ameerpet and Mallepally (Hyderabad District) and authenticated by plant taxonomist Dr. Ramana (Department of Botany, Osmania University). A voucher specimen (0360-OUH) has been deposited in the herbarium for future reference.

Preparation of extract: After collection, flowers were shade dried and coarsely powdered. Approximately 500 gm of powdered flowers were extracted using 99% pure methanol (Carbinol –SD Fine Chemicals) in a soxhlet apparatus. The extract was concentrated under reduced pressure and stored in an air tight container in a refrigerator at temperature below 10° C. Dried mass of crude *Tabernaemontana divaricata* flower methanolic extract (TDFME) was weighed

and recorded. The solution of TDFME was prepared using distilled water for the evaluation of anti-ulcer activity.

Experimental animals: Swiss albino mice weighing 20-30gm were used to assess acute toxicity whereas adult male wistar rats weighing 150-200 gm were used to evaluate anti-ulcer activity by pyloric ligation induced gastric ulceration model. The animals were maintained under standard laboratory conditions in polypropylene cages under 12 hr light/dark cycle, controlled temperature (24 ± 2°C), fed with commercial pellet diet and water *ad-libitum* in an animal house approved by Committee for the Purpose and Supervision on Experiments on Animals (Reg no. 1185/a/08/CPCSEA). All the animals were acclimatized to the laboratory environment for 10 days before commencement of the experiments. The experimental protocol was approved by Institutional Animal Ethical Committee, MESCO College of Pharmacy, Mustaidpura, Hyderabad, Andhra Pradesh, India.

Acute toxicity study: Acute toxicity test was performed in mice according to staircase method. Mice divided into four groups with five animals per dose. The dose was increased from 250-1000 mg/kg, b.w p.o. Mice were observed individually after dosing with special supervision given during first 4 hours and periodically thereafter for first 24 hours [28].

Evaluation of anti-ulcer activity:

Experimental Design: Rats were divided into three groups, each consisting 6 and treated with the respective test solutions as given below -

Group I (Control): Distilled water (1 ml/ 100gm, p.o)

Group II (Standard): Omeprazole (8 mg/kg, b.w p.o)

Group III (Test): TDFME (500 mg/kg, b.w p.o)

Pyloric ligation induced gastric ulceration model [29] – Rats were fasted in individual cages for 24 hours. Care was taken to avoid coprophagy. Distilled water, Omeprazole 8 mg/kg and TDFME 500 mg/kg were administered orally 30 mins prior to pyloric ligation. Under light ether anaesthesia, the abdomen was cut open by making a small incision below the xiphoid process. 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 was closed by interrupted sutures. Rats were sacrificed by an over dose of anesthetic ether after four hours of pyloric ligation.

Collection & Measurement of Gastric juice – The stomachs were excised carefully by keeping the oesophagus closed and opened along the greater curvature, luminal contents were removed. The gastric contents were collected and centrifuged at 1000 rpm for 10 min. The centrifuged samples were decanted and volume of gastric juice was noted.

Determination of pH of Gastric juice – 1 ml of the supernatant liquid was diluted to 10 ml using distilled water. The pH of the solution was recorded by digital pH meter.

Estimation of Free and Total Acidities – The above solution was titrated against 0.01 N NaOH using Topfer's reagent as indicator. When the solution turned orange in colour, volume of NaOH was noted that corresponds to free acidity. Further, the titration was continued till the solution regained pink colour. The total volume of NaOH was noted, that corresponds to the total acidity.

Assessment of Ulcer Index – Mean ulcer score for each animal is expressed as Ulcer Index. The stomachs were washed in running water to detect ulcers in the glandular portion of the stomach. The number of ulcers per stomach were noted and severity scoring was done microscopically with the help of hand lens (10X) and scoring was done as per Kulkarni (1999).

0 = Normal Stomach 0.05 = Red Colouration

1 = Spot Ulcers 1.5 = Haemorraghic Streaks

2 = Ulcers > 3 mm but < 5 mm 3 = Ulcers > 5 mm

Calculation of Percentage Protection – The percentage protection was calculated by the following formula:

Percentage Protection = 100 – (Ut / Uc X 100)

Where, Ut = Ulcer Index of treated group and Uc = Ulcer Index of control group

Statistical Analysis: The values are expressed as Mean ± Standard Error of Mean. P< 0.01 was considered significant and is denoted as **. Data of gastric juice, pH & Ulcer score was analyzed by One-way Analysis of Variance whereas free & total acidities were analyzed by Kruskal – Wallis test (non-parametric ANOVA) followed by Dunnett's multiple comparison post-hoc test using GraphPad Instat version 3.10 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com

Results

The percentage yield of TDFME (dried crude extract) obtained was 14%. The acute toxicity study reveals that maximum tolerable dose of TDFME is more than 1000 mg/kg as all the test doses were found to be safe and no mortality or signs of toxicity were observed. In my previous study TDFME exhibited anti-convulsant activity at 500 mg/kg, hence the same test dose was selected [30]. The results of present study are indicated in **Table - 1** & illustrated in **Figures 1-5**.

Standard drug and TDFME significantly (p<0.01) decreased volume of gastric juice from 3.6 ± 0.4761 (Control) to 1.43 ± 0.1801 and 1.55 ± 0.1979 respectively. A significant decrease of equal degree in free and total acidities was observed in both the groups. Similarly the ulcer index

significantly ($p<0.01$) reduced from 6.5 ± 0.6708 (Control) to 1.33 ± 0.4216 and 0.66 ± 0.2789 for the standard and test groups respectively. Likewise both the treatments raised pH significantly ($p<0.01$) from 2.63 ± 0.3658 (Control) to 6.36 ± 0.4485 (Standard) and 4.75 ± 0.5246 (TDFME). Percentage protection produced by standard and test were 89.84% and 79.53% respectively. The test extract influenced all the parameters positively and magnitude of effect was comparable to that of standard. Thus, from the above results it is evident that TDFME possesses gastroprotective effect.

Table - 1. Effect of treatments on various parameters under study.

Groups	Volume of Gastric Juice	pH of Gastric acid	Free Acidity	Total Acidity	Ulcer Score
Group - I Control - Distilled water	3.6±0.4761	2.63±0.3658	35.33±7.424	76.66±14.063	6.5±0.6708
Group - II Standard - Omeprazole 8 mg/kg	1.43±0.1801***	6.36±0.4485***	3.66±0.4944**	9.0±0.8563*	0.66±0.2789***
Group - III Test – TDFME 500 mg/kg	1.55±0.1979***	4.75±0.5246**	4.5±0.8466*	8.16±2.60**	1.33±0.4216***

Note: Data is expressed as Mean ± SEM; n = 6 per group. * indicates $p< 0.05$, ** indicates $p< 0.01$ & *** indicates $p< 0.001$.

Figures 1-5 indicating effect of TDFME in comparison with control & standard on various parameters of pyloric ligation induced gastric ulceration model.

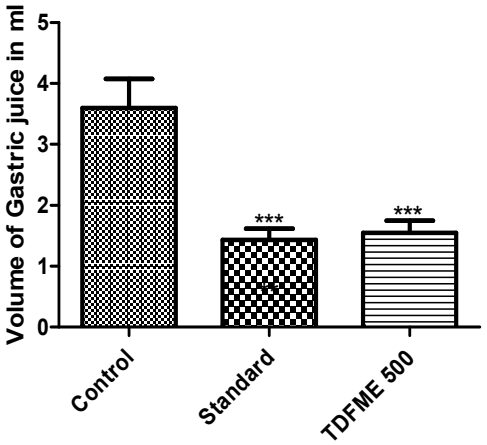

Fig.1 - Effect of various treatments on volume of gastric juice

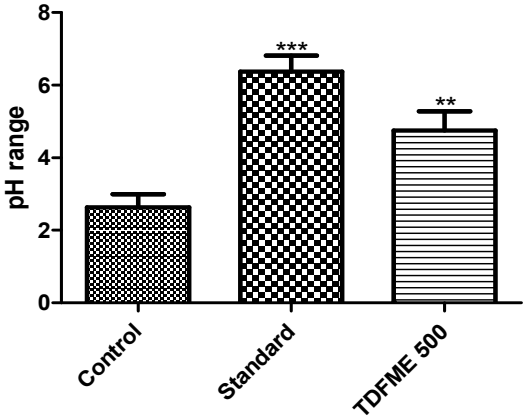

Fig.2 - Effect of various treatments on pH of Gastric juice

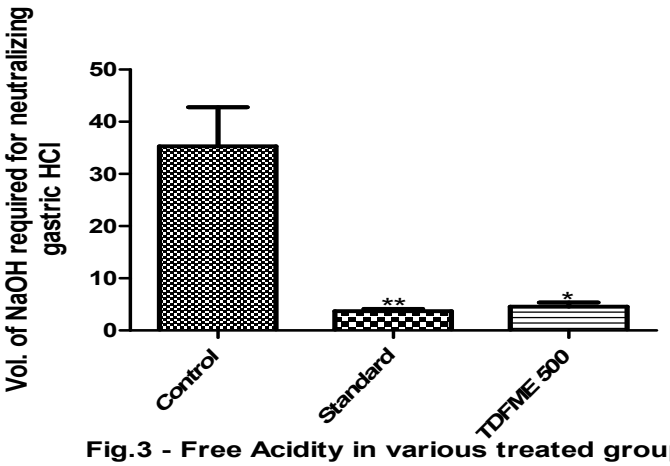

Fig.3 - Free Acidity in various treated groups

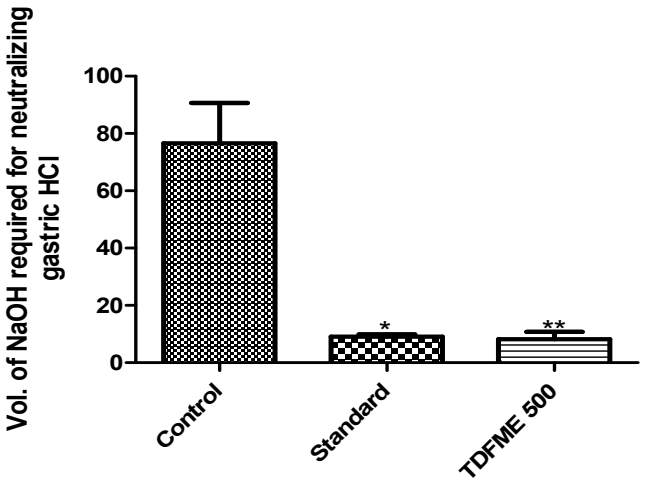

Fig.4 - Total Acidity in various treated groups

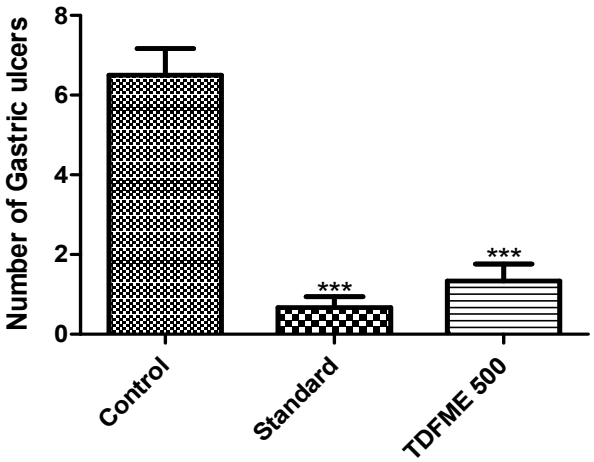

Fig.5 - Ulcer index in various treated groups

Discussion

Plants belonging to *Tabernaemontana* genus are used in folk medicine for the treatment of high blood pressure, pain, inflammation as well as topical application for healing wounds. *T. divaricata* exhibits different roles in CNS, cardiovascular, gonadotropic, anti-tumor, anti-infectious, anti-oxidant activities and enhances cholinergic activity in nervous system [18]. Most common medicinal uses of crude *T.divaricata* extract involves its anti-microbial action against infectious diseases such as gonorrhea, leprosy, syphilis, as well as anti-parasitic action against diarrhoea, dysentery, worms and malaria [11]. The beneficial effects of TDFME can be attributed mainly to the bioactive phytochemicals, alkaloids and flavonoid present in it. Alkaloids are responsible for the medicinal properties of the plant. Alkaloidal component of *T.divaricata* could play important role in its pharmacological activites [18]. The decrease in

volume of gastric juice, its pH & acidity can be due to Isovoacristine, an indole alkaloid isolated from flowers, leaves & roots [21,31]. It is reported to have anti-histaminic effect in an in-vitro study on guinea pig ileum [32]. Apparicine and Isovoacrisitine have been indicated to possess chemotherapeutic potential (anti-microbial & anti-inflammatory activities) [18]. TDFME may be effective against *H.pylori* infections. Hence, it is worth to screen & establish its efficacy against *H.pylori*.

The positive effect of TDFME on ulcer index could also be due to Kaempferol reported to be present in *T.divaricata* flowers [33]. It is reported to exhibit gastroprotective effect [34]. The gastroprotective effect is attributed to the anti-oxidant property, which is linked to the flavonoids and polyphenolic compounds [34-36]. These compounds most likely inhibit gastric mucosal injury by scavenging stress-generated oxygen metabolites [37]. Extensive damage to the gastric mucosa by stress leads to increased neutrophil infiltration into ulcerated gastric tissue. These neutrophils which are a major source of inflammatory mediators, inhibit gastric ulcer healing by mediating lipid peroxidation through the release of highly cytotoxic and tissue damaging reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants. Supression of neutrophil infiltration during inflammation was found to enhance gastric ulcer healing [38]. Further, there is a possibility of synergism between alkaloidal contents and the flavonoid for the same.

Conclusion

In conclusion, the present study demonstrated that *Tabernaemontana divaricata* Flower Methanolic Extract (TDFME) possesses acid neutralizing, anti-secretory and ulcer preventive properties and thereby produces significant gastroprotective effect at a dose of 500 mg/kg. Further studies are required to identify responsible bioactive molecules and to confirm underlying mechanism of action.

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