Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (2):55-60
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Assessment of gastroprotective activity of *Tabernaemontana divaricata* extract in rats

Chanchal N. Raj[1*], A. Balasubramaniam[2] and Sayyed Nadeem[2]

[1]Department of Pharmacy, Karpagam University, Coimbatore, Tamilnadu, India
[2]Technocrats Institute of Technology-Pharmacy, Bhopal, M. P., India

---

ABSTRACT

Present study concerns with the evaluation of gastroprotective effects of ethanolic and aqueous extracts of Tabernaemontana divaricata leaves against ethanol/HCl, aspirin and indomethacin induced gastric mucosal injury in rats. Different groups of rats in each model were treated with ethanolic (TDEE) and aqueous extracts (TDAE) of Tabernaemontana divaricata leaves at the test doses of 100, 200 and 400 mg/kg, p.o. and standard drug omeprazole (20 mg/kg, p.o.). Both the extracts of Tabernaemontana divaricata leaves have provided a significant gastroprotective effects which has been confirmed by determining and comparing the ulcer index in the test group with vehicle control group. The ulcer index in the test group (treated animals) was found to be significantly less in all the models compared to vehicle control animals. Among the extracts, TDEE at higher dose i.e. 400 mg/kg, p.o. was found to have a significant gastroprotective effects in all the models, further, which has been confirmed by a significant decrease ($P < 0.001$) in ulcer index in the test groups which is comparable to standard drug omeprazole ($P < 0.001$). Thus the present study supports the traditional folklore claim and reveals that the leaves of Tabernaemontana divaricata possess Gastroprotective activity.

Keywords: Gastroprotective, *Tabernaemontana divaricata*, omeprazole, ulcer, ulcer index and extracts.

INTRODUCTION

For more than a century, peptic ulcer is one of the most common gastrointestinal diseases which have been a major cause of morbidity and mortality [1, 2]. Peptic ulcers, also known as “ulcus pepticum” are a common disorder of the entire gastro intestinal tract (GIT) which is exposed to gastric acid and pepsin and occurs mainly in the stomach and the proximal duodenum [3].

The etiology of peptic ulcers is not exactly known but it may be develop due to an imbalance between aggressive factors such as hydrochloric acid (HCl), pepsin, leukotrienes (LTs), refluxed bile, reactive oxygen species (ROS), *H.pylori* and the defensive factors such as gastric mucus, high mucosal blood flow and high mucosal turnover rate that work towards maintenance of mucosal integrity [4,5] and bicarbonate secretion, surface active phospholipids, prostaglandins (PGs), nitric oxide, and innate resistance of mucosal cells [6].

Agents currently available for the treatment of gastric ulcers includes antacids (systemic and nonsystemic) and act by either reducing gastric acid secretion such as H2 blockers, anti muscarinic agents, proton pump inhibitors, acting as physical barriers such as colloidal bismuth , sucralfate, subcitrate or increasing the mucous and bicarbonate secretion such as prostaglandin analogues, carbenoxolone [7]. Even though these agents are effective in healing of gastric ulcers and reduced the morbidity rates, but produce many adverse effects such as relapse of the disease, and expensive too [8,9]. Considering the above facts there is an urgent need to develop some novel therapeutic agents which may have less adverse effects in treating the ulcers.

Herbs are a rich source of therapeutically active principles possessing several antioxidants. Above facts has widen the interest in identification and scientific validation of therapeutically active agents that have been used as traditional folklore medicines in the treatment of gastric ulcers and related diseases.

Tabernaemontana divaricata (Family: Apocynaceae, synonym *Ervatamia coronaria*) shrub or small tree, usually glabrous, distributed in tropical countries as a garden plant and found in Konkan, North Kanara, Western ghats in Malabar, throughout North India and Travencore. [10-12]. In traditional medicine *Tabernaemontana divaricata* is used to treat various diseases like ulceration, vomiting, epilepsy, abdominal tumours, eye infections, fractures, fever, headache, inflammation, mania, oedema, leprosy, diarrhea [13]. It is also used as anthelmintic, antihypertensive, aphrodisiac, emmenagogue, purgative, remedy against poisons and tonic to the brain, liver and spleen [14, 15]. Therapeutically active constituents from *T. divaricata* include alkaloids, terpenoids, steroids, flavonoids, tannins, phenyl propanoids, phenolic acids etc. Considering the traditionally reported activity associated with the plant *Tabernaemontana divaricata* (TD), it was planned to study the gastro protective effects of leaves extract (viz: ethanolic and aqueous).

MATERIALS AND METHODS

Plant material

The leaves of *Tabernaemontana divaricata* (TD) were collected in January, 2010, from Bhopal, M.P., India. The plant was identified and authenticated by Dr. D. V. Amla, Deputy Director, National Botanical Research Institute, Lucknow, India, and a voucher specimen No. Tit/NBRI/CIF/141/2009 was deposited in Department of Pharmacognosy and Phytochemistry, TIT-Pharmacy, Bhopal.

Preparation of extract

The leaves were dried in shade and stored at 25ºC, powdered, passed through sieve no.40. The dried powdered leaves of TD (500g) were first defatted with Petroleum Ether (60- 80°C) and later extracted with ethanol and distilled water separately by maceration for 5 days. After completion of the extraction, the solvent was removed by distillation and concentrated *in vaccuo* (40°C) to yield ethanolic and aqueous extract respectively.

Preliminary phytochemical screening of TD

The preliminary phytochemical investigation was carried out with ethanolic and aqueous extracts of leaves of *T. divaricata* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods [16-17].

Animals

Male wistar rats weighing 200±20 g were provided by the animal house of TIT Pharmacy, Bhopal, from the stock originally purchased from, National Institute of Nutrition, Hyderabad, India. Animals were made available with the standard animal feed and water supply *ad libitum* before the experiments. The animal studies were approved by the Institutional Animal Ethics Committee (Reg. no. 831/bc/04/CPCSEA), New Delhi, India. For each experimental study rats were starved for 24h with access to water only.

Drug and chemicals

Indomethacin (Torrent Research Centre, Gandhinagar), ethanol, HCl LR, aspirin (Himedia Laboratories, Mumbai) and omeprazole (Kopran Pharma Ltd. Mumbai) were used in present study.

Acute toxicity study

Acute toxicity study was carried out for the extracts of TD following Organization of economic co-operation and development (OECD) guidelines (OECD guideline, 2001) [18]. The extract was dissolved in distilled water in a dose of 2 g/kg body weight and orally administered to overnight-fasted, healthy rats (n = 6).The animals were observed continuously for 24 h for mortality.

Ethanol/ HCl induced gastric ulceration in rats (Gastric cytoprotection methods)

Gastric cytoprotective effects of *Tabernaemontana divaricata* was evaluated by ethnol/HCl induced ulceration model in rats. Starved rats with free access to water were treated with different doses of *T. divaricata* (100, 200 and 400 mg/kg, p.o.). Group I was kept as vehicle control (10 ml/kg, normal saline) while group II was treated with omeprazole (20mg/kg, p.o.) a standard drug. Group III- V were treated with ethanolic extract of *T. divaricata* leaves (TDEE) (100, 200 and 400 mg/kg, p.o.) and group VI – VIII with aqueous extract of *T. divaricata* leaves (TDAE) (100, 200 and 400 mg/kg, p.o.). One hour after the drug administration the animals received 1ml of a mixture of 70% alcohol+5% HCl as ulcerogenic agent. The animals were sacrificed 1 h after the ulcerogenic dose of ethanol/HCl mixture by cervical dislocation. The stomach removed, cut opened along the greater curvature washed

with normal saline and observed for the severity of the ulcers. Ulcer index and % ulcer protection were calculated by using the methods described earlier [19, 20].

$$\text{Percentage protection} = \frac{(\text{Ulcer index of control group} - \text{Ulcer index of treated group})}{\text{Ulcer index of control group}} \times 100$$

Acetylsalicylic acid (ASA) induced gastric ulcer

24 h fasted rats were treated with following treatment schedule as: Group I received 10 ml/kg of normal saline (control group), group II , Omeprazole (20 mg/kg body weight, p.o.), Group III- V, ethanolic extract of *T. divaricata* leaves (TDEE) (100, 200 and 400 mg/kg, p.o.) and group VI – VIII with aqueous extract of *T. divaricata* leaves (TDAE) (100, 200 and 400 mg/kg, p.o.). One hour later 200mg/kg per oral of Acetyl salicylic Acid (Aspirin) was administered as an ulcerogenic agent. The animals were sacrificed 4 h after Aspirin dosing; stomach was removed and observed for percent protection of ulcerative lesions [3, 21, 22].

Indomethacin induced gastric ulcer

Male Wistar rats (200±20 g) were deprived of food for 24 h with free access to water prior to the experiment. In indomethacin induced gastric ulceration method Group I was kept as vehicle control (10 ml/kg, normal saline) while group II was treated with omeprazole (20mg/kg, p.o.) a standard drug. Group III- V were treated with ethanolic extract of *T. divaricata* leaves (TDEE) (100, 200 and 400 mg/kg, p.o.) and group VI – VIII with aqueous extract of *T. divaricata* leaves (TDAE) (100, 200 and 400 mg/kg, p.o.) following 1 h before administration of 20 mg/kg per oral of indomethacin. Six hour after indomethacin administration the animals were sacrificed, their stomach removed and examined for ulcer protection [19, 23]. Ulcer score: the numbers of ulcers were counted using magnifying lenses. Each ulcer was then measured with a vernier caliper to assess the diameter. Ulcer index was determined by scoring method of Suzuki et al. [24].

Statistical analysis

The results are expressed as mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) after Tukey's multiple comparison test. $P < 0.05$ was considered statistically significant in all the cases.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of TD

Phytochemical screening of TD revealed the presence of alkaloids, tannins, resins, proteins, amino acids, flavonoids, saponins, phenols, glycosides, steroids, tri-terpenoids.

Effect of *Tabernaemontana divaricata* on Ethanol/ HCl induced gastric ulceration in rats

The ethanolic and aqueous extract of leaves of *Tabernaemontana divaricata* was evaluated for cytoprotective action against ethanol/HCl induced ulceration in rats. Severe ulceration is produced after the oral administration of ethanol/HCl and pretreatment of rats with *Tabernaemontana divaricata* extracts (viz: ethanolic and aqueous) offer a better protection against Ethanol/HCl induced gastric ulceration as compared to the control group. TDEE dose dependently reduced the incidence and severity of ulceration in ethanol/HCl induced ulcer model and offered a percentage protection of 29.15, 38.99 and 46.71 % at 100, 200 and 400 mg/kg respectively (**Table 1**). Among the extracts of TD, TDEE offered a better protection than TDAE when compared to standard drug omeprazole ($P < 0.001$, 52.27 %) while TDAE shows its maximum gastroprotective effect at higher dose only i.e. 400 mg/kg (3.86± 1.05, 25.48%, $P<0.05$).

Table 1: Effect of *Tabernaemontana divaricata* on Ethanol/ HCl induced gastric ulceration in rats

Group	Treatment	Dose	Ulcer index (mean ± SEM)	% Protection
Group I	Control(normal saline)	10 ml/kg, p.o.	5.18 ± 0.75	
Group II	Omeprazole	20 mg/kg, p.o.	2.48 ± 1.75***	52.27
		100 mg/kg, p.o.	3.67 ± 1.75*	29.15
Group III-V	TDEE	200 mg/kg, p.o.	3.16 ± 1.65*	38.99
		400 mg/kg, p.o.	2.76 ± 0.14***	46.71
		100 mg/kg, p.o.	4.17 ± 0.50 ^{ns}	19.49
Group VI-VIII	TDAE	200 mg/kg, p.o.	4.00 ± 2.05 ^{ns}	22.77
		400 mg/kg, p.o.	3.86 ± 1.05*	25.48

Values are expressed as mean ±S.E.M. (n = 6). Ulcer index calculated as compared to control group. ^{ns} not significant $P > 0.05$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (One-way ANOVA followed by Tukey's post hoc test).

Effect of *Tabernaemontana divaricata* on Aspirin (ASA) induced gastric ulceration in rats

The results for the study of *Tabernaemontana divaricata* leaves extracts (viz: ethanolic and aqueous) on aspirin induced ulceration are depicted in **Table 2**. *Tabernaemontana divaricata* leaves extracts at higher dose significantly reduced the ulceration produced by aspirin. The animals treated with TDEE and TDAE at higher doses i.e. 400 mg/kg significantly decreases the ulcer index by 1.99 ± 0.36 ($P < 0.001$) and 2.52 ± 0.12 ($P < 0.05$) respectively when compared with diseased control rats. TDEE at the dose of 400 mg/kg afforded 36.21 % ($P < 0.001$) protection against the ulcer, which is comparable to Omeprazole (20 mg/kg, p.o.) exhibiting 45.83 % ($P < 0.001$) protection against aspirin induced gastric ulceration. Thus, TD exhibits a dose dependent protection against the ulcer induced by aspirin.

Table 2: Effect of *Tabernaemontana divaricata* on Aspirin (ASA) induced gastric ulceration in rats

Group	Treatment	Dose	Ulcer index (mean ± SEM)	% Protection
Group I	Control(normal saline)	10 ml/kg, p.o.	3.12 ± 0.5	
Group II	Omeprazole	20 mg/kg, p.o.	1.69 ± 0.7***	45.83
		100 mg/kg, p.o.	2.68 ± 0.87*	14.10
Group III-V	TDEE	200 mg/kg, p.o.	2.48 ± 0.77*	20.51
		400 mg/kg, p.o.	1.99 ± 0.36***	36.21
		100 mg/kg, p.o.	2.99 ± 0.71^{ns}	4.16
Group VI-VIII	TDAE	200 mg/kg, p.o.	2.97 ± 0.34 ^{ns}	4.80
		400 mg/kg, p.o.	2.52 ± 0.12*	19.23

Values are expressed as mean ±S.E.M. (n = 6). Ulcer index calculated as compared to control group. ^{ns} *not significant P > 0.05,* *** *P < 0.001,* ** *P < 0.01,* * *P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).*

Effect of *Tabernaemontana divaricata* on Indomethacin induced gastric ulceration in rats

Animal pretreated with *Tabernaemontana divaricata* leaves extracts (TDEE and TDAE) exhibits gastroprotective effects against the indomethacin induced ulceration, as compared to control animals. Among the extracts of TD, the protection was statistically significant in TDEE at all the test doses viz. 100, 200 and 400 mg/kg (26.40, 33.42 and 48.03 % respectively). Omeprazole (20 mg/kg) offered a significant protection (64.88 %, $P<0.001$) as compared to control group (**Table 3**) while TDAE showed its maximum gastroprotective effect (ulcer index 2.45 ± 0.7**,** percentage protection 31.17 %, $P < 0.01$) at higher dose i.e. 400 mg/kg. Thus TD acts in a dose dependent manner in indomethacin induced ulceration.

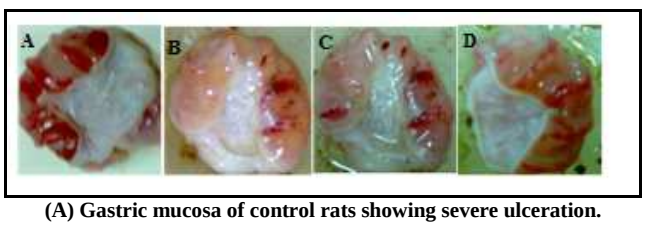
Table 3: Effect of *Tabernaemontana divaricata* on Indomethacin induced gastric ulceration in rats

Group	Treatment	Dose	Ulcer index (mean ± SEM)	% Protection
Group I	Control(normal saline)	10 ml/kg, p.o.	3.56 ± 0.6	
Group II	Omeprazole	20 mg/kg, p.o.	1.25 ± 0.5***	64.88
		100 mg/kg, p.o.	2.62 ± 0.4*	26.40
Group III-V	TDEE	200 mg/kg, p.o.	2.37 ± 0.5**	33.42
		400 mg/kg, p.o.	1.85 ± 0.7***	48.03
		100 mg/kg, p.o.	3.00 ± 0.4 ^{ns}	15.73
Group VI-VIII	TDAE	200 mg/kg, p.o.	2.97 ± 0.78^{ns}	16.57
		400 mg/kg, p.o.	2.45 ± 0.7**	31.17

Values are expressed as mean ±S.E.M. (n = 6). Ulcer index calculated as compared to control group. ^{ns} *not significant P > 0.05,* *** *P < 0.001,* ** *P < 0.01,* * *P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).*

The results of TD leaves extracts obtained are comparable to standard drug and the most significant results by both TDEE and TDAE along with standard and control are depicted in **Figure 1**.

Figure 1: Histological section of rat's stomach showing ulcer lesions



(A) Gastric mucosa of control rats showing severe ulceration.
(B) Gastric mucosa of Omeprazole group showing less intense ulceration.
(C) Gastric mucosa of TDEE treated group showing mild ulceration.
(D) Gastric mucosa of TDAE treated group showing less ulceration.

From the **figure 1** it has been ascertained that the Omeprazole treated rats shows normal mucosa. Among the extracts of TD, TDEE have a prominent role as gastroprotective agent compare to TDAE.

Tabernaemontana divaricata leaves extracts were evaluated for its gastro protective effects employing asprin, alcohol/HCl and indomethacin induced ulcer models which represent some of the most common causes of gastric ulcer in humans.

Many factors and mechanisms have been involved which influence gastric ulceration and causes the gastric mucosal damage. In the present study different models have been used to induce gastric ulcer involving, depletion of gastric wall, mucin mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production [25]. NSAID's like indomethacin and aspirin inhibits prostaglandin synthesis thus causes gastric mucosal damage by decreasing prostaglandin levels [26]. The effect of ethnolic extract of *Tabernaemontana divaricata* (TDEE) was significant in protecting gastric mucosa against aspirin and indomethacin induced ulcers at all the test doses.

Ethanol/HCl increases superoxide anion, hydroxyl radical production and lipid peroxidation in the gastric mucosa and also reacts with most of the cell components and other reactive metabolites [27]. This result into structural and functional changes in cells that eventually causes enhanced oxidative damage.

Ethanol/ HCl and NSAID's are responsible for cytodestructive damage in the gastric mucosa of rats [28]. *Tabernaemontana divaricata* was found to be effective against both the models viz ethanol/ HCl and NSAID's induced ulceration, thus, exhibiting a cytoprotective action.

Results of the study revealed cytoprotection as the major mechanism responsible for the anti-ulcer activity of *Tabernaemontana divaricata* exhibiting significant anti-ulcer effects but not the antisecretory effect. Though the exact active constituent has not been reveled which may responsible to have the anti-ulcer effects of *Tabernaemontana divaricata* but various studies done in the past supports the involvement of flavonoids as an anti ulcerogenic agent in various experimental models of gastric and duodenal ulcer. Thus the study establishes a significant antiulcer and cytoprotective effect of *Tabernaemontana divaricata* leaves extract. However, further studies are required to establish its exact mode of action and the active principles involved in its antiulcer effect.

CONCLUSION

Our findings confirmed the gastroprotective activity of *Tabernaemontana divaricata,* specifically TDEE. From the study it may be concluded that the test drug can be replaced as an alternative agent in preventing and treating the ulcer. However, further studies are needed to evaluate the safety profile of the plant as safe and therapeutic antiulcer agent.

Acknowledgement
The authors would like to thank the management of TIT group of institution, Bhopal and Karpagam University, Coimbatore, for providing necessary facilities to carry out study.

REFERENCES

[1] PC Dandiya; SK Kulkarni. Introduction to Pharmacology, Vallabh Prakashan, New Delhi, **2005**; pp. 247.
[2] ZP Lima; JA Severi; CH Pellizzon, ARMS Brito; PN Solis; A Cáceres; LM Girón,; W Vilegas; CA Hiruma-Lima. *J. Ethnopharmacol*., **2006**, 106, 29-37.
[3] Vandana Panda; Madhav Sonkamble. *Functional Foods in Health and Disease,* **2012**, 2(3), 48-61.
[4] JL Wallace; DN Granger. *FASEB J*., **1996**, 10, 731-740.
[5] D Bandyopadhyay; K Biswas; M; Bhattacharyya, RJ Reiter; RK Banerjee. *Curr. Mol. Med.,* **2001**, 1, 501-513.
[6] DJ Cullen; GM Hawkey; DC Greenwood. *Gut.,* **1997**, 41 (4), 459-462.
[7] HP Rang; MM Dale; JM Ritter; RJ Flower; Henderson G. In Pharmacology, 7th ed., Elsevier Churchill Livingstone, Edinburgh, UK, **2003;** pp. 575-584.
[8] BM Srikanta; MN Siddaraju; SM Dharmesh. *World J. Gastroenterol*., **2007**, 13(39), 5196-5207.
[9] B Maitya; S Chattopadhyay. *Curr. Bioactive Comp*., **2008**, 4, 225-244.
[10] KM Nadkarni. Indian Materia medica, Popular book depot, Bombay, **1954**; Vol. I, pp. 516-518.
[11] P Sharma; PM Mehta. Dravyaguna vignyan, The Chowkhamba Vidyabhawan, Varansi, **1969**; Part II & III, pp. 586.
[12] KR Kirtikar; BD Basu. Indian Medicinal Plants, Periodical Experts, **1975**; Vol. II, pp. 1052- 1053.
[13] A Ghani. Medicinal Plants of Bangladesh; Chemical constituents and uses, Asiatic Society of Bangladesh, Dhaka, **2003**; 381, pp. 1-16.
[14] Khan Mohammed Safwan Ali. *Malaysian J. Pharm. Sci.,* **2010**, 1, 104-105.
[15] AFR Hoernle. The Bower manuscript. Archaeological Survey of India, New Imperial Series, Superintendent of Government Printing, Calcutta, India, **1893-1912**; Vol.22, pp. 18-20,

[16] KR Khandelwal. Practical Pharmacognosy, 6th ed., Nirali Prakashan publisher, Pune, **2006**; pp. 15- 23.
[17] CK Kokate. Practical Pharmacognosy, 4th ed., Vallabh Prakashan publisher, New Delhi, **1994**; pp.110-116.
[18] OECD Guidelines—"Guidance document on acute oral toxicity testing" (2001) series on testing and assessment no. 24, Organization for economic co-operation and development, OECD environment, health and safety publications, Paris. (www.oecd.org/ehs) accessed on 12th January **2007**.
[19] Parmar NS; Desai JK. *Indian J. Pharmacol.*, **1993,** 25, 120-135.
[20] R Singh; J Madan; H Singh Rao. *Phcog. Mag*., **2008**, 4, 232-235.
[21] SK Kulkarni. Handbook of experimental pharmacology, 3 ed, M. K. Jain, Pitampura, Delhi, **2005**; pp. 168-170.
[22] MN Ghosh. Fundamental of experimental pharmacology, 3 ed, Kolkata, **2005;** pp. 180,197.
[23] M Asano; Y Kuribaysshi; Y Ryokawa; T Hashizume; A Akashi. *Arzneim-Forsch/Drug Res*., **1990,** 40 (1), 276-281.
[24] Y Suzuki; M Hayashi; M Ito; I Yamagami. *Jap. J. Pharmacol.*, **1976**, 26, 471-480.
[25] U Bandyopadhyay; D Das; D Bandyopadhyay; M Bhattacharjee; RK Banerjee. *Cur. Sci*., **1999,** 76(1), 55-56.
[26] DA Brodie. *Am. J. Dig. Dis*., **1966,** 11, 231-241.
[27] D Bagchi ; O Carryl ; M Tran; R Krohn; DJ Bagchi; A Garg; M Bagchi; S Mitra; S Stohs. *J. Appl. Toxicol*., **1998,** 18, 3-13.
[28] A Robert; JE Nezmin; C Lancaster; AJ Hanchar. *Gastroenterogy*, **1979**, 76, 439-443.