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COMPARATIVE EVALUATION OF ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACTS OF STEMS AND LEAVES OF *TABERNAEMONTANA DIVARICATA*

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ABSTRACT

Objective:*To evaluate the anti-ulcer activity of ethanolic extracts of stems and leaves of Tabernaemontana divaricata in animal models.* ***Methods:*** *The effect was evaluated in pylorus ligation induced gastric ulcer, ethanol induced gastric ulcer, stress induced gastric ulcer and indomethacin induced gastric ulcer. The extracts were administered orally at two different doses of 200 mg/kg and 400 mg/kg. Omeprazole was used as standard. The anti-ulcer activity of T. divaricata was evaluated with the help of ulcer area and histopathological examination. Preliminary phytochemical screening and acute toxicity studies of T. divaricata were also carried out.* ***Results:*** *The healing of ethanol induced gastric ulcer was increased by both doses of the extracts. In pylorus-ligated rats, the extracts showed significant decrease in ulcer index, total acidity and increase in gastric mucosal content. The extracts also reduced the ulcer index in stress induced, ethanol and indomethacin induced gastric ulcers. Maximum anti-ulcer activity was found in leaves followed by stem. Administration of 2000 mg/kg extract did not show any acute toxicity in animals. Preliminary phytochemical analysis identified the presence of alkaloids in ethanolic leaf and stem extracts of T. divaricate.* ***Conclusion:*** *Etiology of ulcers produced in different ulcer models is diverse, since T. divaricata has been found effective in various models depending on its antiulcerogenic activity. T. divaricata and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence.*

KEY WORDS
Ethanolic extract, Ulcer index, T.divaricata, Omeprazole

INTRODUCTION:

PLANT PROFILE

Tabernaemontana divaricata commonly known as crepe jasmine is a beautifully shaped ever green shrub which forms symmetrical 6ft high mounds of glossy foliage. The species name divaricata means at an obtuse angle, since the branches tend to grow almost parallel to the ground giving shrub an attractive horizontal aspect, like many members of apocynaceae family. The stems of crepe jasmine extend a milky latex when broken. The large shiny leaves are deep green and 6 or more inches in length and 2 to 5 c.m in width. Blooms in spring but flowers appear sporadically all year.

Chemical constituents include alkaloids, terpenoids, steroids, flavanoids, phenyl propanoids, phenolic acids and enzymes. Since 1974, 66 different alkaloids are identified include indole type voacristine, voacangine, coronaridine, vobasine, dregamine. In folk medicine used for treatment of wide range of disorders like pain, inflammation, healing wounds, cuts, boils, oedema and fever. *T. divaricata* was reported to possess antioxidant, antinociceptive, anxiolytic, antidiabetic, antimicrobial, antifertility activities.

PROCUREMENT OF PLANT MATERIAL

For the present investigation the plant *Tabermontana divaricata* was collected in Warangal district of

Telangana. It was identified & authenticated botanist, prof K.S.Raju K.U. Warangal. A voucher specimen has been kept in our laboratory for future reference.

The collected plant material was thoroughly checked for presence of any foreign organic matter and then leaves, and stems were taken separately thoroughly dried under shade and powdered mechanically and sieved through N0.20 mesh sieve. The finely powdered leaves and stems were kept in airtight container until the time of use.

Extraction Procedure for Leaves and Stems

The extraction was carried out by cold maceration and mechanical shaking method. The solvent used was 95%

ethanol about 60grams of powder was soaked with 600ml of 95% ethanol for 12hr and then macerated at room temperature using a mechanical shaker for 4hrs. The extract was filtered off and marc was again soaked with the same volume of 95% ethanol for 12hrs and then further extracted for 4hrs and filtered. The filtered were then combined concentrated at 400C. Ethanol extracts of stems and leaves of *T. divaricata* at doses 200mg & 400mg.

Animals Used

Albino rats (180 to 200 gm) of either sex was maintained in a 12h light/dark cycle at a constant temperature 25^{0}C with free access to feed and water. All the animals were procured from Sairam Agency Hyderabad and maintained in the heightened environmental conditions (25±2ºC) 12h/12h light/dark cycles in the animal house All animals were fasted prior to the experiment, all experiments were carried out according to the guidelines of CPCSEA. Ethical committee clearance was obtained from IAEC (Regd. no. 1663/PO/Re/s/2016/CPCSEA).

Acute toxicity study

The acute toxicity of extracts of *T. divaricata* was determined as per OECD guidelines No.423 (acute toxic class method). It was observed at the extract was not lethal to the rats even at 2000mg/kg dose, hence 1/10th (200mg/kg) & 1/5th (400mh/kg) of this dose were selected for study.

Anti-ulcer activity

Ethanol Induced gastric ulcer

Animals were randomly divided 6 groups each of six rats. Group 1 treated with 4% v/v aqueous tween 80 (10mg/kg). Group 2&3 treated with ethanolic leaf extract of *T. divaricata* (200/400mg/kg) respectively for 14 days. Group 4&5 treated ethanolic stem extracts and group 6 treated with omeprazole (20mg/kg), were administered 30min prior to induction of gastric ulcer, on 14th day gastric ulcers were induced with ethanol at a dose of 8ml/kg to all groups by oral administration. The animals were anesthetized after 6hrs with ether and stomachs were excised along greater curvature and ulcer index for each rat was taken as mean ulcer score.

indo methacin Induced gastric ulcer

Animals were divided into six groups each of six rats. Group 1 treated with 4% v/v aqueous tween 80 (10mg/kg). Group 2&3 treated with ethanolic leaf extract of *T. divaricata* (200/400mg/kg) respectively for 14 days. Group 4&5 treated ethanolic stem extracts and group 6 treated with omeprazole (20mg/kg), were administered 30min prior to induction of gastric ulcer, on 14th day gastric ulcers were induced with indomethacin (400gm/kg) administered to all group after fasting for 24hrs. The animals were scarified 4hr after treatment with the ulcerogenic agent to assess the anti-ulcer activity and ulcer index were examined on the dissected stomach.

Pyloric ligation Induced gastric ulcer

Animals were divided into four groups each of six rats. Group 1 treated with 4% v/v aqueous tween 80 (10mg/kg). Group 2&3 treated with ethanolic leaf extract of *T.divaricata* (200/400mg/kg). Group 4&5 treated ethanolic stem extracts of *T.divaricata* (200/400mg/kg) respectively for 14 days and group 6 treated with omeprazole (20mg/kg), were administered 30min prior to induction of gastric ulcer, on 14th day, all group rats 1hr after drug administration. Animals were allowed to recover and stabilized in individual case and were deprived of water during post. Operative period after 4hr of surgery, rats were scarified by cervical dislocation and ulcer index

were examined on the dissected stomach as described below.

Cold Restraint stress induced ulcers.

Animals were divided into four groups each of six rats. Group 1 treated with 4% v/v aqueous tween 80 (10mg/kg). Group 2&3 treated with ethanolic leaf extract of *T.divaricata* (200/400mg/kg). Group 4&5 treated ethanolic stem extracts of T. (200/400mg/kg) respectively for 14 days and group 6 treated with omeprazole (20mg/kg), were administered 30min prior to induction of gastric ulcer, on 14th day, one hour after drug treatment the experimental rats were immobilized by strapping the hind limbs on wooden plank and kept for 1hr 30 min at temperature of 3-5^{0}C one hour later the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa, immediately after the animals were sacrificed their stomachs were dissected out cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant if any. The sum of the length (mm) of all lesions for each stomach was used as ulcer index (UI) and the percentage of inhibition (%I) was calculated as described by ngwlefack etal using formula %I=USC-UST/USC*100 Where USC=Ulcer surface area in control and UST=USA in treated animals.

Statistical Analysis

The data were expressed as mean ± SEM. The significance of difference among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Preliminary Phytochemical Screening

By performing various chemical tests it was found that ethanolic leaf and stem extracts of *T.divaricata* showed positive results for alkaloids, flavonoids, saponins, glycosides, steroids, carbohydrates and phenolics.

Acute Toxicity Studies

Acute toxicity study in which the animals treated with the ethanolic extracts of leaf and stem of *T.divaricata* at higher dose of 2000mg/kg did not manifest any significant normal signs behavioural changes body weight changes or macroscopic findings at any time of observation there was no mortality in the above mentioned dose at the end of the 14 days of observation.

Effect of ethanolic extracts of *T.divaricata* on gastric ulcer induced by ethanol

The ethanolic extracts of *T.divaricata* showed significant anti-ulcer effect against ulcers induced by ethanol in a dose dependent manner in ethanol induced ulcer model, ethanolic extracts of *T.divaricata* at a doses of 200 and 400mg/kg body weight showed protective effect of 54.65% and 66.45% respectively whereas Omeprazole showed protection index of 66.92% at a dose of 20mg/kg body weight.

Effect of Ethanolic Leaf and Stem extracts of *T.divaricata* on gastric ulcer induced by indomethacin

The ethanolic leaf and stem extracts of *T. divaricata* showed significant anti-ulcer effect against ulcers induced by indomethacin in a dose dependant manner. In induced ulcer model ethanolic extract of *T. divaricata* at dose of 200 and 400 mg/kg body weight showed protective effect of 54.32% and 67.89% respectively, whereas Omeprazole showed protection index of 69.68% at dose of 20mg/kg body weight.

Effect of Ethanolic Leaf and Stem extracts of *T.divaricata* on gastric ulcer induced by Pylorus ligation

The ethanolic leaf and stem extracts of *T.divaricata* showed significant anti-ulcer effect against ulcers induced by pylorus ligation in a dose dependent manner in PL induced ulcer model. Ethanolic leaf and stem extracts of *T.divaricata* at a dose of 200 and 400 mg/kg body weight showed protective effect of 55.84% and 62.68%, whereas Omeprazole showed protection index of 68.42 % at dose of 20mg/kg body weight.

Effect of Ethanolic Leaf and Stem extracts of *T. divaricata* on gastric ulcer induced by cold restraint stress

The ethanolic leaf and stem extracts of *T. divaricata* showed significant anti-ulcer effect against ulcers induced by cold restraint stress in a dose dependent manner in the gastric ulcer induced by cold restraint stress. Ethanolic leaf and stem extracts of *T. divaricata* at a dose of 200 and 400 mg/kg body weight showed protective effect of 49.64 and 56.38 % respectively, whereas Omeprazole showed protection index of 62.11% at dose of 20mg/kg body weight in above models.

DISCUSSION AND CONCLUSION

The result of this study shows that the ethanolic extracts from leaf and stem of *T. divaricata* exert protective effects against ethanol, indomethacin pylorus ligation and cold restraint stress induced gastric mucosal damage. The anti-ulcer effect of *T. divaricata* was tested against gastric lesions by ethanol the experimental model related to lesion pathogenesis of ethanol induced gastric mucosal injury *in vivo*, *T. divaricata* presented the mucosal lesions induces by ethanol.

Results in the present study also indicate similar alternations in the antioxidant status after ethanol induced ulcers. The gastric mucosal protection against ethanol can be mediated through a number of mechanisms that include enhancement of gastric mucosal defence through increase in mucus and bicarbonate production, reducing the volume of gastric acid secretion or by simply ventralyzing the gastric acidity.

ELTB may either reduce the gastric acid secretion or enhance the barrier defence of the mucosal wall, ELTB dose dependant inhibition in ethanol induced gastric lesions. Histopathological studies suggest that the ethanol damage to the gastrointestinal mucosa starts with micro vascular injury namely disruption of the vascular endothelium resulting increased vascular permeability edema formation and epithelial lifting.

Their antiulcerogenic potency was tested against indomethacin induced ulcer, indomethacin is a cyclo oxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barriers as well know that prostaglandins synthesized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogenesis. Thus, when the ulcers lesions are induced by indomethacin the cytoprotective effect of the anti-ulcer agent can be mediated through endogenous prostaglandins. The results obtained show that the mean ulcer index was significantly reduced in the ethanolic extracts from leaf and stem extracts of *T. divaricata* treated groups compared to their respective controls *T. divaricata* extracts may be stimulate the secretion of prostaglandins or possess prostaglandins like substances.

In order to probe the effectiveness of *T. divaricata* extracts in preventing gastric ulcer and also assess their antisecretory activity, they were tested against pylorus ligation and cold restraint stress induced ulcers are results of auto digestion of the gastric mucosal barrier probably due to excess production and accumulation of HCL in the stomach. Gastric acid is an important factor for genesis of ulceration in pylorus ligated rats. The activation of the vagus vagal reflux by stimulation of gastro protective effect of omeprazole is mediated through block of acid secretion by inactivation of H+/Ic appase. The result of this study shows that the ethanolic extracts from leaf and stem of *T. divaricata* exert protective effects against ethanol, indomethacin pylorus ligation and cold restraint stress induced gastric mucosal damage. Further our results fortify the pharmacological importance of ETB as an antiulcer agent. Etiology of ulcers produced in different ulcer models is diverse, since ETB has been found effective in various models depending on its anti-ulcerogenic activity. ETB and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence.

RESULT DATA:

Effect of ELTB and ESTB on ulcer index by pyloric ligation, ethanol and Indomethacin induced ulcers in rats.

Treatment	Ulcer index			
	Pyloric ligation	Ethanol	Indomethacin	Cold restrain stress
Vehicle Control	4.56±0.23	4.83 ±0.46	4.32 ±0.14	4.41 ±0.23
ELTB 200 mg/kg	3.34±0.54*	3.53±1.16*	3.52±0.98*	3.45±0.67*
ELTB 400 mg/kg	2.48±0.43*	2.95±1.08*	2.46±0.98*	2.65±0.87*
ESTB 200 mg/kg	2.35±0.25*	2.34±0.15*	2.34±0.42*	2.65±0.23*
ESTB 400 mg/kg	1.55±0.87**	1.78±0.36**	1.56±0.87**	2.42±0.34**
Omeprazole 200mg/kg	1.68±.27**	1.76±0.16**	1.38±0.21**	1.94±.0.24**

Values are expressed as Mean ± SEM, n= 6, *p < 0.05 and ** p < 0.01 when compared with vehicle control group. (Statistically analysed by one-way ANOVA followed by Dunnet's t-test.).

Treatment	% Inhibition of ulceration			
	Pyloric ligation	Ethanol	Indomethacin	Cold restrain stress
Vehicle Control	-	-	-	-
ELTB 200 mg/kg	43.15	40.68	41.16	42.15
ELTB 400 mg/kg	52.23	58.09	50.04	49.45
ESTB 200 mg/kg	55.84	54.65	54.32	49.64
ESTB 400 mg/kg	62.68	66.48	67.89	56.38
Omeprazole 200mg/kg	68.42	66.92	69.68	62.11

Data are represented as mean±SEM. Statistical analysis was done by one way ANOVA followed by dunnets multiple comparison test P<0.01 and P<0.001 as compared to content presence receptors in the anti-gastric mucosa in the hyper secretion model of pylorus ligature believed to increase gastric acid secretion. The current data clearly demonstrated that ELTB in a dose dependent manner decreased hydrogenical mechanism which has relationship to antisecretory activity.

To further confirm its anti-ulcerogenic effect we have evaluated the efficacy of ELTB against cold restraint stress induced ulcer model. Gastric ulceration induced by stress is probably mediated by the presence of acid, increase in gastric motility most cell degranulation, decreased gastric mucosal blood flow, decreased prostaglandin synthesis and augmented excretion of glycoproteins in the mucus, moreover, stress induced ulcer can be prevented partially or entirely by vagotomy vagal over activity has been suggested to be the principle factor in stress induced ulceration any of these factors could play role in genesis of stress induced ulcers oral administration of ethanolic extracts of T.b showed dose dependant inhibition of gastric ulceration induced by cold restraint stress. The ethanolic extracts of T.b at a dose of 400 mg/kg showed similar activity to that of omeprazole proton pump inhibitor, which is used to heal stomach & duodenal ulcers.

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