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# EVALUATION OF ETHANOLIC EXTRACT OF ROOTS OF *TRIANTHEMA TRIQUETRA* FOR ANTI-ULCER ACTIVITY IN ALBINO RATS

**Syed Safiullah Ghori* and Tahmeena Humaira**

Department of Pharmacology, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Telangana, India.

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***Corresponding Author**
**Syed Safiullah Ghori**
Department of Pharmacology, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500001, Telangana, India.

## ABSTRACT

The aim of the present investigation was to evaluate the antiulcer activity of *Trianthema triquetra* roots ethanolic extract in Wistar albino rats using NSAIDs & Ethanol induced ulcer models. Plant material was collected from Tirupati A.P, during the month of June 2014. The roots were made free from dust and foreign material and dried under shade at room temperature. After a week the roots were powdered and passed through a sieve. The powder was weighed (500 gm) and was extracted by successive solvent extraction process. The yield of ethanolic extract of trianthema triquetra was found to be 7.31% W/W. Phytochemical screening was carried out for the detection of the phytoconstituents by simple qualitative methods. The dosing was designed as per the acute toxicity study reported earlier. The anti-ulcer activity was performed by NSAID and ethanol induced ulcer model at two different doses, 200mg/kg and 400mg/kg. Wistar rats weighing(130-150gm) of either sex were used for the study. There was significant reduction of ulcers in the test groups observed in both NSAID and ethanol induced ulcer models. TTEE exhibited anti-ulcer activity in both curative and prophylactic experimental models which provides the evidence of its use as a potent anti ulcer drug.

**KEYWORDS:** *Trianthema triquetra* roots ethanolic extract (TTEE); NSAID,Ethanol, Intraperitonial.

## INTRODUCTION

Ulcer is an acute inflammatory lesion. It may be defined as the gradual destruction of the stomach, duodenum or both by acid gastric juice and is caused by disruptions of the gastric mucosal surface and repair system.[1] Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases. Non-steroidal anti-inflammatory drugs (NSAIDS) such as indomethacin, aspirin and ibuprofen are know to cause gastric ulcer, especially when abused[2] Trianthema triquetra belonging to the family Aizoaceae is an annual herb which is traditionally used in indigenous medicine for gastric disorders and jaundice. Therefore the present study was conducted to evaluate anti-ulcer activity of Trianthema triquetra root on different ulcer models in albino rats. Due to the reported side effects of available antiulcer drugs, focus have been shifted towards natural products as the new sources of antiulcer agents. With the growing interest in natural medicine, various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcer.[3] Furthermore, medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers. Trianthema triquetra root has been reported to show several pharmacological properties. This plant has so far not been screened for anti-ulcer activity.

## MATERIAL AND METHODS

The roots of *Trianthema triquetra* plant were collected from Tirupathi and authentified by Dr.k Madhava shetty, Department of Botany, Sri Venkateshwara University, Tirupathi. Omeprazole was procured from Dr. Reddy laboratory, Diclofenac sodium from Novartis Mumbai, Anaesthetic ether from Sigma Solvents and Pharmaceuticals, Mumbai, Absolute alcohol, Chloroform and Formalin from Nice-cochin. All the drugs and chemicals used were of analytical grade. The animals required for pharmacological activity were procured from animal house of syncorp institute Hyderabad.

### Preparation of plant extract

The collected fresh roots were dried in shade (2 days) and were grinded into coarse powder with the use of grinder. The powder obtained was weighed separately and transferred to a round bottomed flask and then subjected to continuous heat extraction with soxhlet apparatus using 90% ethanol for 24 hours.[4] Then the extract of ethanol was concentrated. Extract

obtained was dried by placing it on a big petriplate on electric water bath (70ºC) and then kept in an oven at 30ºC for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators.

**Preliminary phytochemical studies**

Ethanolic extract of the root of *Trianthema triquetra* was subjected to chemical tests for the identification of active constituents.[5]

**Evaluation of Anti -Ulcer Activity**

Two animal models (NSAIDs & Ethanol induced) were employed to evaluate the Anti-ulcer activity of *Trianthema triquetra* root extract.

**NSAIDs – induced ulcer[6]**

Healthy male Wistar albino rats were weighing between 160-200gm were taken for the study. The animals were divided in to four groups containing, 6 animals as follows.

Group – I (Control): Diclofenac 20mg/kg, oral

Group – II (Standard): Diclofenac 20mg/kg &Omperazole 20mg/kg, oral

Group – III Ethanolic extract of Diclofenac 20mg/kg &*Trianthema triquetra* 200mg/kg orally.

Group – IV: Diclofenac 20mg/kg &Ethanolic extract of *Trianthema triquetra* 400mg/kg orally.

The animals in all the groups were kept fasting for 24 hr. After that animals of all groups received diclofenac sodium (20mg/kg). The oral feeding of water and diclofenac sodium was continued for 3 days. The animals of II group received Omperazole (20mg/kg). After 1 hour of oral administration of diclofenac. The groups III and IV received Trianthema triquetra root ethanolic extract at doses of 200 and 400 gm/kg orally after 1 hour of oral administration of diclofenac sodium. On 4th day the animals were sacrificed, stomach were removed and cut along the greater curvature to calculate the ulcer score and to measure the ulcer index.

**Ethanol induced ulcer model[7]**

The ulcers were induced by administering ethanol. All the animals were fasted for 24 hours before administration of ethanol. The Albino rats of male sex were divided into four groups, each consisting of six rats. First group represented the control group which received ethanol, Second group received omeprazole 20mg/kg and ethanol & Third and Fourth groups received

ethanolic extract of *T. triquetra* 200mg/kg and 400mg/kg and ethanol orally. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (0.5ml/100g) orally, after 45 min of test dose and omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized after 1hr with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored.

**Histopathology**

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the anti-ulcerogenic activity of *Trianthema triquetra*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-µm thick sections were cut using a rotary microtome. These sections were stained with haematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes[8]

**Statistical analysis**

The results were expressed as the mean ± SEM for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnet's test. Results were considered to be statistically significant at $P<0.05$.

**RESULTS**

**Acute toxicity study**

The Maximum safe dose of ethanolic extract of trianthema triquetra was 2000 mg/kg b.w. in rats[9]

**Phytochemical Evaluation**

The yield of ethanolic extract of trianthema triquetra was found to be 7.31% W/W. Preliminary phytochemical analysis revealed that the plant possessed phytoconstituents like alkaloids, tannins, Phytosterols, flavonoids.(tab no-1&tab no-2)

**Table No. 1: Percentage yield of ETT.**

| S.no. | Extract | Colour of extract | Consistency | Yield(%W/W) |
|---|---|---|---|---|
| 1. | Ethanolic | Dark reddish | Semi-solid | 7.31 |

**Table 2: Preliminary phytochemical screening.**

| Phytoconstituents | Presence or Absence |
|---|---|
| Carbohydrates | + |
| Glycosides | + |
| Fixed oils and fats | + |
| Gums & mucilage | - |
| Potein & amino acids | - |
| Saponins | + |
| Tannins | +++ |
| Phytosterols | + |
| Flavonoids | +++ |
| Alkaloids | ++ |

**Effect of ethanolic extract of *Trianthema triquetra* on Ethanol – induced ulcer in rats.**

Ethanol produced massive gastric ulcers in all the rats under study. Most of the ulcers were superficial in nature. There was bleeding in the stomach. Adhesion and dilation were also noted in stomach. Ulcer index was 11.92±0.85. Pretreatment of rats with root of ***Trianthema triquetra*** gave significant ($p<0.001$) protection for the animals from ethanol induced ulcers by 40.35, and 49.24 respectively. Omeprazole gave more protection (56.54) to the rats from ethanol induced gastric ulcers.(tab no-3)

**Effect of ethanolic extract of *Trianthema triquetra* on NSAIDs –induced ulcer in rats.**

In NSAID induced ulcer model the root extract at doses of 200 and 400 mg/kg showed significant gastro protective activity 49.49 % and 63.03 % compared with standard drug omeprazole showed 72.99 % (tab-4).

**ETHANOL INDUCED ULCER**

**Table.no. 3: Effect of ethanolic extract of *Trianthema triquetra* on Ethanol – induced ulcer in rats.**

| Treated groups | Ulcer Index | % Inhibition of Ulcer |
|---|---|---|
| Control (Ethanol 5ml/kg) | 11.92±0.85 | - - - - |
| Standard (Omeprazole 20mg/kg) | 5.18±0.34*** | 56.54% |
| ETT 200mg/kg | 7.11±0.51*** | 40.35% |
| ETT 400mg/kg | 6.05±0.67*** | 49.24% |

All the values were mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***$p$< 0.001, as compared to control group.

**NSAIDS INDUCED ULCER**

**Table.no.4: Effect of ethanolic extract of *Trianthema triquetra* on NSAIDs – induced ulcer in rats.**

| Treated groups | Ulcer Index | % Inhibition of Ulcer |
|---|---|---|
| Control (Diclofenac 20mg/kg) | 17.80±1.18 | - - - |
| Standard (Omeprazole 20mg/kg) | 4.80±0.07*** | 72.99 |
| ETT 200mg/kg | 8.99±0.62*** | 49.49 |
| ETT 400mg/kg | 6.58±0.47*** | 63.03 |

All the values were mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, ***$p$< 0.001, as compared to control group.

**Histopathological Studies**

**a) Group I Control**

Necrosis and agranulocytosis areas were gastric lesions were predominant over vast surface area, perforations with complete mucosal destruction were seen.

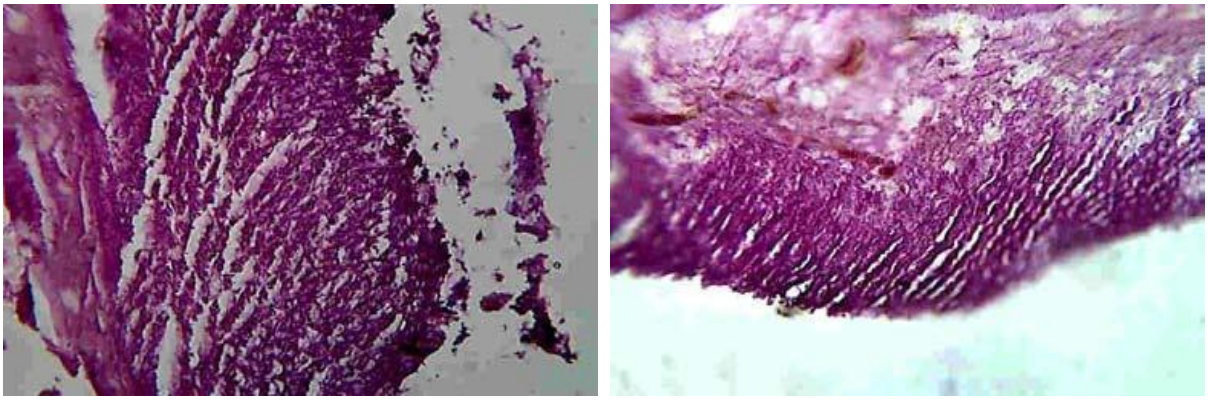
**b) Group II Standard (Omeprazole)**

No congestion, no necrosis and gastric lesions. Superficial mucosal layer and muscularis mucosa unaffected.

**c) Group III ETT 200mg/kg**

Less congestion and muscalaris mucosa remained unaffected.

**d) Group III ETT 400mg/kg: Less ulcer sites**



**DISCUSSION**

Peptic ulcer and gastritis have been associated with multi pathogenic factors and could be due to disturbances in natural balances between the aggressive factors (acid, bicarbonate, pepsin)

and maintenance of the mucosal integrity through the endogenous defense mechanism (defensive mechanisms of mucus, mucosal turnover and blood supply (mucosal barrier)[10] Generally various non-specific methods are used to restore these imbalances including regular food intake, adequate rest and avoidance of ulcerogenic agents (e.g. Tobacco, Alcohol and Coffee). Their aims are to attenuate and possibly block the gastric acid secretion or to enhance the mucosal defense mechanisms.[11] The latter can be achieved through increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis. In addition, there are also drugs, such as proton pump inhibitors, histamine (H2)-antagonists, anti-cholinergics and antacids, used in the treatment of ulcers. Despite the availability of many pharmaceutical products for the treatment of gastric ulcers in the market as mentioned above, their success were limited by presence of several adverse effects (e.g. Anaphylaxis reactions, Gynecomastia, Hematopoietic changes, Thrombocytopenia, Acute interstitial nephritis, Nephrotoxicity and Hepatotoxicity).[12] The reported side effects of available antiulcer drugs, focused have been shifted towards natural products as the new sources of antiulcer agents. With the increasingly growing interest in natural medicine, various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcer. Furthermore, medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers. trianthema triquentra root has been reported to exert several pharmacological properties such as a traditional medicine to relieve ulcer.. This plant has so far not been screened for anti-ulcer Activity. Thus, we take this opportunity to report the preliminary findings on anti-ulcer potential of trianthema triquentra root ethanolic extracts for the first time here. The present study demonstrated that ethanolic extracts significantly reduced gastric ulceration as indicated by the reduction in ulcer index in the ethanol and NSAIDS induced model.

## CONCLUSION

In conclusion, the present study provided preliminary data for the first time that the roots of *Trianthema triquetra* possesses significant anti-ulcer activity in animal models. It has gastric anti-secretory and acid neutralizing effects that are comparable to reference drug Omperazole. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavanoids and tannins. Further studies are required to confirm the exact mechanism underlying the ulcer healing and protective property of the extracts and to identify the

chemical constituents responsible for it. The extract showed antiulcer potential in both curative and prophylactic experimental models.

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