Available online on 15.12.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Gastro-protective effects of methanol extract of *Tephrosia calophylla*

Ramesh C[1]* and A. Prameela Rani[2]

[1]Department of Pharmacology, East West College of Pharmacy, Bangalore, India

[2]Department of Pharmaceutics, ANU College of Pharmaceutical Sciences, Guntur, India

ABSTRACT

Objective: The present research work was designed to investigate gastro protective potentials of methanol extract of *Tephrosia calophylla*.
Methods: The aerial parts of *Tephrosia calophylla* were dried under shade, powdered and deffated with petroleum ether and then marc left over was subjected to methanol extraction using soxhlet apparatus. Antiulcer activity of methanol extract was determined against stress induced and aspirin induced ulcers in experimental animal models. The total number of ulcers formed, ulcer index, percentage inhibition, ulcerated area, protected area, pH and Total acidity were parameters in the study.
Results: Methanol extract of *Tephrosia calophylla* have significantly reduced the total number of ulcers formed, ulcer index, ulcerated area and total acidity in therapeutic groups compare to vehicle control and there by significantly increased percentage inhibition of ulcers and protected area which was evident by significant rise in pH of gastric content. The effect of extracts was dose dependent and results were comparable to that of standard drug omeprazole.
Conclusion: The results obtained from the present work suggest that the methanol extract of *Tephrosia calophylla* possess significant anti-ulcer potentials against experimentally induced ulcers in albino rats.

Keywords: *Tephrosia calophylla*, Anti ulcer activity, Ethanol, Aspirin Ulcer index, pH, total acidity, Percentage inhibition and percentage of protected area.

Article Info: Received 18 Oct 2018; Review Completed 30 Nov 2018; Accepted 03 Dec 2018; Available online 15 Dec 2018

Cite this article as:

Ramesh C, Prameela Rani A, Gastro-protective effects of methanol extract of *Tephrosia calophylla*, Journal of Drug Delivery and Therapeutics. 2018; 8(6-s):141-145 **DOI:** http://dx.doi.org/10.22270/jddt.v8i6-s.2100

***Address for Correspondence:**

Mr. Ramesh C, Asso. Professor, Department of Pharmacology, East West College of Pharmacy, No-63, I Bhartah Nagar, Off Magadi Road, Vishwaneedam (PO), Bangalore-560091

INTRODUCTION

Peptic ulcer which includes both gastric and duodenal ulcers is one of the most prevalent gastrointestinal tract diseases that affect a wide range of people worldwide[1]. Due to its high morbidity and mortality rates, peptic ulcer disease has been one of the leading causes of gastrointestinal surgery over a century. The pathophysiology of peptic ulcer disease was attributed to the imbalance between the offensive factors (e.g. acid, pepsin, *Helicobacter* infection) and the defensive ones (e.g. bicarbonate, mucin, prostaglandins, nitric oxide and growth factors)[2]. The use of non-steroidal anti-inflammatory drugs (NSAIDs), irregular diet, emotional stress, excessive alcohol use and smoking are all the principal etiological factors associated with the peptic ulcer[3]. Nowadays, the drug treatment of ulcer is commonly focused on the suppression of acid secretion and the enhancement of gastric protection[4]. However, more and more clinical evaluation on the drug treatment showed that tolerance was developed and also incidence of relapses as well as side effects were increased, which made the efficacy of the treatment be arguable. Many of the existed medicines have limitations, especially when they were used against the ulcers with complex etiologies[4].

The aim of treating peptic ulcer disease is to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently, efforts are on research of a suitable treatment from natural product sources. A large number of species and herbs have been evaluated by various researchers for their anti-ulcer effects to achieve a favourable outcome[5]. A wide range of drug is currently available for the treatment of gastric ulcer which includes proton pump inhibitor, H_2 blocker, antacid, and anti-cholinergic. The most common adverse effect of these drugs are hypergastrinemia, hypersensivity, gynecomastia, impotence, arrhythmia and blood dyscaris such as thrombocytopenia and enteric infection (clostridium deficile)[6]. Hence till now there is no truly satisfactory medicine for the management of peptic ulcer and plant drugs are proven as effective and safe drugs for the management ulcers[6].

About 600 commercial preparations with claimed liver protecting activity are available all over the world. About

100 Indian medicinal plants belonging to 40 families are used for herbal formulation[7]. The *Tephrosia villosa*Linn. is native to India and it is medicinally important and used in traditional system for the treatment of liver ailments[8]. The *Tephrosia villosa*Linn. is commomly known as Shankhpushpi and used in ayurvedic system of medicine as memory enhancer, neuroprotective[9] and treatment many ailments. The leaves of this plants contains alkaloids, flavonoids, tannins and phenols[10] and scientifically proved for its anti-diabetic[11] antiulcer[9], anti-anxiety[12], antioxidant[13] and many other pharmacological activities. The phyto-constituents of plant leaves are capable of reducing liver toxicity due to their antioxidant properties but, the plant has not been scientifically investigated for evaluation of hepato-protective activity[14]. In view of this, the present study was undertaken to investigate the hepatoprotective activity of aqueous extracts of *Tephrosia villosa* (TVME) leaves against thiocetamide induced liver damage in rats.

The *Tephrosia* is a genus of plant, pantropical taxa with about 400 species distributed chiefly in Asia, Africa, Australia and America[7]. About twenty-four species of *Tephrosia* were recorded in India. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellant, larvicidal, piscicidal, antimicrobial and anticancer properties[8,9,10,11]. The *Tephrosia calophylla* belongs to the genus was essentially used for the management of diabetes, ulcers, cancer, hyperlipidemia, hepatotoxicity and renal problems in the folklore medicine but doesn't have the scientific evidence for the same[12,13,14]. Hence the present study was designed to assess the anti ulcer activity of the *Tephrosia calophylla* against animal models of ulcer.

MATERIALS AND METHODS

Plant material

The areal parts of *Tephrosia calophylla* have been collected from Sri Venkateshwara university, Tirupati, India and dried under shade. The leaves were identified and authenticated by Dr. Madhava chetty Asst.Prof. Dept. of Botany and speciman herbarium were preserved at institute herbarium library. The leaves part were separated from other parts, washed, cleaned and dried for further use.

Preparation of extract

The shade dried leaves were pulverised into powder and sieved through No. 22 mesh. About 350 g (appx.) of coarse powder was subjected to successive solvent extraction using petroleum ether, benzene, chloroform and methanol in soxhlet's apparatus[15].

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the methanolic extract of *Tephrosia calophylla* had been conducted as per procedure prescribed by Khandelwal [16].

Drugs and chemicals

All the chemical and reagents used in the present study were of analytical grade and procured from following sources.

- Ethanol and Asprin from Sigma-aldrich chemical Pvt. Ltd., Bangalore.
- Tween80 was obtained from Nice chemicals Bangalore.
- All the other solvents and chemicals used for extraction and physiochemical investigation were as of analytical grade purchased from S.D fine chemicals Pvt. Ltd. Bangalore.

Animals

The healthy albino wistar male rats were procured from Sri Venkateswara Enterprises, Bangalore housed under standard conditions of temperature (22 ± 10C), relative humidity (55 ± 10%), 12 hr light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee, IJAHSM, Bangalore (Ref.no.IJAHSM/IAEC/2014/03) with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute Oral Toxicity Studies

The OECD guidelines 423 (up and down procedure) were used to determine acute oral toxicity for methanol extract of *Tephrosia calophylla*. A starting dose used was 2000 mg/kg body weight p.o. of extract (TCME) was administered to 3 male rats, observed for 14 days. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, and observed for 14 days[17].

Evaluation of anti ulcer activity

The methanol extract of *Tephrosia calophylla* was evaluated against stress and aspirin induced ulcers and study design in both studies consisting of six groups of six animals in each group as follows.

Groups	TREATMENT
Group I: Normal	Treated with Normal Saline(2ml/kg)
Group II: Vehicle control	Induced with ulcers and treated with 2% tween 20
Group III: Standard	Induced with ulcers+ Treated with omeprazole (10 mg/kg, p.o.)
Group IV: TVME (100mg/kg)	Induced with ulcers+ Treated with ethanol extract of *Tephrosia calophylla* (100 mg/ kg p.o)
Group V: TVME (200mg/kg)	Induced with ulcers+ Treated with ethanol extract of *Tephrosia calophylla* (200 mg/ kg p.o)
Group VI: TVME (400mg/kg)	Induced with ulcers + Treated with ethanol extract of *Tephrosia calophylla* (400 mg/ kg p.o)

Evaluation of anti ulcer activity against stress ulcers[18,19]

Stress ulcers were induced by forcing the Wistar albino rats of either sex to swim in the glass cylinder containing water to the height of 35 cm maintained at 25 °C for 3 h. After the drug treatment animals were allowed to swim in cold water for 4 h. After this the animals were killed with high dose of anesthetic ether. Stomach of each rat were opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (10×). Gastric juice collected into centrifuge tubes and centrifuged at 1 000 r/min for 10 min and the volume were noted. The number of ulcers were scored and percentage of protection and ulcer index were calculated.

Evaluation of anti ulcer activity against stress ulcers [18,20,21]

All the experimental animals were kept for overnight fasting and gastric ulcers were by administering cold absolute asprin (150 mg/kg p.o) .All the extracts and standard drug omeprazole were administered orally prior to the administration of aspirin. One hour later, the animals were sacrificed by cervical dislocation and the stomachs were removed. Gastric acid was collected and its pH was determined. Stomachs were opened along the greater curvature and gently rinsed with water for subsequent scanning. The number of ulcers was scored and percentage of protection and ulcer index were calculated.

RESULTS

Preliminary phytochemical study

The percentage yield of the TPME was found to be 8.15 % w/w. The preliminary phyto-chemical investigation of the methanol extract of *Tephrosia calophylla* reveals the presence of alkaloids, glycosides, poly phenols, flavonoids, tannins, steroids, and carbohydrates in the plant.

Acute toxicity studies

The methanol extract of *Tephrosia calophylla* was safe up to dose of 2000 mg kg^{-1} b.w. and caused neither mortality nor any signs of clinical abnormality in the tested animals during the observation period of 14 days after administration of highest dose. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, no changes were observed for 14 days. As per the results obtained in acute oral toxicity study doses were selected as 100, 200 and 400mg/kg on the ratio $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ respectively.

Evaluation of anti ulcer activity

Anti- ulcer activity against stress induced ulcers

In the present study of stress induced ulcer model, control animal have shown significant (P<0.001) no of ulcers and ulcer index compare to normal animals which have shown no ulcers and ulcer index. Administration of Standard drug omeprazole and TPME at medium and high dose have significantly (P<0.001) reduced number of ulcers formed and ulcer index when compare to vehicle control. Due to the reduction in number of ulcers and ulcer index, percentage of protection was significantly increased in therapeutic groups treated with standard drug and TPME. Effect of TPME at 100mg/kg was not significant (See table No 1)

The significant increase in formation of ulcerated area and total acidity were observed in vehicle control animals due to stress. But there was significant (P<0.001) decrease in ulcerated area and total acidity found in omeprazole and TPME (200mg/kg and 400mg/kg) treated animals compare to vehicle control group. Hence the percentage of protected area was significantly increased in animals treated with omeprazole and TPME (See table No 1)

Table 1: Effect of Methanol extract of Tephrosia calophylla on Stress induced ulcers

Group	Number of ulcers	Ulcer Index	Percentage of inhibition	Percentage of Ulcerated Area	Percentage of Protected Area	pH	Total Acidity
Group I: Normal	0	00	100	0	100	3.776±0.1151	40.48±0.6388
Group II: Vehicle control	6.400±0.5099	28.56±1.776	00	69.54±1.238	30.46	1.906±0.09745	72.81±1.691
Group III: Standard	1.600±0.2449	3.930±0.3035	86.239±6.391	21.90±1.690	78.1	3.822±0.1680	42.69±1.349
Group VII: TCME(100mg/kg)	4.800±0.4899	18.22±1.580	36.204±2.692	48.31±1.812	51.69	2.450±0.1112	49.65±1.823
Group VIII: TCME (200mg/kg)	1.800±0.5831	13.11±0.6922	54.09±3.65	38.56±1.072	61.44	3.798±0.09184	45.23±2.576
Group IX: TCME (400mg/kg)	1.000±0.4472	5.240±0.5592	81.652±2.59	19.09±0.8325	80.91	4.106±0.08382	37.02±1.065

Values are mean ± S.E.M, n=6 symbols represent statistical significance.
^{ns} p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control.
^{ns} p>0.05, $^{+}$ p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.001 normal control vs positive control.

Anti ulcer activity against aspirin induced ulcers

Administration of aspirin has shown significant (P<0.001) no of ulcers and ulcer index in vehicle control animals in the present study compare to normal animals which have shown no ulcers and ulcer index. Administration of Standard drug omeprazole and TPME at medium and high dose have significantly (P<0.001) reduced number of ulcers formed and ulcer index when compare to vehicle control. Due to the reduction in number of ulcers and ulcer index, percentage of protection was significantly increased in therapeutic groups treated with standard drug and TPME. (See table No 2)

The formation of significant ulcerated area was observed and total acidity was significantly increased in vehicle control animals due to the administration of aspirin. But there was significant (P<0.001) decrease in ulcerated area

and total acidity found in omeprazole and TPME (200mg/kg and 400mg/kg) treated animals compare to vehicle control group. Hence the percentage of protected area was significantly increased in animals treated with omeprazole and TPME (See table No 2).

Table 2: Effect of Methanol extract of Tephrosia calophylla on Aspirin induced ulcers

Group	Number of ulcers	Ulcer Index	Percentage of inhibition	Percentage of Ulcerated Area	Percentage of Protected Area	pH	Total Acidity
Group I: Normal	0	00	100	0	100	3.866±0.1080	41.54±0.6759
Group II: Vehicle control	7.2±0.5831	28.49±2.243	--	70.23±1.392	29.77	1.808±0.1022	75.85±2.486
Group III: Standard	1.8±0.2000	4.216±0.5368	85.20±3.77	24.37±2.106	75.63	3.592±0.2051	41.69±1.008
Group VII: TVME(100mg/kg)	5.200±0.2000	21.69±1.409	23.868±2.831	48.64±1.044	51.36	2.256±0.05904	55.43±3.991
Group VIII: TVME (200mg/kg)	2.400±0.5099	13.49±0.4953	52.65±2.561	38.62±0.8450	61.38	3.684±0.05644	46.75±3.466
Group IX: TVME (400mg/kg)	1.800±0.4899	4.792±0.2540	83.18±4.672	20.17±0.8464	79.83	4.080±0.06124	36.53±1.376

Values are mean ± S.E.M, n=6 symbols represent statistical significance.
ns p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control.
ns p>0.05, + p<0.05, ++ p<0.01, +++p<0.001 normal control vs positive control.

DISCUSSION

The causative factors that may produce ulcer in human being are several they are stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion, spicy food among others. In most of the cases, the exact causative factor of ulcer is unknown but it is generally accepted that it is the result of an imbalance between aggressive factors and defensive factors that maintenance mucosal integrity through the several endogenous mechanism. Peptic ulcer is leading cause of mortality and morbidity in developing countries, characterized by imbalance between aggressive gastric luminal factor and defensive mucosal barrier. This disease is mainly associated with increase in gastric acid secretion. Numerous factors like diet, smoking, drugs like aspirin and infection are responsible for augmentation of ulcers. Still, no therapeutic intervention has been found successful. So, in the present study efforts has been made to review and to explore various animal models to find out a suitable medication for the treatment of peptic ulcer [22,23,24]..

Tephrosia calophylla are employed in the treatment and management of the ulcers in folklore medicine but still no complete curative treatment is available. So this review has been designed to explore the effects of *Tephrosia calophylla* extracts for the treatment of peptic ulcer against various ulcer models like pyloric ligation; ethanol and aspirin induced ulcer models.

In the present study, oral administration of methanol extract *Tephrosia calophylla* at 200mg/kg and 400mg/kg significantly inhibited gastric ulcer formation in both selected ulcer models when compared control and effect was comparable to standard omeprazole.

Aspirin is commonly used for inducing ulcer in experimental rats' due to its intense gastric mucosal damage. Studies suggest that the Aspirin damage to the gastrointestinal mucosa starts with micro vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting [25,26,27].

Aspirin is an NSAID its abuse will induce gastric ulceration by inhibition of gastric cyclo-oxygenase resulting in the formation of prostacyclin, which is predominant prostanoid produced in the gastric mucosa. The ulcers produced can be prevented by exogenous PGE_2 and PGI_2 [28,29]

The significant decrease in gastric ulcer, ulcer index, ulcerated area and total acidity was observed animals in pretreated methanol extract compared to vehicle control group. In the present study we also found that there is significant rise percentage inhibition of ulcer formation, protected area and pH of gastric content. The anti ulcer potentials of plant extracts were almost similar to standard drug omeprazole used in the present study.

The stress produces stimulates vagus that increases release acetyl choline which ultimately produces nitric oxide responsible for the development of ulcers in the stomach due to free radical nature. The other ulcerogenic aspirin used in the present study directly irritate GIT mucosa and acts as free radical results in the formation of peptic ulcers. Hence the drugs those possess antioxidants can be good approaches for the treatment of ulcers due to the presence flavonoids and phenolic compounds. In this regard, the study can be performed to evaluate the antioxidant properties of the plant to determine the possible mechanis[30,31].

The methanol extract of *Tephrosia calophylla* possess significant anti ulcer property against stress and aspirin induced ulcers. The further investigation should be performed to isolate and evaluate specific constituents responsible for anti ulcer activity.

CONCLUSION

The present study was undertaken for the investigation of antiulcer activity of methanol extracts of *Tephrosia calophylla* against stress and aspirin induced ulcers in animal model. From the results obtained from the study, it can be concluded that methanol extract of the plant exhibited a strong gastroprotective activity against experimentally induced ulcers. However further investigation required establishing the clear mechanism of action of the extract and also to isolate individual phyto constituents present in *Tephrosia calophylla* that may be responsible for these beneficial therapeutic effects.

ACKNOWLEDGEMENTS

The authors of manuscript are thankful to The Principal and management of East West College of Pharmacy, Bangalore and The Principal and management of ANU College of Pharmaceutical Sciences, Guntur for providing facilities to conduct this research work.

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