Anti-Ulcer Effect of Aqueous Extract of Gynura cusimbua (D.Don) S.Moore on Swimming Stress Ulcer Model in Albino Rats

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ABSTRACT

BACKGROUND
Gynura cusimbua (D.Don) S.Moore. (Family: Compositae) which is commonly known as purple passion and is locally known as "terapaibi" is a traditionally and routinely used home remedy among the indigenous population of the state of Manipur, India. It has been used in various ailments and diseases like- control of bleeding from wounds and cuts, expediting wound healing, relief of common headache, faster healing, prevention of gastric ulcer and also for decreasing inflammation.

METHODS
The present study was carried out to evaluate the anti-ulcer and gastro-protective property of Gynura cusimbua against the formation of gastric/peptic ulcer from exposure to stress by using a suitable experimental animal model that included subjecting the experimental animals to prolonged swimming in cold water as a stress factor to induce gastric ulcer. The division of animals (albino rats) was made into 5 groups that included 6 animals each, namely- Group I (Control- 2% gum acacia in distilled water), Group II (Standard- Omeprazole 2 mg/Kg bodyweight), Group III (Test drug- 500 mg/Kg body weight), Group IV (Test Drug- 1000 mg/Kg bodyweight) and Group V (Test Drug- 2000 mg/Kg body weight). The experimental animals in the different groups were administered the specified drugs orally using a gavage tube. The administration of drugs was done 30 minutes before they were subjected to a 3-hour stress activity of swimming in cold water and the anti-ulcer activity was evaluated thereafter using ulcer index as the evaluating parameter.

RESULTS
The test drug, aqueous extract of Gynura cusimbua, showed significant ($p<0.05$) reduction in ulcer index at doses of 2000 mg/Kg. body weight when compared to control. The test drug showed a dose dependant decrease in the formation/development of stress ulcer from the experiment, when compared to the control animal, justifying its age long persistent and continued use in the traditional medicine/healing practices for the treatment of gastric ulcer.

CONCLUSIONS
This study adds data to the anti-inflammatory activity and an earlier study on the anti -ulcer effect of Gynura cusimbua in aspirin plus pylorus ligation model for gastric ulcers and further strengthens the safety of the plant extract for its traditional use in the treatment of peptic ulcer as well as research into the extraction, refinement and development of a novel chemical entity with anti-ulcer property.

KEY WORDS
Gynura cusimbua, Omeprazole, Anti-Ulcer, Swimming Stress, Albino Rats, Terapaibi, Folklore Medication

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DOI: 10.14260/jemds/2019/567

Financial or Other Competing Interests: None.

How to Cite This Article:
Tewari B, Medhabati M. Anti-ulcer effect of aqueous extract of Gynura cusimbua (D. Don) S.Moore. on swimming stress ulcer model in albino rats. J. Evolution Med. Dent. Sci. 2019;8(33):2602-2606, DOI: 10.14260/jemds/2019/567

Submission 13-06-2019,
Peer Review 04-08-2019,
Acceptance 10-08-2019,
Published 19-08-2019.

BACKGROUND

Traditional medicine has been a key player and a stakeholder in world health and continues to be used in treating a variety of medical conditions, ailments and complaints, and this use of medicinal, herbal and aromatic plants, whether used in its most basic indigenous form to make potions and elixirs, or in the latest researched and refined modern form of herbal and plant based cosmetic products and medicines is increasing steadily. A major chunk of the population amounting to 70% and 95% from the developing countries in Latin America, South East Asia, the Indian subcontinent, almost all of Africa, the Far East European nations and the Middle East, use traditional and herbal based medicine, for managing the health. They also use it as a sole means to primary health care for addressing their daily and common health care needs.[1] Moreover the consumers and users of the traditional medicines as well as the common public are in the misconception that "natural" is always "safe" which in turn leads to the propagation of a belief that considers that all remedies made from natural origin are safe, harmless and carry absolutely no risk. However, toxicity is inherent to some medicinal plants and hence are dangerous on long term use and can have many interactions with other forms of medications.[2] But on the contrary a certain group of medicinal plants that have a long history of use in traditional medicines following passing and sharing of the experience of using such medications from generations to generation, has not only demonstrated the safe nature of the plant but has also lead to refinement of efficacy of many traditional medicines and practices. However scientific study with data and well established facts are essential to provide concrete supporting evidence of the safety and efficacy of the traditional medicine, so as to promote further research in the field.[3] Gynura cusimbua (D.Don) S.Moore locally famous as Terapaibi in Manipur is a herb belonging to the Compositae (Asteraceae) family and is found commonly valley as well as the hills of Manipur. It also is found in the foothills of the entire Himalayan region. This tall and succulent herb has been used as a traditional medicinal plant for control of bleeding from wounds and cuts, expediting wound healing, relief of common headache, decreasing inflammation. And as an anti-ulcer agent for centuries.[4] Fresh leaf and shoot from the plant are cooked and the soup is taken a glassful after food every day for a week to cure stomach ulcer. [5] Previously the test plant, Gynura cusimbua, has been studied for the anti-inflammatory effect using the carrageenan induced paw oedema model with favourable results.[6] Additional the plant has been studied for the antiulcer effect using the aspirin plus pylorus ligation model in albino rats which revealed that it has significant anti-ulcer properties.[7] Gynura procumbens a close relative of the test plant Gynura cusimbua has been studied by Mahmood AA et al,[8] for the anti-ulcer effect using ethanol induced ulcer in the albino rats as an experimental model. The results have shown to be a positive reduction in the ulcer area. Stress plays a major role in the pathogenesis of gastric ulcer in a man and are classified as stress induced gastritis or ulcers. These ulcers can be commonly seen in patients who are suffering from stressful conditions such as sepsis, shock, severe trauma, head injuries, massive burns, and can develop acute and erosive changes in the gastric mucosa or furthermore develop frank ulceration with/without bleeding. [9] Therefore, the present study was undertaken in continuation to evaluate the anti-ulcer property of the plant in a suitable animal experimental model for stress ulcer.

METHODS

Plant Material and Extract Preparation

The fresh leaves of Gynura cusimbua were locally collected from Lamphelphat, Imphal West during the rainy months of July-August in 2011. The authentication and identification of the plant was done by Prof. P. K. Singh from the Department of Life Sciences, Manipur University, Canchipur, Manipur, India. The leaves were cleaned, dried under shade and powered by a mechanical grinder. The method described by Verma SCL and Agarwal [10] was followed for the preparation of the aqueous extract. Soxhlet Apparatus was used to extract 50 grams of fresh powdered leaves using distilled water. A brownish extract was obtained which was further processed by evaporating, drying in the shade, scrapping out and weighing for storing in a glazed porcelain jar for experimental use.

Acute Toxicity Testing

OECD guidelines was followed to perform the acute toxicity testing on the experimental albino rats. Three animals were used for each step. The starting dose selected for testing was 300 mg/Kg body weight. The animals were fasted overnight, then were weighed and the test dose was administered as a single dose by gavage using a stomach tube. Animals were then observed individually for any sign of toxicity. The observation was done once during the first 30 minutes, then periodically during the first 24 hours and then daily thereafter for a total of 14 days. The same procedure was carried out for doses of 2000 mg/Kg and 5000 mg/Kg body weight of the test substance, for testing the toxicity status. [11]

Experimental Animals

Thirty male albino rats weighing between 150-200 grams were obtained from Central Animal House, RIMS, Imphal. They were housed in standard rat cages, fed standard pellet diet with water ad libitum and maintained at a 24–28° C temperature with a 12-hour day and night cycle. The protocol of the experiment was approved by the Institutional Animal Ethics Committee, RIMS, Imphal, Manipur, India.

Experimental Design

The rats were subdivided into five groups of six animals in each group, namely-control, standard, test I, test II and Test III. The animals were fasted for 24 hours before starting the experiment. The drugs were suspended in 2% gum acacia and was administered orally 30 minutes before to the animals were subjected to stress, by gavage using a stomach tube. The volume of the medicaments was kept constant at 2 ml/100 gm of body weight of the animal. The results, of the test groups, obtained were then compared with those of the control and the standard drugs for establishing statistical significance. One-way ANOVA followed by Dunnett's t test was used for establishing the statistical significance.

Group	Drug	Dose Administered P.O.
I (Control)	2% Gum acacia in distilled water	2 ml/100g p.o.
II (Standard)	Omeprazole	2 mg/Kg, p.o
III (Test)	Aqueous extract of Gynura cusimbua	500 mg/Kg
IV (Test)	Aqueous extract of Gynura cusimbua	1000 mg/Kg
V (Test)	Aqueous extract of Gynura cusimbua	2000 mg/Kg

Table 1. Division of the Albino Rats into Groups as per the Drugs Administered

Test of Swimming Stress Ulcer

Swimming stress ulcer method[12] as described by Parmar NS and Desai JK was used. After thirty minutes following the administration of the drugs, the animals were subjected to swimming stress. The animals were forced to swim inside the vertical cylinders (Height 30cm, diameter 15cm) containing cold water up to 15cm height for 3 hours. After 3 hours they were removed from the cylinders and then sacrificed by a blow on the head. The animals were dissected carefully, and the stomachs were removed. The removed stomachs were then opened along the greater curvature to reveal the changes in the gastric mucosa. The ulcers of the gastric mucosa grossly appear as haemorrhagic lesions mostly in the long axis along the mucosal folds of the stomach. Gastric mucosa of each experimental rat was examined and assessed for damage. A planimeter under a dissecting microscope (1.8x) was used to measure the length and width of the ulcer in millimetres. The measurement of the ulcerated area was done by counting the small squares measuring 2 × 2 mm that covered the length and breadth of each ulcer. The sum total of the areas in sq.mm for all lesions in each stomach assessed and was applied in the calculation of the total ulcer area (UA) wherein the sum of small squares × 4 × 1.8 = UA (mm^2) as described by Kauffman and Grossman.[13]

The ulcer index was then determined by the method of Ganguly AK and Bhatnagar OP.[14]

Ulcer Index = 10/X

Where, X = Total mucosal area/Total ulcerated area.

RESULTS

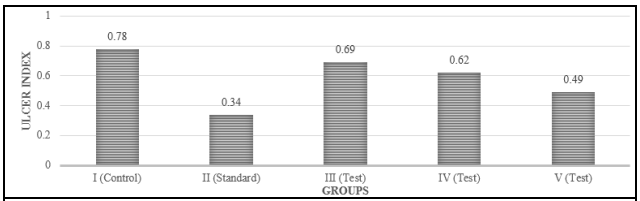
The aqueous extract of Gynura cusimbua (D.Don) S.Moore. (AEGC) was found to be safe in the doses tested. There was no mortality or moribund state seen in the treated animals, up to a dose of 5000 mg/Kg body weight per oral.

Group	Drug	Dose Administered P.O.	Ulcer Index	F	p	df
I (Control)	2% Gum acacia in distilled water	2 ml/100 g	0.78±0.68	30.728	<0.01	4,25
II (Standard)	Omeprazole	2 mg/Kg	0.34±0.02***			
III (Test)	Aqueous extract of Gynura cusimbua	500 mg/Kg	0.69±0.02			
IV (Test)	Aqueous extract of Gynura cusimbua	1000 mg/Kg	0.62±0.01###			
V (Test)	Aqueous extract of Gynura cusimbua	2000 mg/Kg	0.49±0.01*, ###,$$$			

Table 2. The Anti-Ulcer Effect of Aqueous Extract of Gynura cusimbua (D.Don) S.Moore. (AEGC) on the Ulcer Index, in the Swimming Stress Induced Ulcer Model in Albino Rats

The values are mean ± SEM, n = 6 in each group, ***p<0.001 and *p<0.05 when compared to control, ###p<0.001 when compared to test dose (500 mg/Kg b. w.), $$$p<0.001 when compared to test dose (1000 mg/Kg b.w.)

The table shows a significant (p<0.05) reduction in ulcer index at doses of 2000 mg/Kg. Body weight when compared to control. The test drug showed a dose dependent reduction in the ulcer index. The test drug at doses of 1000 mg/Kg body weight and 2000 mg/Kg body weight produced a significant (p<0.001) reduction in the ulcer index when compared to the test drug dose of 500 mg/Kg body weight. The test drug at the dose of 2000 mg/Kg body weight produced a significant (p<0.001) reduction in the ulcer index when compared to the test dose of 1000 mg/Kg body weight. The reduction in the ulcer index by the test drug was lower than the reduction in the ulcer index by the standard drug, Omeprazole.



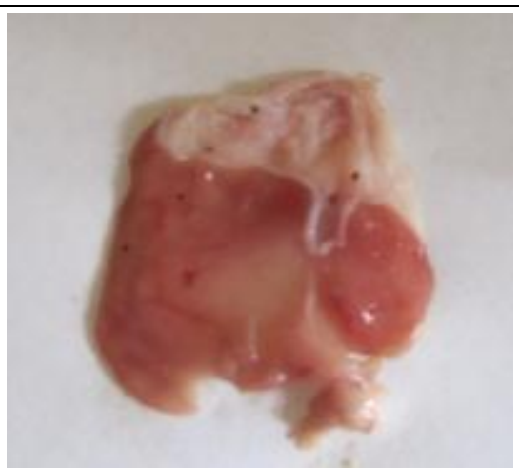
Bar Graph 1. Anti-Ulcer Effect of the Aqueous Extract of Gynura cusimbua on Ulcer Index in the Swimming Stress Induced Ulcer Model in Albino Rats

Figure 1. Leaves of Gynura cusimbua (D.Don) S.Moore

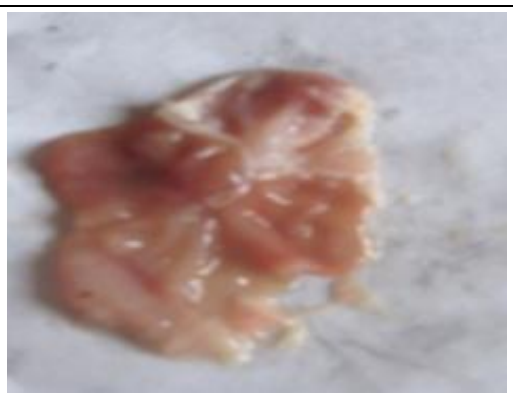
Figure 2. Flowers of Gynura cusimbua (D.Don) S.Moore

Figure 3. Extraction in Soxhlet Apparatus

Stomachs showing swimming stress induced ulcer in albino rats in the control, standard and test groups-



Control



Standard

Test I

Test II

Test III

DISCUSSION

The antiulcer activity of the aqueous extract of Gynura cusimbua (D.Don) S.Moore. Was studied by Swimming stress induced ulcer as described by Parmar NS and Desai JK. [11] Omeprazole, a proton pump inhibitor at a dose of 2 mg/Kg body weight was used as the standard drug in this study as was done by other workers like Shenoy AM et al,[15] Manoharan P et al,[16] Bhave AL et al. [17] The test drug at doses of 500 mg/Kg, 1000 mg/Kg and 2000 mg/Kg body weight was evaluated for its anti-ulcer activity. In swimming stress induced ulcer model, the ulcer index of the control group was 0.78±0.68 which was comparable with the findings of Debnath PK et al.[18] The test drug (AEGC, 2000 mg/Kg) treated group showed significant decrease in the ulcer index after 3 hours of forced swimming in cold water when the values were compared to the control group ($p<0.05$). The test drug, aqueous extract of Gynura cusimbua, when given prior to stress did not show comparable efficacy with the standard omeprazole 2 mg/Kg, in reducing the occurrence of stress induced ulcers/reduction of ulcer index in albino rats in swimming stress ulcer model. The reduction in ulcer index was consistent with the previous study evaluating the antiulcer effect of aqueous extract of Gynura cusimbua in aspirin plus pylorus ligation model in albino rats. [8] This anti- ulcer/ gastro protective activity can be attributed to the presence of several phytochemicals in Gynura cusimbua, chiefly flavonoids which are a consistent presence in the plants of genus Gynura, family asteraecea as determined by Ibrahim MH and Kong YC et al,[19] Akhi TM and Adib M et al[20] and Hassali HA et al.[21] Additionally the anti-inflammatory activity of the test drug, as established in a previous study could further help in reducing the occurrence of gastric ulcer and reducing the ulcer index in the experimental animals.[6] The test for toxicity showed no acute moribund or mortality, consistent with the findings of Mahmood AA et al. [8]

CONCLUSIONS

The present study establishes that the aqueous extract of the leaves of Gynura cusimbua produces a significant dose dependent reduction of the ulcer index in the tested animals under conditions of stress without any obvious side effects and therefore justifies it's use in the traditional medical and healing practices of the people of Manipur, India. The anti-

inflammatory activity of the plant along with the ant-ulcer activity is a good combination of activities and hence can be researched further to evaluate its use as a safe and an effective analgesic anti-inflammatory, or in a combination use with an analgesic to mitigate the gastric ulcer generating side effects of modern day analgesics. We can thus conclude that the research with plant sources can return good and novel treatment modalities as well as chemicals/compounds for treatment of gastric ulcer. This study also helps in reinforcing the fact that stressful conditions are also strong and indispensable factors of gastric ulcer formation, the studies on which, considerable progress has been made. Owing to recent technological advances and a spurt of interest in the research of plant sources for development of medication for various ailments has led to the contribution of more than 50% of new chemical entities being developed in the recent years. Efforts therefore should now be directed towards isolating and characterizing the active principles in the various traditional and ayurvedic medicines and describing the relationship between the activity and the structure of these chemical entities. In addition, further study to establish data for sub-acute and chronic toxicity needs to be done. The data so obtained will further strengthen the safety of the plant extract for its use in the treatment of various ailments.

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