International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

EVALUATION OF ANTIULCER ACTIVITY OF PLANT CHROZOPHORA PLICATA

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ABSTRACT

The herbal medicines derived from various plants extracts are being increasingly utilized to treat a wide variety of clinical diseases[1]. Besides their action as gastroprotectives, flavonoids also act in healing of gastric ulcers[2]. The study was designed to investigate the anti-ulcer effect of chloroform extract of *Chrozophora plicata* leaves (600mg/kg) against experimentally induced ulcer models in albino rats. In the present study, anti-ulcer activity was assessed by using pylorus ligation induced and indomethacin (40mg/kg) induced Ulcer [3]models in albino rats. The antiulcer activity was assessed by determining and comparing gastric volume, acidity, ulcer score and ulcer index in control, test extract and standard (ranitidine 10mg/kg) treated rats by using both pylorus ligation and indomethacin induced ulcer models. Pre-treatment of animals with the Chloroform extract of *Chrozophora plicata* (600mg/kg)) orally significantly reduced formation of ulcers induced by pylorus ligation and indomethacin models. The percentage inhibitions of ulcer with test extract being 60 ±1.12 % and 57.18 ± 1.32 % respectively whereas standard ranitidine has shown percentage inhibitions of ulcer to an extent of 100± 0.42% and 68.52±1.07% in both ulcer models. It is evident from literature that *Chrozophora plicata* leaves possess flavonoids[4]**.** The results obtained indicate that *Chrozophora plicata* possesses antiulcer principles.

Key words: *Chrozophora plicata,* Flavonoids, Antiulcer activity

INTRODUCTION

A lesion of the skin or mucous membrane such as the one lining the stomach are duodenum that is accompanied by formation of pus and necrosis of surrounding tissue, usually resulting from inflammation or ischemia[5]. A peptic ulcer, also known as peptic ulcer disease (PUD), [6] is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. It is defined as mucosal erosions equal to or greater than 0.5 cm. Ulcers can also be caused or worsened by drugs such as aspirin, indomethacin ibuprofen, and other NSAIDs. The phytochemical profile of this plant chrozophora plicata reveals the presence of sterols, alcohols, hydrocarbons. flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, alkaloids, cyanogenic glucosides and glucosinolates. It is evident from the literature that flavonoids posseses antiulcer properties[7]. Since there are no reports of isolation of active antiulcer principles of *Chrozophora plicata,* the present study is planned to exploit the antiulcer activity of herbal plant named ***Chrozophora plicata,*** Family: *Euphorbiaceae* by using experimentally induced ulcer models in rats.

MATERIALS AND METHOD

For this study, the leaves of *chrozophora plicata* were collected from the surrounding gardens of the gajwel (mandal), medak dist. After the plant materials authenticated by botanist, sample specimens of leaves have been deposited at the museum of the college. Fresh mature leaves were

shade dried at room temperature, coarse powdered and extracted with chloroform by soxhlet's extraction method. Thereafter, the extracts were concentrated using electric water bath to obtain semisolids crude extract. The percentage yields of the leaf extract were found to be 7.8% and 9.7% respectively. The extract was stored in airtight container in refrigerator below 10ºc. Appropriate concentration of stock solution of extract were prepared using distilled water and used for the following studies.

Preliminary phytochemical screening[8]: Preliminary phytochemical tests were performed for the extract of Chrozophora plicata to detect the presence of phytochemicals by following the standard methods described in the practical pharmacognosy of kokate and khandelwal. The results have been tabulated in table 1.

Experimental animals: Albino rats (180-225g) were used in the experiments. They were procured from sainath agencies, musheerabad. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 ± 2ºc), relative humidity (45-55%) and 12hr dark/light cycle. The animals were fed with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum***.** The study protocol was approved from the institutional animal ethics committee (IAEC) before commencement of experiment (1230/a/08/CPCSEA).

Determination of acute toxicity: The chrozophora plicata chloroform extract was studied for acute toxicity study at a dose of 5 mg/kg, 50mg/kg, 300 mg/kg, and 2000 mg/kg P.O in albino mice for each dose 3 mice are used (up and down procedure, OECD guidelines No. 425). The extract was found safe to all the animals 12 mice. The mice are subjected to a dose of 5000 mg/kg. Even at 5000mg/kg no mortality is seen, but few symptoms of CNS depression such as sedation and drowsiness is seen in all the mice at 5000mg/kg. Hence a dose of 3000mg/kg is selected as safer dose and 1/5th of 3000mg/kg i.e. 600mg/kg is selected for our study.

Effect of chloroform extract of Chrozophora Plicata leaves on Pylorus ligation induced ulcers in albino rats[9]: The experiment was performed on albino rats (150 – 200gms) of either sex from Sainath agencies, Musheerabad. The animals were grouped housed in colony cages at an ambient temperature of 26+2˚C and, relative humidity (45 - 55%), with a 12h/12h light dark cycle and access to food and water ad libitum. Food was restricted during experiments. Ranitidine (10 mg/kg) and chloroform extract of Chrozophora plicata (600mg/kg) are prepared in 2% acacia suspension. Weigh and mark the animals. Divide the animals into three groups control(C), test (T) and standard (S) each consisting of 1 animal. Anaesthetize the overnight fasted rat with anaesthetic ether. Secure the rat on the operating table. Give an incision of 1cm long in the abdomen just below the sternum. Expose the stomach, pass a thread around the pyloric sphincter and apply a tight knot. While putting the knot care should be taken so that no blood vessel is tied along the knot. Close the abdomen wall by putting the sutures. Clean the skin from any blood spots and bleeding. Apply collodion over the wound. Keep the rat in separate cage and allow it to recover. To standard (S) group of rat inject ranitidine (10mg/kg). After 45minutes of ranitidine administration, perform pyloric ligation as described above. To test (T) group of rat inject Chrozophora plicata leaf extract. After 45minutes of leaf extract administration perform pyloric ligation as described above. After 4 hrs of pyloric ligation sacrifice both the animals by decapitation. Open the abdomen and tie the oesophageal end (cardiac end) of the stomach. Cut and remove the entire stomach body of the animal. Open the stomach along the greater curvature and wash it slowly under the running tap water. Put it on slide glass and observe under 10 x magnifications for ulcers. Score the ulcers as below **0 =**Normal coloured stomach, **0.5** =Red colouration, **1.0** =Spot ulcer, **1.5** = haemorrhagic streaks, **2.0** =Ulcers ≥ 3 ≤ 5, **3.0=**Ulcers > 5

Mean ulcer score for each animal is represented as Ulcer index.

The stomachs were isolated and the content of the stomachs were collected and centrifuged. The volume of the gastric juice was measured and this was used for estimation of free acidity and total acidity.1ml of centrifuged and filtered gastric secretion was titrated against 0.01N Sodium hydroxide using Topfers reagent as indicator. Note the volume of NaOH which corresponds to the free acidity (orange colour as end point) and titrate further till the solution regains pink colour. Note the volume of NaOH which corresponds to the total acidity. Acidity can be expressed as: Acidity = vol.of NaOH × Normality × 100/0.1 mEq/100g

Effect of chloroform extract of Chrozophora Plicata leaves on Indomethacin induced ulcers in rats[10-12]: Albino rats weighing 180-225 g were used for experiment. They are randomized into three groups of one animal each. Food was withdrawn 18 h and water 1h before the experiment. Group I (control) received only indomethacin (40 mg/kg.) group II (standard) received ranitidine (10 mg/kg.) and groups

III was pretreated with chrozophora plicata leaves extract (600mg/kg.) p.o. 45 minutes later, groups II and III were administered with indomethacin. Four hours after indomethacin administration, animals were killed by decapitation method. The stomachs were removed and open along the grater curvature. Microscopic examination was carried out by observing with 10X magnifications and the presence of lesion was scored. Scoring of ulcer will be made as follows:

Normal stomach…….. (0)
Red coloration …… (0.5)
Spot ulcer…… (1)
Hemorrhagic streak…….. (1.5)
Ulcers …….. (2)
Perforation …… (3)

Mean ulcer score for each animal will be expressed as ulcer index.

Statistical analysis: The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using One way ANOVA, Followed by Dunnett's test where $P<0.001$, $P<0.01$ and $P<0.05$ was considered statistically significant.

RESULTS

Results of the preliminary phytochemical investigation of chloroform extract of Chrozophora plicata leaves extract are shown in table no.1. The results obtained (Table II) by using pylorus ligation method of anti-ulcer model in rats indicates that the ulcer score in control animals are 1.0 and 1.5 which is an indication of ulceration, whereas the chrozophora plicata extract treated animals has shown less ulcer score i.e.0.5 and percentage protection from ulcer is 60±1.12 in comparision with control animals which is a clear indication of anti-ulcer activity of chrozophora plicata leaf extract (600mg/kg). The chrozophora plicata chloroform extract at 600 mg/kg produced significant gastro-protective effect. The extract had a percentage ulcer protection of 57.18±1.32 against indomethacin induced ulcers (Table III) whereas the protection is less than that of standard ranitidine which is 68.52±1.07.

DISCUSSION

Flavonoids present in the leaves of Chrozophora plicata are also known to exhibit antioxidant activities as well as scavenge superoxide radicals .Furthermore, from this study, it could also be suggested that Chrozophora plicata could also increase up regulation or increase in COX1 and COX2 which inturn would lead to increase in prostaglandin synthesis. Although the exact mechanism is not known. However, it could be concluded that Chrozophora plicata (600mg/kg) has both anti-ulcer and anti-oxidant properties probably by increasing defensive gastric mucosa.

CONCLUSION

The results of the present study indicates that the chloroform extract of Chrozophora plicata leaves at 600mg/kg possessed significant anti-Ulcer effect and thus supports the use of Chrozophora plicata leaves in treatment of Ulcer. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach and administration of indomethacin (40mg/kg) increases severity of ulceration. Chrozophora plicata leaf extract had significantly reduced ulcer index to an extent of 0.5 and 0.85 in both ulcer models suggesting that this leaf extract is having antisecretory property.

ACKNOWLEDGEMENTS

The authors are grateful to staff and department of Vijaya College of Pharmacy & Bhaskar college of Pharmacy, Hyderabad for providing the facilities for our Research.

Table I. Results of Phytochemical investigation of chloroform extract of chrozophora plicata

Phytoconstituents	**Chloroform extract of chrozophora plicata**
Carbohydrates	-
Steroids	-
Glycosides	-
Flavonoids	+++
Alkaloids	++
Tannins	+++

- Absent; + + present; + + + present with more clarity

Table II. Anti ulcer activity of chrozophora plicata chloroform leaf extract (600mg/kg) by using pylorus ligation method

Animal group	Ulcer Score	Ulcer index	%Protection
control	1.0, 1.5	1,25	-
Chrozophora plicata extract	0.5	0.5	60 ± 1.12**
Standard (Ranitidine 10mg/kg)	0	0	100± 0.42***

P<0.001***, P<0.01** and P<0.05* was considered statistically significant

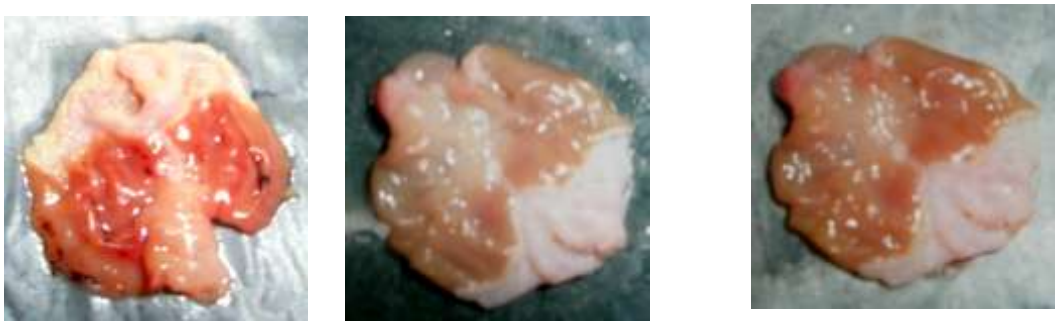
Table no.III: Effect of Chrozophora plicata chloroform leaf extract on indomethacin induced ulcers:

Animal group	Ulcer index	%Protection
Control(indomethacin40mg/kg)	3.63 ± 0.431	-
Chrozophora plicata extract+ indomethacin	0.85±0.35***	57.18 ± 1.32**
Standard (Ranitidine 10mg/kg) + indomethacin	0.18±0.27***	68.52±1.07***

P<0.001***, P<0.01** and P<0.05* was considered statistically significant

a) Control group b) Standard group c) Chrozophora plicata (600mg/kg)

Figure 1: ***Pylorus ligation induced ulcer model in rats and treatment with test extract and standard drug***.



control Standard(ranitidine10mg/kg) Chrozophora plicata(600mg/kg)

Figure 2*: Indomethacin induced stomach ulcers in rats and treatment with test extract and standard drug*.

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