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THE ANTI-ULCEROGENIC ACTIVITY OF THE CRUDE METHANOLIC EXTRACT OF MONECHMA CILIATUM SEEDS

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ABSTRACT

This study was carried out to evaluate the anti-ulcerogenic activity of the methanolic extract of Monechma ciliatum seeds to validate its traditional uses in treatment of stomach disturbances. The methanolic extract of Monechma ciliatum seeds was screened for its anti-ulcerogenic activity against induced-ulcer in four different models. The phytochemical screening of the plant showed that flavonoids, sterols, alkaloids, triterpenes, tannins and saponins are the main constituents of the plant. The results showed that the methanolic extract of Monechma ciliatum seeds at a dose 300mg/kg markedly decreased the incidence of ulcer induced by oral administration of ethanolic HCl in mice by 81.86% ulcer inhibition where as sucralfate showed 50.54% ulcer inhibition, as well as it significantly inhibited ulcer induced by water immersion stress in rats by 86.92%ulcer inhibition, whereas omeprazole showed 100% ulcer inhibition. Also the extract reduced ulcer index compared with the control (10.66±0.53 – 14.72±0.65) in aspirin-induced ulcer. At a dose 400µg/ml the extract elevated stomach pH{H^+} to (0.0015) compared with pH {H^+} (0.0050) induced by histamine(2 µg/ml). The results obtained from this study revealed that the methanolic extract of M.ciliatum seeds possesses anti-ulcerogenic activity.

KEYWORDS: Anti-ulcer, Monechma ciliatum, Water immersion stress induced ulcer.

INTRODUCTION

Peptic ulcer is one of the most prevalent disease. Man kind lived with it since ancient times. Peptic ulcers are defined as breaks or erosions or sores extend from mucosal surface to submucosa. It encompasses gastric ulcers (Gus) and duodenal ulcers (DUs) which share many common features in term of pathogensis, diagnosis and treatment. It is chronic inflammatory conditions in which injury to stomach and duodenal is caused by offending factors that disturb the gastric mucosal parrier and thus promote ulcer development. These conditions include ulcer secondary to the use of conventional nonsteroidal antiinflamatory drugs (NSAIDs),gastric infection with Helicobacter pylori bacterium, ulcer due to Zollinger-ellison syndrome (ZES) due to gastrin producing tumer, gastro-esophageal reflux disease (GERD), benign & malignant peptic ulcer, stress related mucosal injuries &injuries due to other factors e,g alcohol consumption, sigarette smoking, spicey diet etc.

Treatment of symptoms related to gastric ulcer or gastritis with medicinal plants are quite common in traditional medicine worldwide since ancient Egyptian, Greek, Roman and in traditional Chinese medicine,

Monechma ciliatum (Acnthaceae) is a native of tropical Africa and India. In Sudan it grows in Kassala, Kordofan, Bahrelghasal and Bahreljebel states. It is used to treat stomach disturbances and used as carminative for children in such areas beside it contains an essencial oil which emits sweet and pleasant odor that used in traditional Sudanese fragrance and cosmetics.

The present study is an attempt to evaluate the traditional use of Monechma ciliatum to treat gastric disorders.

MATERIALS AND METHODS

Plant

The seeds of Monechma ciliatum were collected from Medicinal and Aromatic Plants Research Institute (MAPRI) farm and authenticated by MAPRI herbarium staff.

Extraction

The seeds of Monechma ciliatum were freed from foreign parts and coarsely powdered, then 10g were extracted with 100 ml of 80% methanol in a shaker for 1hr., filtered and

concentrated under reduced pressure, kept under fan to get the solid mass (9.73%) that kept frozen for pharmacological investigation.

Animals

Albino wister rats, healthy males of (200-250g)

Swiss albino mices of both sex (30g) wt.

Which were fed on wheat, grain, grass, oil, and meat, water ad libidum, in the animal house of (MAPRI)

METHODS

General phytochemical screening of the methanolic extract was carried out using the method described by Martinez & Valencia (1999) Sofowora (1993), Harborne (1984) and Wall et al (1952).The results obtained revealed the presence of triterpenes, sterols, tannins, alkaloids, flavonoids and saponins.

Evaluation of antiulcerogenic activity against Hcl-ethanol induced ulcer in mices

The experiment was performed as described by Yesilada et al. (1997). Swiss albino mice of either sex were divided into three groups, each group consists of six animals. Group 1 received 1.0ml/kg per.os. 1%Sodium carboxymethylcelulose (SCMC) as vehicle control, group2 received 100mg/kg, p.o. sucralfate as standard control. Group 3 received 300mg/kg, p.o. methanolic extract of test plant. After 1hr all the animals were treated with 0.2ml of Hcl ethanol mixture p.o. (0.3M hydrochloric acid and ethanol 60%) to induce gastric ulcer. Animals were killed by cervical dislocation with anesthesia, 1hr. after administration of Hcl-ethanol mixture and the stomach was excised. The ulcer lesions were counted by counting the red spots on the stomach surface of each rat in the group (n=6). Mean lesion index for each group was calculated. Percentage of ulcer inhibition was calculated for each group on comparison with vehicle control group.(Mean lesion index of treated group/(Mean lesion index of untreated group x 100).

Evaluation of antiulcerogenic activity using experimentally water immersion stress induced ulcer in rats

Stress ulcers were induced by forcing the Wistar albino rats of either sex to swim in the glass cylinder (Bhattacharya and Bhattacharya, 1982 and Alder, 1984) containing water to the height of 35 cm maintained at 25 °C for 3 h. animals were fasted for 24 h prior to the experiment and divided into three groups each group consist of six animals. Group 1 received

1.0 ml/kg p.o. 1% (SCMC) as vehicle control, Group 2 received 20 mg/kg, p.o. Omeprazole as standard control, Group 3 received 300 mg/kg, p.o. ethanolic extract of test plant. After the drug treatment animals were allowed to swim in water for 3 h. thereafter, the animals were killed by anesthetic ether. The stomach of each animal was cut longitudinally along the greater curvature. The ulcer lesions were counted. Mean lesion index for each group was calculated. Percentage of ulcer inhibition was calculated for each group in comparison with vehicle control group.

Evaluation of untiulcerogenic activityagainst the effect of gastric acid secretion (PH) in rats.

The experiment was performed as described by Esplugues et al., (1990) and Lippe et al.,(1989). In this experiment male wister rat(250-300) was anaesthetized with urethane.A soft polyethene catheter (inner diameter 0.8mm) was inserted into the stomach through the oesophagus and was connected to a peristaltic pump for infusion of warm saline at 37c.Another polyethylene canula (internal diameter 3mm) was inserted via duodenum into the stomach and tied in place for collection of gastric outflow. The temperature was maintained at 37c. The stomach was flushed with worm (37c) saline to remove all solid contents. Then the stomach was been perfused at a rate of 0.9ml/min. One hour later, 9ml fractions (at 10min intervals) of gastric perfusate were collected and their ph were determined by digital Ph-meter After stabilization of gastric acid output, the gastric acid secretion was stimulated calculated as (H).by passing histamine 2 µg/ml through the stomach. Then the influence of certain doses of the test extract on the secretogogues induced gastric secretion examined by passing different concentrations before the stimulants. The readings of ph-meter were scored and

Evaluation of antiulcerogenic activity against Aspirin-induced ulcers in rat stomach and duodenum

This experiment was prepared according to Selye and Szabo (1973), Robert et al., (1974) and Scarpignato, Tramacere and Zappia (1987), peptic ulcer can be induced in fasting male rats by specific H_2 receptors stimulation through intravenous injection of a single large dose of an ulcerogenic agent that stimulate the secretion of HCl from the parietal cells.Also ulcer can be induced by oral administration of ulcerogenic drug to fasting rat that decrease the strength of gastric mucosa. In this experiment ulcers were induced in the stomach of fasting rats by oral administration of aqueous suspension of acetyl salicylic acid (150 mg/kg).

Twenty four male rats were housed in a suitable environment of lighting, temperature, food and water supply for a week to be acclimatized. Then the rats were been housed individually in cages. On the day before the experiment, the rats were divided in four groups each of six. and were marked. The rats were fastened from food for 24 hrs but free access to water was allowed. On the day of experiment, the rats were weighed individually and calculated doses of the test extract 300mg/kg, 150mg/kg, 75mg/kg, in a volume of 2 ml/kg was administered orally by oral gavage to three groups while the fourth group was given normal saline. 45 minutes later aqueous oral suspension of aspirin 150mg/kg was administered to all rats and rats were returned to their cages. Twenty–four hours later, rats were killed by dislocating the necks and the abdomens were opened to expose the stomach and duodenum. Both organs (stomach and duodenum) were removed and opened carefully (the stomach opened along the greater curvature and washed with saline interiorly clean. Using a were identified high power magnifier, red spots (representing ulcer lesions) were identified and counted.

Histopathological studies

The stomach tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethano (l 50-100%) cleared in xylene and embedded in paraffin sections (4-5µm) were prepared and then stained with hematoxylin and eosin (H.E) dye for photomicroscopic observations.

Statistical analysis of results

Results were expressed as mean ± S.E.M and were analysed statistically by one –way Anova followed by Tukeys multipie comparison test using SPSS software students version.

RESULTS

Table 1: Effect of Omeprazole and ME of M.ciliatum seeds against water immersion stress induced- ulcer in rats.

Treatment	Dose(mg/kg)	Mean ulcer score	%ulcer inhibition
Control	1%SCMC	10.17±2.982	
Omeprazole	20mg/kg	00±00***	100%
M.ciliatum	300mg/kg	1.33±0.715**	86.92%

N=6 **P<0.02 ***p<0.007

Table 2: Effect of ME 0f M.ciliatum seeds against HCL/ethanol induced- gastric lesions in mice.

Treatment	Dose(mg/kg)	Mean lesion index	%Ulcer inhibition
Control	1%SCMC	30.33±10.938	
Sucralfate	100mg/kg	15.0±5.825*	50.54%
M.ciliatum	300mg/kg	5.5±3.106*	81.86%

N=6 *=p<0.05

Table 3: Effect of ME of Monechma ciliatum seeds on Aspirin-induced ulcer in rat

Treatment	Number of ulcer Lesion (mean ±S.E.M)
Aspirin150mg/kg(control)	14.72±0.65
M.C.75mg/kg+Asp.150mg/kg	14.44±0.65
M.C.150mg/kg+Asp.150mg/kg	13.24±0.47
M.C.300mg/kg+Asp.150mg/kg	10.66±0.53*

N=6 *=P0.005

Table 4: Effect of methanolic extract of M.ciliatum seeds on gastric acid secr**etion (pH).**

Treatment	pH(M±S.E.M)	$[H^+]$
Normal saline	3.75 ± 1.9	0.000177
2 µg/ml Hist.	2.30 ±0.99	0.00501
100µg/ml.M.ciliatum+2 µg/ml Hist	2.50 ±1.75	0.00316
200µg/ml.M.ciliatum+2 µg/ml Hist.	2.55± 1.94	0.0028
400 µg/ml.M.ciliatum+ 2 µg/ml .Hist	2.82 ±1.83 *	0 .0015

N=10 *=P<0.05

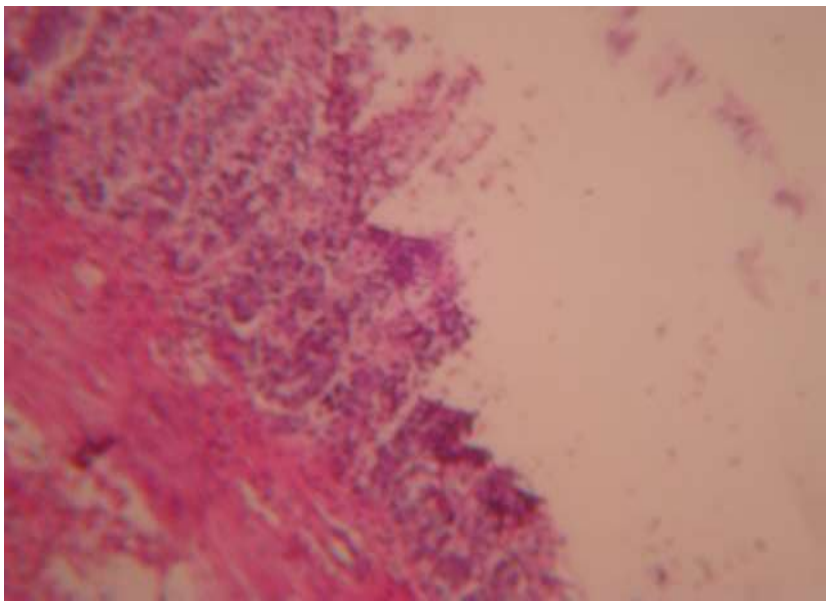

Figure 1: Photomicrograph showed mucosal sloughing with mucous necrosis and mild leukocyte infiltration,of rat treated with ethanolic HCl at a dose 1.66ml/kg (H&E satin), X100.

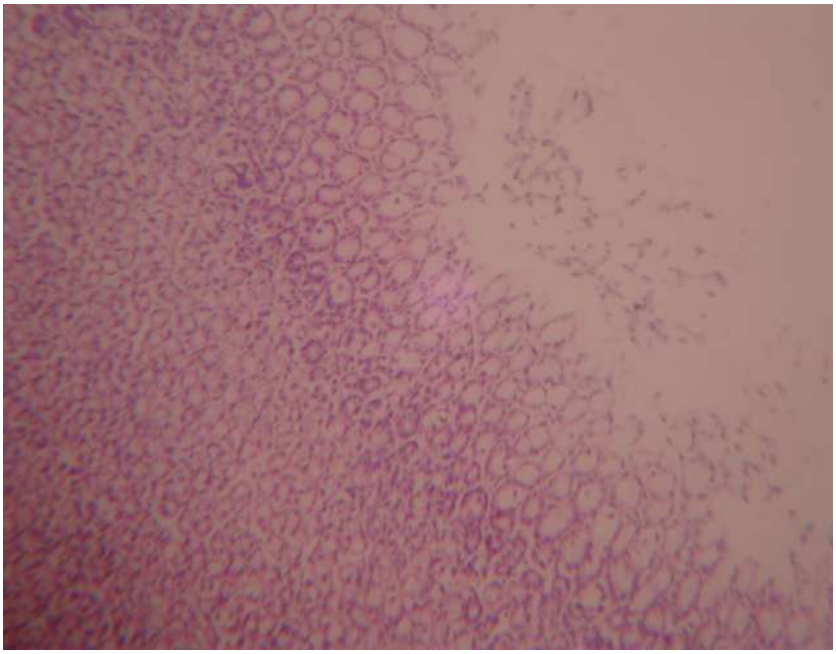

Figure 2: Photomicrograph of the gastric mucosa showed mild erosions of rat treated with methanolic extract of Monechma ciliatum at a dose 300mg/kg after it had been treated with ethanolic HCl at a dose 1.66ml/kg (H&E stain), X100.

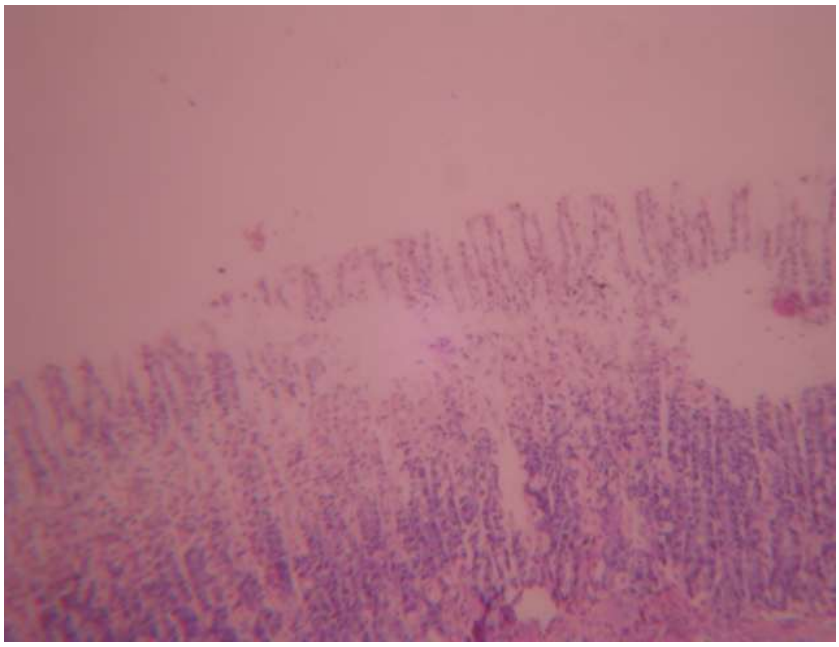

Figure 3: Photomicrograph showed focal detachment of the mucosal surface, of fasting rat inforced to swim 3hours in the glass cylinder containing water to the height of35cm at 25°C (H&E stain), X100.

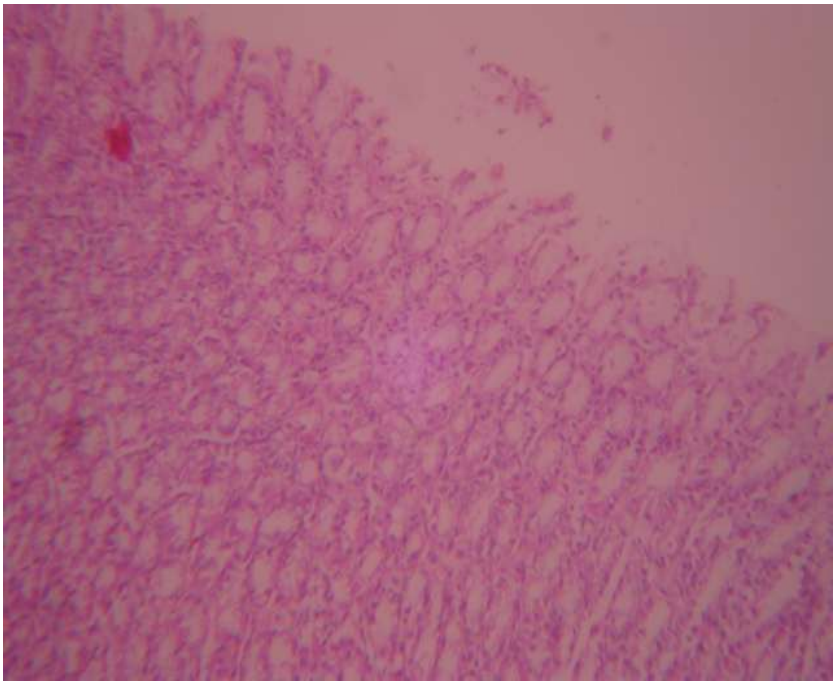

Figure 4: Photomicrograph showed minimal changes on the gastric mucosa,of fasting rat treated with methanolic extract of Monechma ciliatum seeds at a dose 300mg/kg after it had been inforced to swim 3hours inthe glass cylinder containing water to the height of35cm at 25°C (H&E stain), X100.

DISCUSSION

The methanolic extract (ME) of Monechma ciliatum (M.C) seeds, which was subjected to antiulcerogenic test in four different models inhibited ulcers induced by water immersion stress in rats (Table 1).Immersion of rats in cold water provides both emotional stress and physiological stress which in turn induces ulcer in rats stomach. Anti-ulcer activity of ME of M.ciliatum seeds in this model may be due to proton pump inhibition. Also ME of M. ciliatum showed gastroprotective activity as it decreased the incidence of ulcer induced by ethanolic HCl in mices with an inhibitory effect more than that obtained by sucralfate (Table 2). The gastroprotective effect obtained by MEof M.C seeds may be due to mechanism of enhancement of defensive factors e.g mucin and bicarbonate secretion and prostaglandin synthesis and thus strengthing the mucosal parrier.

Aspirin is one of noxious agents that aggravate acid secretion which in turn leads to ulcer formation and gastric mucosal injury. The gastric damage induced by aspirin may possibly be due to leukotrienes production and involvement of libooxygenase. The gastroprotective effect against gastric damage in this model may be due to protection against cycloxygenase and leukotrienes pathway possibly by stimulation of prostaglandin synthesis and/or stimulation of

mucin and bicarbonate secretion which in turn protect gastric mucosa.ME of M. ciliatum seeds showed a remarkable reduction in incidence (10.66±0.53) of gastric ulcer that induced by aspirin in aspirin-induced ulcer in rats compared to that of the control (14.72±0.65) (Table 3)

The antiulcer activity is recognized by reduction of acid secretory parameters (i.e total acid or free acid) suggesting that acid inhibition accelerates ulcer healing as stated by (Nunes, et al at 2009) gastroprotective and antiulcerogenic effects are related to the inhibition of gastric acid secretion and increase of mucosal defensive factors such as prostaglandins and mucin.

The M E of M.C seeds elevated rat stomach pH compared with that obtained by histamine (Table 4), hence it suppressed gastric acid secretion.

The phytochemical constituents of M.C may account for its gastroprotective and antiulcer activity.

The histopathological diagnosis confirmed the experimental induction of ulcer(Figure 1&3) as well as gastroprotective and anti-ulcerogenic activity of the M E of M.C seeds (Figure 2&4).

CONCLUSION

From the results obtained, it can be concluded that the methanolic extract of M.C seeds possess antiulcerogenic activity in rats.These results seem to support the traditional use of it.

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