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Research Article

STUDY ON PHYTOCHEMICAL PROFILE AND ANTIULCEROGENIC EFFECT OF *MALACHRA CAPITATA (L.)* IN ALBINO WISTAR RATS

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ABSTRACT

The aim of this study is to evaluate the anti-ulcer activity of aqueous extract of roots of *Malachra capitata (L.)*. The aqueous extract of *Malachra capitata (L.)* (AMC) was investigated for its anti-ulcer activity against pylorus ligation and ethanol induced gastric ulcers in rats. Ranitidine (50mg/kg,p.o.) and misoprostol (100ug/kg,p.o.) were used as standard drugs. A significant ($p<0.01$) anti-ulcer activity was observed in both the models. Both does of *Malachra capitata (L.)* produced gastric anti-secretory effect in pylorus ligated rats and also showed gastric cytoprotective effect in ethanol induced gastric ulcers. Pylorus ligation showed significant ($p<0.01$) reduction in gastric volume. Free acidity, ulcer index as compared to control. It also showed significant ($p<0.01$) reduction in ulcer index in ethanol induced model with respect to control. This present study indicates that, *Malachra capitata (L.)* roots extract have potential anti-ulcer activity in this tested models.

Key Words: *Malachra capitata (L.)*, Pylorus Ligation, Ethanol Induced Gastric Ulcers.

INTRODUCTION

Peptic ulcer disease (encompassing gastric ulcer and duodenal ulcer) affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs [1]. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection [2,3]. There has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Modern approach to this includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog [4]**.** Development of tolerance and incidence of relapses and side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new antiulcer drugs, which includes herbal drugs.

Malachra capitata (L.) is a herb belongs to family: Malvaceae. Description: Mostly erect, coarse, annual or perennial herb 1-2 m tall, throughout densely whitish- or yellowish-tomentose with stellate hairs and usually also moderately to copiously hispid with simple or stellate hairs to 2 mm long; roots long-petioled; stipules lanceolate, 5-15 mm long; blades orbicular to ovate, 2-10 cm long, palmately sinuate to 3-, 5-, or 7-lobed, lobes mostly obtuse, crenate to serrate, the base obtuse or truncate; flowers in axillary, pedunculate, bracteate heads, bracts 1-2 cm long, stipitate and subtended by paired, filiform bracteoles, conduplicate, suborbicular to ovate, obtuse or acute, entire or once or twice dentate, obtuse to cordate at base, prominently veined and whitish basocentrally; involucral bracts wanting; calyx tubular-campanulate, 4-8 mm long, 5-lobed to below middle, lobes ovate-lanceolate, white with brownish or reddish nerves; petals yellow, obovate, 10-15 mm long, slightly exceeding stamina column; mericarps 3-3.5 mm long,

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muticous, reddish veined, puberulent; seed obovoid-cuneate, about 2.5 mm long, black, whitish-pubescent about hilum. The root of the *Malachra capitata* (L.) is traditional remedies for the many disease condition such as pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer, healing of wounds [5-9]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the constituents and anti-ulcer profile of the aqueous extract of *Malachra capitata (L.)* (AMC) is being reported here.

MATERIALS AND METHODS

Collection and authentication of plant material

The Plant material of *Malachra capitata (L.)* roots was collected from Tirunelveli District, in the Month of August 2011. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The roots of the *Malachra capitata* (L.) are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at 35^{o}C for 4 days. The dried roots of *Malachra capitata* was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of aqueous extract of *Malachra capitata* was found to be 10.5 % w/w. The aqueous extract was administered to the animals by suspending each time in 1% CMC.

Phytochemical Screening

The phytochemical examination of aqueous extract of *Malachra capitata (L.)* was performed
by the standard methods [9].

Experimental animals

Adult Wistar rats of either sex weighing 180-250 gms were used in pharmacological and toxicological studies. The inbred animals were taken from the animal house and maintained in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at 22±1˚C with humidity at 55±5%. They were fed balanced rodent pellet diet from Poultry Research station, Nandanam, Chennai-35 and tap water ad *libitum* throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).

Acute toxicity study

The acute toxicity of aqueous extract of *Malachra capitata (L.) roots* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [10].

ANTIULCER ACTIVITY

Pylorus ligated model

Wistar rats of either sex weighing 180 to 250 g were divided into five groups of six animals each.

Group I - Control animals which are pretreated with suspended 1% CMC, ml/100g.

Group II- Pylorus ligated animals which are pretreated with 1% W/V CMC, 1ml/100g.

Group III - Pylorus ligated animals which are pretreated with AMC (200mg/kg p.o) suspended in 1%w/v CMC.

Group IV - Pylorus ligated animals which are pretreated with AMC (400mg/kg p.o) suspended in 1%w/v CMC.

Group V - Pylorus ligated animals which are pretreated with Ranitidine (50mg/kg, p.o) suspended in 1%w/v CMC.

PROCEDURE

Groups III and IV animals were treated with AMC and group V were treated with ranitidine for the respective 5 days daily. On day 6th after the last dose, all the groups were kept for 18h fasting and care was taken to avoid coprophagy [11]. Animals were anaesthetized using ether the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully in the abdomen and the wound was sutured by interrupted sutures. After 6 hr53, stomachs were dissected out and cut open along the greater curvature and the content were drained into small beakers, centrifuged and then subjected to analysis for gastric volume, free acid, total acid and protein estimation [12].

The mucosa was flushed with saline and the stomach was pinned on frog board and the ulcer score was calculated.

Ethanol Induced Ulcer

Wistar rats of either sex weighing 180 to 250 g were divided into five groups of six animals each.

Group I - Control animals which are pretreated with suspended 1% CMC, 1ml/100g.

Group II - Ethanol (1ml/200g p.o) induced ulcer which are pretreated with 1% W/V CMC, 1ml/100g.

Group III - Ethanol (1ml/200g p.o) induced ulcer which are pretreated with AMC (200mg/kg /p.o) suspended in 1%w/v CMC.

Group IV - Ethanol (1ml/200g p.o) induced ulcer which are pretreated with AMC (400mg/kg /p.o) suspended in 1%w/v CMC.

Group V - Ethanol (1ml/200g p.o) induced ulcer which are pretreated with Ranitidine (50mg/kg/p.o) suspended in 1%w/v CMC.

Procedure

Groups III and IV animals were treated with CCEP and group V animals were treated with ranitidine for the respective 14 days. On day 15th after the last dose, all the groups were kept for 24h fasting and care was taken to avoid coprophagy56. The 100 % ethanol (1ml/200g p.o) was administered to the groups II, III, IV and V animals and after 1 h animals were sacrificed by cervical dislocation and stomachs were dissected out and cut open along the greater Curvature and examined for ulcer [13].

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%*I*) was calculated as described by Nguelefack et al. [14] using the following formula:

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

BIOCHEMICAL ESTIMATIONS

Pylorus ligated model

Determination of gastric volume

After sacrificing the rat, the stomach portion was removed. The gastric contents were transferred in to the centrifuge tube, and centrifuged at 1000 rpm for 10 minutes. The supernatant liquid was then transferred to a measuring cylinder, and the volume was measured.

Determination of pH of gastric content

1 ml of the gastric juice was collected, and pH was directly measured by using pH strip [15].

Determination of free acidity and total acidity

The total volume of gastric content was measured. The gastric contents were centrifuged and filtered. One ml of the gastric juice was pipetted out and the solution was titrated against 0.1N sodium hydroxide using 2 to 3 drops of Topfer's reagent as indicator, to the endpoint when the solution turned to yellowish orange colour was observed. This indicated the volume of NaOH required neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The difference between the two readings indicated the volume of NaOH required neutralizing the combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice [16].

Acidity was calculated by using formula;

$$\text{Acidity} = \frac{\text{Vol. of NaOH X Normality of NaOH}}{\text{Vol. of Gastric juice used}} \text{ m. Eq. /dl.}$$

Estimation of total proteins

Reagents

Alkaline copper reagent

Solution A: 2% sodium carbonate in 0.1N sodium hydroxide

Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate. 50 ml of solution A was mixed with 1 ml of solution B just before use. Folins phenol reagent. One volume of folins reagent was diluted with two volumes of distilled water just before use.

Standard bovine serum albumin

20 mg of bovine serum albumin was dissolved in 100 ml of distilled water. Few drops of NaOH was added to it aid complete dissolution of bovine serum albumin and to avoid frothing, it was allowed to stand overnight in a refrigerator.

Procedure

The dissolved proteins in gastric juice were estimated in the alcoholic precipitate obtained by adding 90 % of alcohol with gastric juice in 9: 1 ratio respectively. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken in another test tube. To this 4 ml of alkaline copper reagent was added and kept for 10 minutes. Then 0.5 ml of phenol reagent was added and again 10 minutes was allowed for color development. Reading was taken against blank prepared with distilled water at 640 nm. The protein content was calculated from standard curve prepared with bovine albumin and has been expressed in terms of µg/ ml of gastric juice [17].

Statistical Analysis

The data represents Mean ± SEM. Results were analyzed statistically using one way ANOVA followed by Dunnet's test. The minimum level of significance was set at ($P<0.05$).

RESULTS

Phytochemical investigation

The results of preliminary phytochemical investigation of the aqueous extract of *Malachra capitata (L.)* roots (AMC) shows the presence of carbohydrates, phenols, flavanoids, glycosides, terpenes, alkaloids, tannins, and Saponins.

Acute toxicity study

Acute toxicity study in which the animals treated with the AMC at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at

any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of AMC on gastric ulcer induced by pylorus ligation

Gastric volume

A significant (P<0.01) increase in the gastric volume level is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.01) decrease in the gastric volume level and ranitidine treated group have shown a significant (P<0.01) decrease in the gastric volume level when compared to the Pylorus ligated group. The results were shown in Table 1.

pH

A significant (P<0.01) decrease in the pH level is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.05) and (P<0.01) increase in the pH level and ranitidine treated group have shown a significant (P<0.01) increase in the pH level when compared to the Pylorus ligated group. The results were shown in Table 1.

Table 1. Effect of AMC on acid secretary parameters in pylorus ligation induced gastric ulcer

Groups	Gastric Volume (ml/100g)	pH	Number of Ulcer	Ulcer severity score	Free acidity (mEq/dl)	Total acidity (mEq/dl)	Total protein (µg/ml)
I Control (CMC)	2.367 ± 0.15	2.92 ± 0.27	0.17±0.14	0.16 ±0.12	4.58± 0.34	5.45 ± 0.0291	317.80 ± 8.01
II Pylorus Ligated	7.033± 0.28^{a**}	2.21 ±0.11^{a**}	4.54±0.23^{a**}	9.57± 0.62^{a**}	7.57± 0.22^{a**}	8.38 ± 0.21a**	486.12 ± 6.45^{a**}
III AMC 200mg/kg	6.333± 0.17^{b**}	2.44 ± 0.17^{b*}	3.52±0.31^{b*}	7.23 ±0.25^{b**}	6.48±0.12^{b**}	7.88 ±0.35b*	384.62 ±5.36^{b*}
IV AMC 400mg/kg	5.817 ± 0.11^{b**}	3.15 ± 0.17^{b**}	2.16±0.14^{b**}	3.47± 0.62^{b**}	4.35± 0.29^{b**}	5.25 ± 0.20b**	317.24 ± 2.45^{b**}
V Ranitidine50mg/kg	3.69 ± 0.15^{c**}	3.54 ± 0.14^{c**}	1.02±0.21^{c**}	1.27± 0.20^{c**}	3.18±0.12^{c**}	4.18 ± 0.12c**	265.55 ± 4.27^{b**}

Comparisons were made between: a- (Group I vs II), b- (Group II vs III, IV), C-(GroupII vs V)
Values are expressed as mean ± SEM of 6 animals. Statistical Significance test for comparison was done by ANOVA followed by Dunnet's test.
Symbols represent statistical significance: ** P < 0.01, * P < 0.05, ns- non a significant.

Table 2. Effect of AMC on ulcer index in Ethanol induced gastric ulcer

Groups	I Control (CMC)	II Pylorus Ligated	III AMC 200mg/kg	IV AMC 400mg/kg	V Ranitidine 50mg/kg
Ulcer index(mm^2)	0.23±0.33	38.54 ±1.24^{a**}	28.53 ± 0.72^{a**}	17.46 ± 1.87^{b**}	9.34 ± 1.7^{C**}

Comparisons were between: a- (Group I vs II), b- (Group II vs III, IV), C-(Group II vs V). Values are expressed as mean ± SEM of 6 animals. Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's test.
Symbols represent statistical significance: ** P < 0.01, * P < 0.05, ns- non a significant.

Ulcer number

A significant (P<0.01) increase in the ulcer number is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P,0.05) (P<0.01) decrease in the ulcer number and ranitidine treated group have shown a significant (P<0.01) decrease in the ulcer number when compared to the Pylorus ligated group. The results were shown in Table 1.

Ulcer severity score

A significant (P<0.01) increase in the ulcer severity score is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.01) decrease in the ulcer severity score and ranitidine treated group have shown a significant (P<0.01) decrease in the ulcer severity score when compared to the Pylorus ligated group. The results were shown in Table 1.

Free Acidity

A significant (P<0.01) increase in the free acidity is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.01) decrease in the free acidity and ranitidine treated group have shown a

significant (P<0.01) decrease in the free acidity when compared to the Pylorus ligated group. The results were shown in Table 1.

Total acidity

A significant (P<0.01) increase in the total acidity is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.05) and (P<0.01) decrease in the total acidity and ranitidine treated group have shown a significant (P<0.01) decrease in the total acidity when compared to the Pylorus ligated group. The results were shown in Table 1**.**

Total protein

A significant (P<0.01) increase in the total protein is observed in the pylorus ligated group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.05) and (P<0.01) decrease in the total protein and ranitidine treated group have shown a significant (P<0.01) decrease in the total protein when compared to the Pylorus ligated group. The results were shown in Table 1.

Effect of AMC on gastric ulcer induced by Ethanol
Ulcer index

A significant (P<0.01) increase in the ulcer index is observed in the ethanol induced group when compared to the control group. AMC 200mg/kg and 400mg/kg treated group have shown a significant (P<0.01) decrease in the ulcer index and ranitidine treated group have shown a significant (P<0.01) decrease in the ulcer index when compared to the ethanol induced group. The results were shown in Table 2.

DISCUSSION

The antiulcer activity of AMC was studied in different models LIKE ulcers induced by pylorus ligation and ethanol. Although the etiology of gastric ulcer is not known in most cases, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms [18].

It is well known that pylorus ligation causes gastric hyper secretion due to poorly understood mechanisms. The activation of the vagus-vagal reflex by stimulation of pressure receptors in the antral gastric mucosa in pylorus ligature model is believed to increase gastric tonus and secretion [19]. Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in the pylorus ligated rats. Pylorus ligated induced ulcers are thought to be caused due to increased presence of acid and pepsin in the stomach. The essential criteria, which determine the status of mucosal defense barrier against the offensive assault of acid-pepsin is the quality and quantity of gastric mucus secretion. Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms including lessening stomach wall friction during peristalsis and acting as an effective barrier to the back diffusion of hydrogen ions [20].

The AMC reduces the gastric volume, ulcer score, free and total acidity of gastric acid secretion of pylorus ligature induced gastric ulceration in rats. From results it is clear that the AMC exhibited a significant gastric acid secretion activity by reducing the secretory parameters when compared with the Pylorus ligated group. AMC pretreatment have shown a significant reduction in protein levels when compared with group II. Hence, the protection by AMC against gastric ulcers induced by Pylorus Ligated appears to be produced by the suppression of pepsin levels and strengthening of mucosal barrier. Further, it is clear that the antiulcer activity against this model was again found to be more with AMC extract.

Oral treatment with ethanol causes focal hyperemia, edema, necrosis and submucosal hemorrhage as well as circulatory disturbances. The extent of ethanol-induced gastric mucosal damage in rats correlates with the number of degranulating mast cells. Since these cells are a source of several neuropeptides and inflammatory mediators, including histamine and leukotrienes [21]. Ethanol induced gastric lesion formation may be due to stress in the gastric blood flow which contributes to the development of hemorrhage and necrotic aspects of tissue injury. Ethanol also increases Na+ and K+ flux into the lumen and increases pepsin secretion along with the histamine release.HCL further deepens the necrosis and increases the tissue injury65. Ethanol induced ulcer are more predominant in the glandular part of the stomach stimulates leukotrienes, 5-lioxygenase pathway, mast cell secretary product and breakdown of reactive oxygen species resulting in the damage of gastric mucosa.Another action promoted by ethanol is its ability to damage the gastric mucosa by mechanical injury [22].

The results further indicate that AMC may enhance gastric mucosal defensive factor, such as mucus and or prostaglandin. Therefore protection afforded by AMC against ethanol induced gastric ulceration could also be due to inhibition of the 5-lipoxygenase pathway or to the antagonistic activity of leukotrienes. Flavonoids are capable of protecting the gastric mucosa from necrotizing substances and possible useful in the therapy of acute and chronic gastric ulcerations. plant-originated flavanoid substances such as Solon- Sophoradin root, seed extract of Amaranth, extract of grapefruit seeds - Citro, and capsaicin present in chilli pepper extract are beneficial and dose-dependent reduction in acute and chronic gastric lesions. Plant-originated flavonoid substances are highly gastroprotective [23].

The Antiulcer effect of the lipid components of M. azedarach fruits which is mainly due to the phytosterol fraction [24,25].Sterols may even protect against peptic ulcer diseases even in the area of prevalence of H.pylori. Phytosterol esters and sterols, in horse gram an herb in the

genus Dolichos cultivated in India for food and fodder were protective for ulcers [26-43]. Phytochemical studies of the aqueous extract of *Malachra capitata (L.)* revealed the presence of flavonoids, sterols and terpenes. Numerous flavonoids have shown reducing gastric acid secretion and protective properties in different experimental model. Phytosterols which have been shown various properties that is necessary for protection against ulcer induction. Since flavonoids and sterols shown to be present in the, aqueous extract of *Malachra capitata (L.)* these constituents may be responsible for the anti-ulcer activity of the aqueous extract of *Malachra capitata (L.)*.

Therefore, it can be concluded that this crude extract have a potential to be used as an antiulcer drug in combination with other drugs or alone. Though the mechanism of the Anti-ulcer action of the plant extract remains to be studied in detail. Isolation of the active constituents may be carried in future.

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