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Original Research Article

Anti-Ulcer Activity of the Ethanol and Aqueous Extracts of *Mikania scandens* in Wistar albino Rats

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Abstract

Background: *Mikania scandens* (family: Asteraceae), known as climbing hempvine, is a twining herb with long petioles, opposite leaves and small monogamous flower heads. It has been used in traditional medicine as an analgesic and anti-inflammatory agent.

Objective: The aim of the present study was to investigate the anti-ulcer activity of ethanol and aqueous extracts of aerial parts of the plant in Wistar albino rats.

Materials and Methods: Ulcer was induced by pylorus ligation method in experimental Wistar albino rats. Gastric juice content, pH, free acidity, total acidity, ulcer index and percentage of ulcer protection were used for evaluation. The aerial parts of the plant were collected, shade dried, powdered and successively extracted with ethanol and water (1:10 w/v in g/mL). The extracts were separately collected using rotary evaporator. Percentage yield of ethanol and aqueous extracts were 17.6 g, (9.8% w/w) and 24.3 g, (15.5% w/w). The crude extracts were tested qualitatively for presence or absence of selected phytoconstituents. The anti-ulcer activity of ethanolic and aqueous extracts was carried for the two selected common doses (100 and 200 mg/kg of b.w per day orally) for each of the extract against the standard drug, [lansoprazole (pure drug suspension)] at a dose of (8 mg/kg) day orally.

Results: The phytochemical study has revealed that both extracts of *M. Scandens* contain flavonoids, alkaloids, steroids, saponins and glycosides. The anti-ulcer activity, the two extracts was found significantly high ($p<0.05$) at the dose of 200 mg/kg orally compared to lansoprazole (8 mg/kg).

Conclusion: The present study of aerial parts of *M.scandens* in ethanolic and aqueous extracts has shown to possess effective anti-ulcer activity.

Keywords: *Mikania scandens*, Asteraceae, phytoconstituents, anti-ulcer activity, lansoprazole.

1. Introduction

Plants have a great potential for producing new drugs for human benefit. Medicinal plants are the elegant source of drugs for conventional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates, ethno pharmacy, and chemical entities for synthetic drugs. They also play important role in healthcare systems due to low cost, effectiveness, better cultural accessibility, compatibility and fewer side effects, and easy availability. They appear to possess moderate efficacy with no or less toxicity as compared to synthetic drugs which make them incredible application for drug development research programs[1-2].

Mikania scandens (L) Willd (Asteraceae) is also known as climbing hemp weed or climbing Boneset. "*Mikania"* is named for Joseph Gottfried *Mikan*, the species 'scandens' comes from the Latin *scandere* meaning 'to climb' [3-5]. It is a perennial herb, rapidly growing herbaceous to semi woody, branching vine. The leaves are simple, acuminate, coarsely denate, 1.5-8 cm wide and 2.5-14 cm long with a deeply cordate base, oppositely arranged at swollen nodes on the stem. The flowers in small heads are clustered in panicles, 5-7 mm long. They have pink, pale purplish or rarely white corolla [6]. Fruit is an achene, oblong, 1.5-2.5 mm long, brownish black, five angled [7]. The ethno botanical uses of the *M.scandens* are to treat itchy skin, also for circumcision wounds and tumours, planted as an ornamental, cover crop, and cattle feed [8-12].

Ulcers, a common gastrointestinal "disorder", is an open sore or lesions of the skin or mucous membrane characterized by sloughing of inflamed dead tissue[13-14]. Peptic ulcer is a chronic, heterogeneous recurrent disease [15]

of multifactorial aetiology. It is denoted by the disorder of mucosal integrity of the oesophagus, stomach, or duodenum [16].

Ulcers are particularly occurs when there is an imbalance between aggressive (hydrochloric acid, pepsin secretion, helicobacter pylori and bile salts) and protective factors [17-18] (mucin, bicarbonate secretion, prostaglandins, proliferation, nitric oxide and growth factors). Other factors commonly intricated are acid pepsin hyper secretion, abundantly uses of non steroidal anti inflammatory drugs (NSAIDs), and due to usage of tobacco, psychological stress, pathological condition Zolliger-Ellision Syndrome (ZES) which indicated a high and uncontrollable production of acid leads to ulcer formation [19-20].

2. Materials and Methods

2.1 Plant Materials

The aerial parts (leaves, stem, flowers) of *M.scandens* were collected during June-July 2013 from rural areas of Midnapore district of West Bengal, India. The species was taxonomically established and authenticated by Scientist-'O' V.P.Prasad, Central National Herbarium, Botanical Garden, Howrah (Voucher no. CNH/57/2014/Tech.II/278). After authentication, the fresh aerial parts collected in bulk from the plant were washed, shade dried at room temperature for 15 days and ground mechanically into coarse powder. The powdered aerial plant materials were loaded into a Soxhlet extractor and extracted with ethanol and water successively. The two extracts of plant were collected separately and the solvent was evaporated using rotary evaporator. After evaporation, the crude extracts of ethanol and water were obtained and stored in a freezer at 8ºc temperature until further use.

2.2 Experimental Animals

Thirty six healthy wistar albino rats of either sex weighing (150-200) gm were selected for this study. The animals were maintained in standard housing conditions (room temperature 24º±2 with 50-60% relative humidity and 12/12 hours light/dark cycle [21-22]. Each group of animals (n=6) had free access to water *ad libitum* a normal diet (Hindustan Unilever). The study was approved (IAE/SKIPS/2014/OCT06/04/RATS-72/MICE-72) by Institutional Animal Ethical Committee (IAEC) with the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA).

2.3 Acute Toxicity Study

The acute toxicity study of extracts was done as per OECD 420 guidelines [23] for different doses between 1000 and 2000 gm/kg orally in mice.

2.4 Pylorus Ligation and Ulcer Induction

This anti-ulcer model was designed by Shay *et al.*, 1945 for the study of the effect of drugs on gastric secretion. The rats were divided into six groups (n=6) and they were kept on fasting for overnight prior to the start of experiment but water was provided *ad libitum*. One hour prior to the pyloric ligation with a thread, each group of animal was treated with the extracts as shown in Table 1 [24-25].

Table 1: Groups of Treatment

Serial No.	Groups
i)	Control (0.2 ml distilled water)
ii)	Standard (lansoprazole 8 mg/kg)
iii)	Ethanol (100 mg/kg)
iv)	Ethanol (200 mg/kg)
v)	Aqueous (100 mg/kg)
vi)	Aqueous (200 mg/kg)

The animals were deprived of both food and water for 4 hours postoperatively and were sacrificed at the end. To observe ulcer lesions, the gastric juice was collected in graduated centrifuge tube and centrifuged at 1000 (rpm) for 10 minutes immediately. Volume of gastric juice and its pH were recorded [26]. The stomachs were cleaned by normal saline and then focused under magnifying lens to note the ulcers.-Mean ulcer score is expressed as ulcer index which has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach [27-28]. The ulcer score was counted as per Table 2.

Table 2: Calculation of Ulcer Index

Ulcer Score	Inference
0	No Visible Ulcers
0.5	Red Coloration
1	Spot ulcers
1.5	Hemorrhagic streaks
2	Ulcer>3mm<5mm
3	Ulcers>5mm

% Inhibition of Ulcer index =

$$\frac{(\text{Ulcer index Control} - \text{Ulcer index Test}) \times 100}{\text{Ulcer index Control}}$$

The pH of the gastric juice was determined by pH meter. The estimation of free acidity and total acidity of gastric juice was carried out as per the standard method. The results were expressed as mean ± SEM. Statistical analysis was represented by one way (ANOVA) followed by Turkey's multiple comparison test. P values <0.05 vs control were considered significant.

3. Results

3.1 Acute Oral Toxicity study

From this study, it was found that both the extracts of *M.scandens* were safe at 2000 mg/kg with no mortality. ($1/20^{th}$ and $1/10^{th}$) of this dose i.e. 100 mg/kg and 200 mg/kg were used in the anti-ulcer study.

3.2 Phytochemical Evaluation

The results of phytochemical evaluation, (Flavonoids with $Fecl_3$ and Shinoda test, Alkaloids with Dragendorffs and Mayer's reagent, Saponin with ability to produce stable foam, Steroid with Libermann-Burchard reagent, Glycosides with Borntrager's test) of the extracts have showed presence of flavonoids, alkaloids, saponins, steroids and glycosides Table 3.

Table 3: Phytochemical Screening of Ethanol and Aqueous Extracts of *M.Scandens*

Serial No.	Phytoconstituents	Ethanol extract	Aqueous extract
1.	Flavonoids	+	+
2.	Alkaloids	+	-
3.	Saponins	-	+
4.	Steroids	+	-
5.	Glycosides	+	-

(+) sign indicates presence and (-) sign indicates absence of phytoconstituent.

3.3 Anti-ulcer activity

The results of anti-ulcer activity study were shown in the Table 4. Both the extracts have shown the decreased volume of gastric juice when compared to control group. The aqueous extract at 200mg/kg has shown a significantly different ($p<0.05$) effect in reducing the volume of gastric juice volume as compared to the standard drug, lansoprazole (8mg/kg) (Figure 1).

Table 4: Anti-ulcer Activity Result of Ethanol and Aqueous Extracts of *M.scandens*.

Sl No.	Gp. Name	Gastric Juice (ml)	P^H	Free acidity	Total acidity	Ulcer index	% Ulcer protection
1.	CNT	6.85 ± 0.523	2 ± 0.00	39.5 ± 2.790	78 ± 3.864	3.66 ± 0.421	0%
2.	STD	4.28 ± 0.535**	4 ± 0.365**	20 ± 2.875**	37.66 ± 3.051**	0.66 ± 0.105**	81.83%
3.	ETH 100 mg/kg	5.35 ± 0.348**	2.83 ± 0.401	31.83 ± 2.182**	65.16 ± 3.646**	2 ± 0.316**	45.44%
4.	ETH 200 mg/kg	4.33 ± 0.455**	3.83 ± 0.307**	25.33 ± 2.458**	50 ± 1.709**	1.33 ± 0.210**	63.63%
5.	AQS 100 mg/kg	5.21 ± 0.382**	3 ± 0.258	32.66 ± 2.060	65.86 ± 3.846*	1.58 ± 0.327**	56.81%
6.	AQE 200 mg/kg	3.91 ± 0.385**	3.16 ± 0.477	21.583± 0.712**	44.16 ± 1.301**	1.16 ± 0.166**	68.19%

Gp.-Group, CNT-Control, Std-Standard, Values are expressed as mean ± SEM; n=6; * =P<0.05 **=P<0.01 when compared to Control.

Compared to control group, both of the tested extracts showed elevated pH indicating their capacity to lower the acidity of the gastric juice. The ethanol extract at 200mg/kg indicates almost equipotent effect as that of the standard drug- (Figure 2).

The oral administration of ethanol and aqueous extracts at a dose of 200 mg/kg in ligated pylorus inhibited the ulcer formation in a dose dependent manner as compared to control. Both the extracts have showed a significant reduction in gastric juice volume, free acidity (Figure 3), total acidity (Figure 4) ulcer score (Figure 5) and ulcer protection (Figure 6).

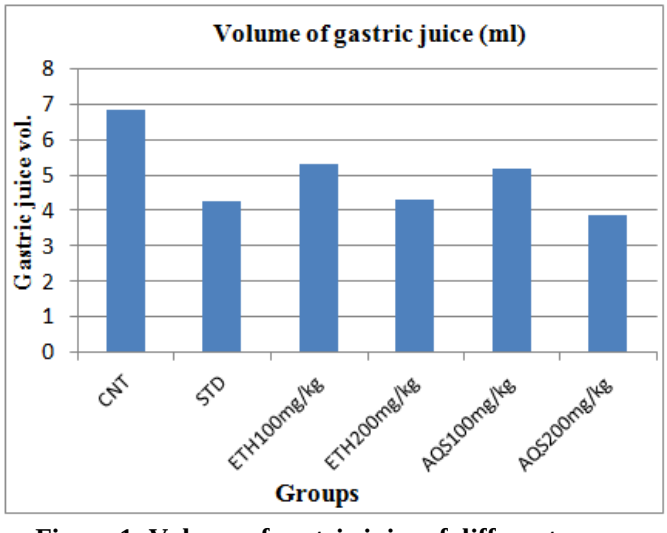

Figure 1: Volume of gastric juice of different groups

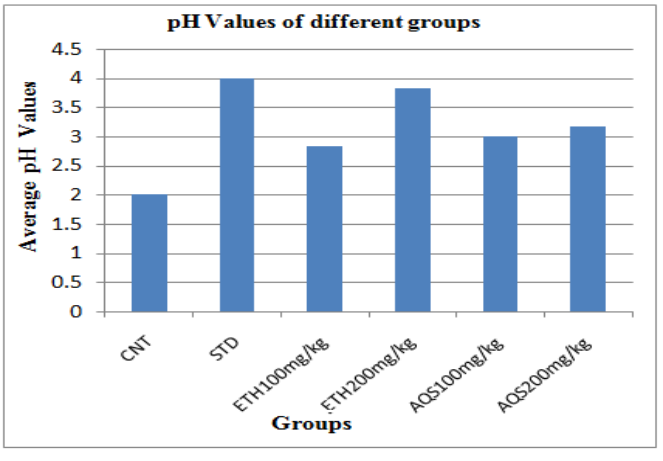

Figure 2: pH of gastric juice of different group

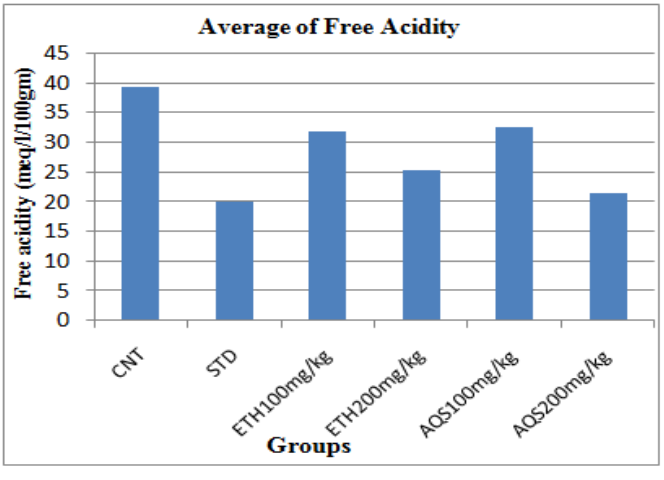

Figure 3: Avg. free acidity of different groups

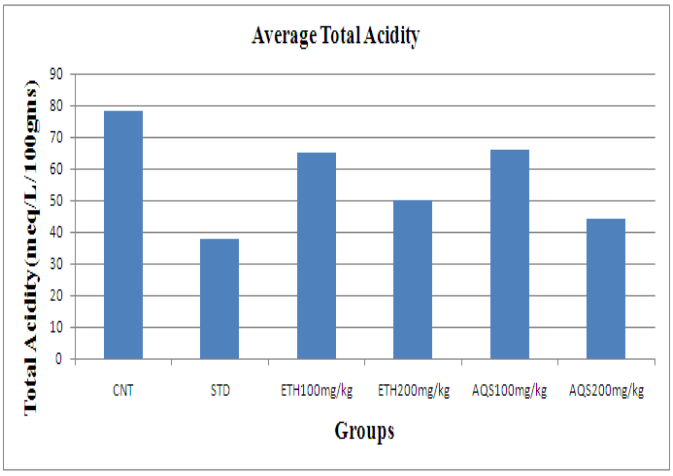

Figure 4: Average Total Acidity of gastric juice of different groups

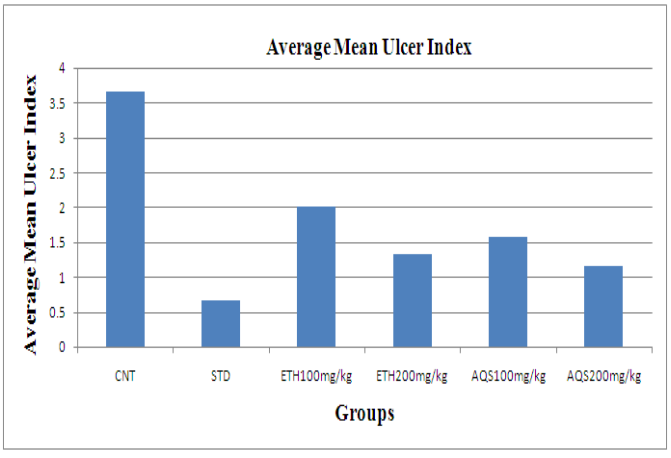

Figure 5: Avg. mean ulcer index of different groups

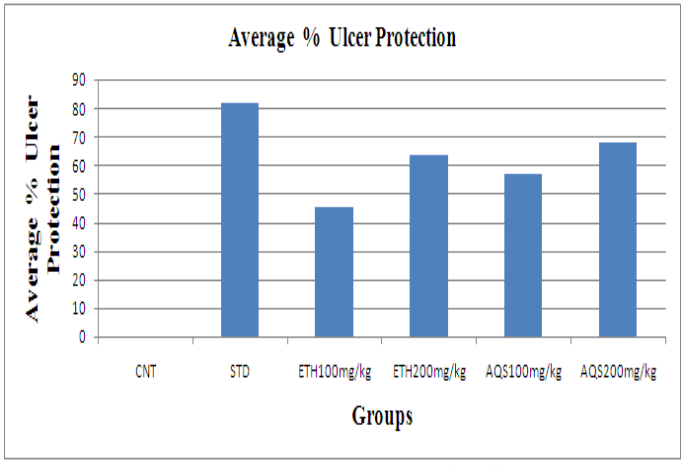

Figure 6: Avg. % ulcer protection of different groups

4. Discussion

The phytochemical investigation of *M. scandens* extracts showed the presence of flavonoids, alkaloids, saponins and steroids. The anti-ulcer activity of *M.scandens* could be ascribed to the presence of flavonoids, alkaloids, saponin and glycosides. Flavonoids are one of the major anti-ulcer agent and may be responsible for this activity. It has suggested that these active components might be able to enhance mucous membrane, bicarbonate and prostaglandin secretion and hinder with the deteriorating effects of reactive oxidants in gastrointestinal lumen. So, the anti-ulcer activity of *M.scandens* may be regarded to its flavonoids content. The results of the present study expressed that the aqueous extract of *M.scandens* at a dose of 200 mg/kg/day may be helpful for the treatment of gastric lesions [29].

The present study has revealed that there was a decrease in the gastric acid secretion and increase in mucus secretion. The ulcer may be due to an imbalance between aggressive factors (gastric acid, gastrin, pepsin) and mucosal integrity maintained by endogenous defense (prostaglandin, mucosa, bicarbonate) mechanisms [30]. The causes of gastric ulcer by pyloric ligation are due to stress-induced increase in gastric hydrochloric acid secretion or stasis and volume of acid secretion are also important factors in the formation of ulcers.

The study has also showed that acid secretion was decreased and gastric pH was raised but there were reports that pepsin acts only at lower pH [31]. Since there is an elevated pH, the pepsin becomes inactive and thereby there is a reduction in digestion of mucosal barrier.

5. Conclusion

On the basis of the results,we may conclude that aerial parts of *Mikania scandens* could have the promising anti-ulcer or acid neutralising or buffering effect.Thus, *M. scandens* could be used as a natural source of anti-ulcer drug preparations in future. Further studies should be carried to isolate and identify the most active principles from the extracts that are responsible for the anti-ulcer activity.

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