ANTIULCEROGENIC ACTIVITY OF MIKANIA CORDATA LEAVES EXTRACT AGAINST ETHANOL-INDUCED GASTRIC ULCER IN RATE AS ANIMAL MODEL

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Abstract
The present study was carried out Mikania cordata leaves was evaluation for their cytoprotective activity against ethanol-induced gastric lesions in rat. Four groups of male Sprague Dawley adult rats each consist of 3 animals. Group-1 animals were pretreated with phosphate buffer saline 5ml/kg as a control, where as Group-2 and Group-3 rats were pretreated with 250ml/kg and 500ml/kg Mikania cordata extract (5ml/kg), respectively. Group-4 rats were pretreated with Ranitidine 150mg/kg as reference. After 30 min all animals were administered absolute alcohol (1.5 ml/rat) orally and after 15 minutes, all rats were sacrificed. Macroscopically, oral administration of absolute ethanol to rats pretreated with PBS significantly produced extensive hemorrhagic lesions of gastric mucosa, whereas animals pretreated with 250mg/kg and 500mg/kg aqueous extract or ranitidine significantly reduced the formation of gastric lesions compared to control group. Microscopically, pretreated rats with aqueous extract or ranitidine showed significantly marked inhibition of gastric lesions and marked reduction of submucosal edema compared to control group. These results strongly documented the beneficial cytoprotective effects of plant extract against ethanol-induced gastric ulcer in rats.

Key words: Cytoprotection, rats, Mikania cordata, ranitidine,

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INTRODUCTION

*Mikania cordata* (Family: Asteraceae) of aqueous leaf extracts have been used in traditional/folk medicine in many countries in the world, tropical reason [1]. Biological tests reported antiulcer [2] analgesic [3]; anti-inflammatory [4] and anticarcinogenic [5] properties of several extracts of *M. cordata*. From the chemical point of view, *M. cordata* has been reported to contain non-volatile compounds: sesquiterpene dilactones, germacranolides, diterpenes and flavonoids[6]. The plant is vigorous and takes up a great amount of potash which must be returned to the soil (green manuring), or the soil will be improverished [7]. In Nigeria a decoction of the plant is taken for cough [8, 9, 10], and the leaf-sap is a remedy for sore eyes [11]. In Ivory Coast sap from the whole plant is crushed with pimento and taken by draught, repeated daily, for cough and bronchitis nad as a vermifuge[12, 13]. Frictions are given in Senegal for fever aches and pain [14]. The leaves are used for headache in Tanganyika [15] and drops of the leaf–sap are instilled in to the eyes or nose in Congo for migraines, conjunctivitis and to prevent vertigo [16]. It is sometimes used for small-pox (also see below) and for jaundice in Ivory Coast [12]. In Tanganyika the leaf-sap is drunk as an anti-malarial and diuretic and also for schistosomiasis [17]. The sap from the entire plant is held in Congo to be antiseptic and is used with vigorous rubbing for psoriasis [16]. A sulphuric acid extract has been found to inhibit growth of Staphylococcus aureus [18]. Realizing the potential use of this plant in ulcer treatment, the present study was undertaken to study the effect of aqueous leaves extract of *Micania cordata* on ethanol-induced ulcer in rats.

MATERIAIS AND METHODS

Collection of plant material: Whole plants of *Micania cordata* were collected from Pabna District of Bangladesh and were identified by the Bangladesh National Herbarium, Mirpur, and Dhaka. One voucher specimen was deposited in Bangladesh National Herbarium. The plant leaves were cut, wash with distilled water and dried in oven 50°C for 5-7 days until fully dried. The leaves were ground to a fine texture or became power from using a grinder and stored at 4°C.

Preparation of plant extract: About 100gm of powder material of leaf were taken in a clean glass container and soaked in 300ml of distilled Ethanol. The container with it contain was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white, cotton material. Then it was
filtrate through Whatman filter paper. The filtrate (Ethanol extract) thus obtained was evaporated under ceiling fan and in a water bath until dried. It rendered a greenish black color. The greenish black color extract was designated as crude extract of Ethanol.

**Ranitidine Preparation:** The reference antiulcer drug, ranitidine, was obtained from Manarat International University, Medical Centre (MIUMC). Each tablet was 150mg, the tablet was dissolved in phosphate buffer saline in a concentration of 10mg ml⁻¹, thoroughly mixed and administered to animal (50mg /kg body weight) 1ml/animal orally.

**Experimental animals:** For the experiment Sprague Dawley adult rats of either sex, 6-8 weeks of age, were collected from Standard Animal House. They were maintained under standard environmental conditions (temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle). The average weight of the rats was between 125-150 gm. The animals were left for 48hours to acclimatize to the animal room conditions and were maintained on standard pellet diet and tap water. All protocols for animal experiment were approved by the institutional animal ethical committee.

**Animal treatment:** We took 12 rats and divided them randomly into four groups, three rats per group. The groups of rats were housed separately and treated as follows:
- **Group-1:** Negative Control, neither drug nor extract were given. Group 1 is treated with Phosphate buffer solution.
- **Group-2:** Treated with *Mikania cordata* extract, Dose: 250mg/kg body weight.
- **Group-3:** Treated with *Mikania cordata* extract, Dose: 500mg/kg body weight.
- **Group-4:** Treated with Ranitidine 2.5mg/kg body weight.

All rats were fasted for 48hours before the experiment but excess water was allowed and just two hours before starting the experiment the water was also removed. Controlled animals (Group-1) each received 5ml/kg phosphate buffer saline by orogastric intubations; whereas treated animal Group-2 and group-3 each received 250mg/kg and 500mg/kg aqueous extract of *Mikania cordata*. (5ml/kg) by orogastric intubations, respectively. Group-4 animal each received 1ml of Ranitidine drug solution, (dose: 2.5mg/kg body weight.)
After 30 minutes of administering extracts/drug/PBS to the respective groups; all the animals were gavaged with absolute alcohol (1.5ml/rat). After 15 minutes, the rats were sacrificed and their stomachs were removed rapidly and fixed in 10% buffer formalin.

**Gross gastric lesions evaluation:** Each stomach was opened along the greater curvature, rinsed in ice-cold PBS and fixed with 10% formalin and examined macroscopically for gastric damage. The length (mm) and the width (mm) of the ulcer on the gastric mucosa were measured by planimeter square (10x10mm) under a dissecting microscope (20X). The ulcer area (UA) was calculated as described by Kauffman and Grossman. The total ulcer area (mm²) of each stomach was recorded and the % protection was calculated as follow:

\[
\% \text{protective} = \frac{\text{UA control} - \text{UA treatment}}{\text{UA control}} \times 100
\]

**Histological examination:** Stomach biopsies were processed and assessed for damage by taking a 5µm section, stained with hematoxylin and eosin and analyzed under light microscopy.

**Statistical analysis of data:** Results were expressed as mean ± M.S.E. The statistical difference between the groups in the term of the mean rate of wound healing was calculated by using student’s t -test.

**RESULT**

Grossly, the result of the current study showed the pretreated rats with *Mikania cordata* extracts or Ranitidine significantly reduced the formation of gastric ulcer induced by total alcohol compared to animals pretreated with BPS and administrated absolute alcohol (Table-1, figure -1&2). Also animals pretreated with aqueous plant extract significantly reduced the gastric lesion compared to rats pretreated with ranitidine (table-1). Histologically, rat’s pretreated with *Mikania cordata* extracted or ranitidine also significantly inhibited the gastric lesion formation and submucosal edema, induced by absolute alcohol compared to animal’s pretreated with PBS. Animals pretreated with aqueous extract significantly inhibit the formation of gastric lesions and submucosal edema compared to animals pretreated with ranitidine (table-1).
Fig.1: Server macroscopic necrosis gastric mucosa Gastric mucosal damage caused by absolute alcohol. Absolute alcohol produced extensive visible hemorrhagic necrosis of gastric mucosa in control group.

Fig.2: Mild macroscopic necrosis of gastric mucosa Cytoprotection of aqueous extracts 500mg/kg against absolute alcohol. Aqueous extract reduce the formation of gastric lesions induced by absolute alcohol.

Table 1: Effect of *Mikania cordata* extracts on ethanol-induced gastric lesions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-treatment</th>
<th>Oral dosage</th>
<th>Ulcer area(\text{mm}^2)</th>
<th>Protection%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>PBS(negativeControl)</td>
<td>5ml/kg</td>
<td>920±2.5</td>
<td>0</td>
</tr>
<tr>
<td>Group-2</td>
<td>250mg/kg <em>Mikania cordata</em></td>
<td>5ml/kg</td>
<td>844.44±1.50</td>
<td>38.69565217</td>
</tr>
<tr>
<td>Group-3</td>
<td>500mg/kg <em>Mikania cordata</em></td>
<td>5ml/kg</td>
<td>720±2.16</td>
<td>71.73913043</td>
</tr>
<tr>
<td>Group-4</td>
<td>Ranitidine 2.5mg/kg body weight</td>
<td>5ml/kg</td>
<td>253±2.38</td>
<td>96.19565217</td>
</tr>
</tbody>
</table>
The present results demonstrate that the aqueous extract of *Mikania cordata* protects the rat gastric mucosa against hemorrhagic lesions produced absolute ethanol. The cytoprotective effect was confirmed by histological examination showing prevention of mucosal lesions and submucosal edema. Absolute ethanol method of including gastric lesions is rapid and convenient way of screening plant extract for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Ethanol-induced gastric ulcer has been widely used for the experimental evaluation of anti-ulcer activity. Disturbances in gastric secretion, damage to gastric mucosa, alteration in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effect of ethanol [19]. Ethanol-induced gastric lesions formation may be due to stasis in gastric blood flow, which concentration to the development of the hemorrhagic and necrotic aspect of tissue injury [20].

It is of interest to note that administration of antioxidants inhibit ethanol-induced gastric injury in the rats [21]. Aqueous extract of *Mikania cordata* was reported to possess’s significant antioxidant activity. Isolated of flavonoids from *Mikania cordata* are known to possess antioxidant activity. It’s likely that the antioxidants property of the *Mikania cordata* could be linked to its gastroprotective effect. It could be conceived that *Mikania cordata* aqueous extract their anti-ulcer activity through the flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents. These results suggest that *Mikania cordata* leaf extract could be beneficial component of preventing ulcer formation induced by ethanol. In conclusion, the anti-ulcer effects of aqueous extract of Mikania cordata appeared to have several important properties that make it useful ideal as a remedy for anti-ulcer. We can suggest that it may be possible to use plant leaf extract as remedy to prevent ulcers. However, further investigations are required to elucidate their extract mechanism of anti-ulcer activity.

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