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Antiulcerogenic Activity of *Ageratum conyzoides* Leaf Extract Against Ethanol-induced Gastric Ulcer in Rats as Animal Model

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Abstract: The aqueous extract of *Ageratum conyzoides* leaves was evaluation for their cytoprotective activity against ethanol-induced gastric lesions in rats. Four groups of male *Sprague Dawley* rats each consist of 6 animals. Group I animals were pretreated with phosphate buffer saline 5 mL kg^{-1} as a control, where as Group II and Group III rats were pretreated with 250 mg kg^{-1} and 500 mg kg^{-1} *Ageratum conyzoides* extract (5 mL kg^{-1}), respectively. Group IV rats were pretreated with cimetidine 50 mg kg^{-1} as reference. After 30 min all animals were administered absolute ethanol 5 mL kg^{-1} orally and 15 min later 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 250 mg kg^{-1} and 500 mg kg^{-1} aqueous extract or cimetidine significantly reduced the formation of gastric lesions compared to control group. Microscopically, pretreated rats with aqueous extract or cimetidine showed significantly marked inhibition of gastric lesions and marked reduction of submucosal edema compared to control group. These results strongly document the beneficial cytoprotective effects of plant extract against ethanol-induced gastric ulcer in rats.

Key words: Cytoprotection, rats, *Ageratum conyzoides,* cimedine

INTRODUCTION

A. conyzoides L. (Family: Asteraceae) is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions[1]. The plant has been used for treatment of several diseases. Githens[2] listed the uses of the plant in folk remedies to include, the use as purgative, febrifuge, treatment of ulcers and wound dressing. In addition to its popular use for skin diseases and wound healing, a decoction of the plant is taken internally to treat diarrhea and to relieve pain associated with navel in children[3]. It is used in the treatment of leprosy and as an oil lotion for purulent ophthalmia[4], treats wounds and fractures[5] and its leaves are used in cases of parasitic infection, rheumatism, headache and colic[3, 6]. In Folk medicine, leaves of *A. conyzoides* are used as anti-inflammatory[3]. Its juice is commonly used for peptic ulcer[7]; gasroprotective and anti-oxidant activity[8]; analgesic[9]; and its oil are reported to possess antibacterial and antifungal activities[10, 11] and spasmolytic[12]. Realizing the potential use of this plant in ulcer treatment, the present study was undertaken to study the effect of aqueous leaves extract of *A. conyzoides* on ethanol-induced ulcer in rats.

MATERIALS AND METHODS

Collection of plant materials: The plant was collected from different localities of Malaysia and identified by comparison with specimens available at the Herbarium of the Forest Research Institute, Kepong, Malaysia. Voucher specimens of the plant material are deposited at Department of Pharmacy, University of Malaya, Malaysia. 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 become powder form using a grinder and stored at 4°C.

Preparation of Plants Extracts: Weighing 40 g of plant powder and mixing it with 800 ml of sterile distilled water in a conical flask using a ratio of 1:20. It was then heated and stirred on a hotplate for 3 hours. After being left to cool, the residue was removed by filtration using a mesh and filter funnel. Rotatory evaporator then extracted the filtered material. Aqueous extract was then submitted to lyophilization by a freeze-dryer, to produce powdered forms of the extract. 500 mg extract were dissolved in 10 mL PBS (50 mg mL^{-1}) and 1000 mg extract were dissolved in 10 mL PBS (100 mg mL^{-1}).

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Cimetidine: The reference antiulcer drug, cimetidine, was obtained from University Malaya Medical Centre (UMMC). Each tablet was 200 mg, the tablet was dissolved in phosphate buffer saline in a concentration of 10 mg mL^{-1}, thourolly mixed and administered to animal (50 mg kg^{-1} body weight) 1 mL/animal orally.

Experimental animals: *Sprague Dawley* adult male rats were obtained from the animal house, Faculty of Medicine, University of Malaya. The rats were divided randomly into 4 groups of 6 rats each. Each rat that weighted between 180 - 200 gm was housed separately (one rat per cage). The animals were left for 48 h to acclimatize to the animal room conditions and were maintained on standard pellet diet and tap water.

Animal treatment: All rats were fasted for 48 h before the experiment but excess water were allowed and just two hours before starting the experiment the water also were removed. Control animals (Group I) each received 5 mL kg^{-1} phosphate buffer saline by orogastric intubations; whereas treated animals Group II and Group III each received 250 mg kg^{-1} and 500 mg kg^{-1} aqueous extract of *A. conyzoides* leaves (5 mL kg^{-1}) by orogastric intubations, respectively. Group IV animals each received 50 mg kg^{-1} cimetidine (5 mL kg^{-1}). Thirty minutes after their pretreatment, all animals were gavaged with absolute ethanol (5 mL kg^{-1}). They were sacrificed 15 min later by diethyl ether and their stomach rapidly removed and fixed in 10% buffered 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 planmeter square (10 X 10 mm) under a dissecting microscope (20X). The ulcer area (UA) was calculated as described by Kauffman and Grossman[13]. The total ulcer area (mm^2) of each stomach was recorded and the% protection was calculated as follow:

$$\% \text{ Protective} = \text{UA control} - (\text{UA treatment})/\text{UA control} \times 100$$

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

RESULTS

Grossly, the results of the current study showed that pretreated rats with *A. conyzoides* leaf extracts or cimetidine significantly reduced the formation of gastric ulcer induced by absolute ethanol compared to animals pretreated with PBS and administered absolute ethanol (Table 1, Fig. 1 and 2). Also animals pretreated with aqueous plant extract significantly reduced the gastric lesion compared to rats pretreated with cimetidine (Table 1). Histologically, rats pretreated with *A. conyzoides* leaf extracts or cimetidine also significantly inhibited the gastric lesions formation and submucosal edema, induced

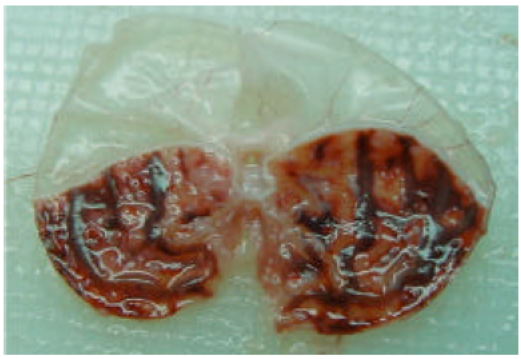

Fig. 1: Sever macscopic necrosis of gasric mucosa Gastric mucosal damage caused by absolute ethanol. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in control group

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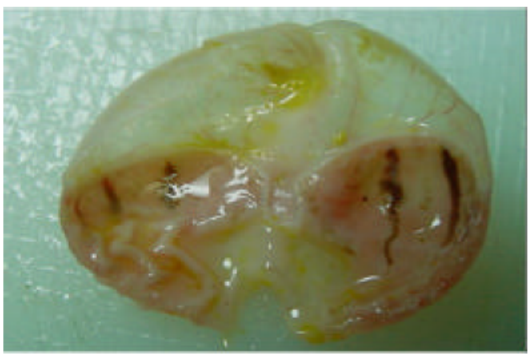

Fig. 2: Mild macroscopic necrosis of gastric mucosa Cytoprotection of aqueous extract 500 mg kg^{-1} against absolute ethanol.
Aqueous extract reduce the formation of gastric lesions induced by absolute ethanol

Table 1: Effects of *A. conyzoides* leaf extracts on ethanol-induced gastric lesions

Groups	Pre-treatment	Oral dosage mL kg^{-1}	Ulcer area (mm^2) (Mean ± S.E. M)	Protection %
1	PBS (control)	5 mL kg^{-1}	987.00±45.38	-
2	*A. conyzoides* 250 mg kg^{-1}	5 mL kg^{-1}	274.33±10.16*	72.21%
3	*A. conyzoides* 500 mg kg^{-1}	5 mL kg^{-1}	168±10.48**	83.00%
4	Cimetidine 50 mg kg^{-1}	5 mL kg^{-1}	380±11.94***	61.50%

*P<0.05 significantly from control (Group 1)
**P<0.05 significantly from control (Group 1) and Group 2
***P<0.05 significantly from control (Group 1), Group 2 and Group 3

by absolute ethanol compared to animals pretreated with PBS. Animals pretreated with aqueous extract significantly inhibit the formation of gastric lesions and submucosal edema compared to animals pretreated with cimetidine (Table 1).

DISCUSSION

The present results demonstrate that the aqueous extract of *A. conyzoides* protect the rat gastric mucosa against hemorrhagic lesions produced by absolute ethanol. The cytoprotective effect was confirmed by histological examination showing prevention of mucosal lesions and submucosal edema. Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts 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, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol[14]. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury[15].

It is of interest to note that administration of antioxidants inhibit ethanol-induced gastric injury in the rats[16]. Aqueous extract of *A. conyzoides* was reported to posse's significant antioxidant activity[8]. The flavonoids kaempferol and quercetin, in addition to several other flavonoids earlier isolated from *A. conyzoides*[8, 17] are known to possess antioxidant activity. It is likely that the antioxidant property of the *A. conyzoides* could be linked to its gastroproptective effect. It could be conceived that *A. conyzoides* aqueous leaf extract exert 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[18]. These results suggest that *A. conyzoides* leaf extract could bebeneficial component of preventing ulcer formation induced by ethanol. In conclusion, the anti-ulcer effects of aqueous extract of *A. conyzoides* appeared to have several important properties that make it useful ideal as a remedy for antiulcer. 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 exact mechanism (s) of anti-ulcer activity.

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