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Gastroprotective effects of Anisomeles *indica* Kuntze

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ABSTRACT

The demands for cheap, anti-ulcer drugs are increasing. In Sri Lanka, there is a folklore belief that a boiled, aqueous extract (decoction) of leaves and stems of *Anisomeles indica* Kuntze (Lamiaceae) possesses gastroprotective properties. The aim of the present study was to scientifically investigate the validity of this claimed gastroprotective effects of *A.indica* in rats using ethanol and indomethacin induced gastric lesion models. The results demonstrated that the decoction of the leaves and stems of *A.indica* at the pre-flowering stage, but not the flowering stage, can offer a significant ($P<0.01$), and dose-dependent, gastroprotection (in terms of length and number of gastric lesions induced by ethanol or indomethacin). The extract of the pre-flowering plant, significantly increased the amount of mucous produced by the gastric mucosa and also significantly decreased the volume of gastric juice secreted, without any alteration in its acidity. This extract also possessed *invitro* antioxidant activity (as judged by TBARS assay). It is concluded that *A.indica* has significant gastroprotective activity that is mediated via an increase in the thickness of the protective mucous layer and by antioxident activity. However, for use of this plant for gastroprotection, it has to be collected during the pre-flowering stage.

KEYWORDS: *Anisomeles indica;* gastroprotection; ethanol; indomethacin,

INTRODUCTION

Anisomeles indica Kuntze (Lamiaceae), is a large, perennial herb that grows commonly as a weed in many parts of Sri Lanka, India, Malaya, China and the Phillipines [1]. A decoction of the leaves and stems of this plant is used by traditional medical practitioners in Sri Lanka and Dutch East Indies for the treatment of a variety of disorders such as pain, rheumatic joints and kidney stones. [2]. The immature stems and leaves of this plant are also used in the preparation of medications used in the Deshiya Chikitsa system of medicine in Sri Lanka to treat fever, fits, inflammation of the upper respiratory tract, wheeze, cough [3,4]. Recent investigations by Dharmasiri et al., [5,6] have confirmed that a decoction of the leaves and stems of this plant contains components with significant analgesic , antihyperalgesic and anti-inflammatory properties, thus rationalizing its ethnopharmacological uses. There is also a folklore belief in Sri Lanka, that a decoction prepared from the leaves and stems of *A.indica* is useful for the treatment of gastric dysfunction. Since this claim has not been scientifically validated, as a part of a programme to discover hitherto unreported pharmacological properties in *A. indica,* which may help to enhance its medicinal value, an investigation was carried out with the objective of determining whether a decoction of the leaves and stems of *A. indica* truly possesses gastroprotective properties.

MATERIALS AND METHODS

Chemicals

Indomethacin was purchased from the State Pharmaceutical Corporation of Sri Lanka. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Animals

Wistar rats (150-200)g from a colony maintained under standardised animal house conditions at the Department of Zoology, University of Colombo were used. All animals had free access to pelleted food and water at all times. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guide lines and rules of the Faculty of the Science, University of Colombo, for animal experimentations.

Collection of plants and preparation of plant extracts

Fresh *A.indica* plants at the pre-flowering and flowering stages were collected separately from a field area around Colombo, Sri Lanka and authenticated by Prof. R.N. de Fonseka, Department of Botany,

University of Colombo, Sri Lanka. A voucher specimen (No. 20-AI) has been deposited in the Department of Zoology, University of Colombo, Sri Lanka. The two categories of plants were boiled separately in distilled water (200 g plant material / litre water) under reflux conditions, for 3 h. After 3 h, the boiled extracts were filtered through cotton wool and each filtrate was further reduced to 100 ml by boiling under reduced pressure. The concentrated extracts were then freeze dried and stored at 4^0 C until required. The freeze dried extracts were dark brown solid masses (yield: 5%). The extracts obtained from the pre-flowering and flowering stages were designated E_1 and E_2 respectively.

Evaluation of protection by A.indica against ethanol-induced gastric lesions

Eighty four rats, deprived of food for 24 h, but provided with water *ad libitum* until 1 h before the start of the experiment, were randomly divided into7 equal groups (n = 12 / group). The rats in groups 1 – 3 were orally treated with 125, 250 and 500 mg/kg of E_1 respectively. The rats in groups 4 – 6 were treated with 125, 250 and 500 mg/kg of E_2 respectively. These doses selected are identical to what has been used previously in showing analgesia, antihyperalgesia and anti-inflammatory activities of this plant [5,6]. Each rat in group 7 (controls) received 1 ml distilled water. One hour later, all rats were orally administered with 1 ml of absolute ethanol. After 1h, all the rats were sacrificed with ether, stomachs removed, fixed with 10% formalin, opened along the greater curvature, and examined for hemorrhagic lesions using a magnifying lens [7]. After counting the number of haemorrhagic lesions, lengths of the linear lesions were measured with a vernier caliper (Philip Harris International Ltd., Lichfield, UK). The percentage inhibition of gastric lesion formation with respect to the controls, mediated by E_1 and E_2 at each of the different doses tested was then calculated.

Stomachs of rats belonging to groups 3 and 7 were then preserved in 10 % buffered formalin, 5 µ sections prepared and then stained with haematoxylin and eosin, for assessment of the histopathological changes that have occurred in the stomach [8] of the control and E_1 treated animals.

Effect of A.indica on indomethacin-induced gastric lesions

In this experiment only the effect of E_1 was investigated because E_2 was shown to be ineffective in protecting against gastric lesions induced by ethanol. Effect of E_1 on indomethacin induced gastric lesions was investigated as described by Bhargava et al. [9].

Twenty four rats that had been fasted for 24h were orally treated with 50 mg/kg of indomethacin in 1ml of 1 % methylcellulose. After 1h, the rats were randomly divided into two equal groups (n=12 / group). Animals of group 1 (control group) were each orally administered 1 ml distilled water, while those in group 2 received 500 mg /kg of E_1. Five hours later, all rats were sacrificed with ether and the stomachs examined as described above.

Effect of E_1 on mucous secretion

Effect of E_1 on gastric mucous secretion was assessed by the method described previously by Ratnasooriya et al, [10]. In this method, the amount of mucous produced was estimated by use of the dye alcian blue. Alcian blue binds reversibly with mucous in the gastric mucosa, and the amount of the dye bound is directly proportional to the amount of mucous present [11].

Twenty rats were starved for 24 h as described previously and then randomly assigned into two equal groups. (n= 10 rats / group). Group 1 served as the control group and these animals were each administered orally, 1ml distilled water. Group 2 animals were treated orally with 500 mg / kg of E_1 (test group). After 1h, all rats were sacrificed with ether and the stomachs excised, opened along the lesser curvature, inverted and rinsed with 0.25 M sucrose solution. These stomachs were incubated in 10 ml aliquots of 0.1% alcian blue solution for 2 h at room temperature (30 ^{0}C). At the end of this time period, the stomachs were removed, washed with 0.25 M sucrose solution and separately incubated in 10 ml aliquots of 0.5 M magnesium chloride solution for 2h at room temperature while shaking at 30 min intervals to elute the alcian blue bound to the mucosa of the stomach. Two hours later, the stomachs were removed and 5 ml portions of each aliquot of magnesium chloride solution containing the alcian blue eluted from each stomach were shaken with 5 ml portions of diethyl ether. The aqueous phase was then separated, centrifuged at 3200xg for 5 min and the absorbance of the supernatant measured at 605 nm. The amount of bound alcian blue was estimated by using a standard calibration curve of absorbance vz. concentration of alcian blue.

Effect of E_1 on acidity and volume of gastric juice secreted

The volume and acidity of gastric juice produced by the experimental rats were estimated as described by Fernandopulle [8]. Twenty rats that had been starved

for 24 h as previously described, were randomly divided into two equal groups (n= 10 rats / group). The rats in the control group (group 1) and the test group (group 2) were orally administered with either 1 ml distilled water or 500 mg/kg of E_1 respectively. One hour later, these rats were laparotomised under ether anaesthesia and the stomachs were carefully ligated with a cotton thread at the pyloric end so that the blood supply to the alimentary tract was not interrupted. The stomachs were then carefully placed back in the abdominal cavities, rats sutured, and allowed to regain conciousness. After 4h, the rats were sacrificed with ether, abdominal cavities opened and the stomachs carefully removed and placed individually in separate petri dishes. The stomachs were cut open and the gastric juices gathered in them were collected into centrifuge tubes and these centrifuged at 3200xg for 5 min.

To determine the acidity, I ml of gastric juice from each sample was diluted up to 5 ml with distilled water and titrated with 0.1 M NaOH in the presence of methyl orange followed by phenol red, and the end points recorded. The amount of free acid present in 1 ml of gastric juice in moles is equal to the number of moles of NaOH spent to reach the end point in the presence of methyl orange. The amount of bound acids present in the gastric juice in moles is equal to the number of NaOH moles required to reach the end point in the presence of phenol red.

Total acidity = free acid + bound acid (moles / litre).

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was performed using Student's t-test, and linear regression analysis. $P \leq 0.05$ was considered as significant.

RESULTS

Effects on ethanol-induced gastric lesions and changes in the stomach histopathology

Treatment with absolute ethanol produced linear and scattered dot-like haemorrhagic lesions on the glandular portion of the stomach. Some of the linear lesions had fused together to form large haemorrhagic patches. The treatment with E_1 resulted in a significant ($P<0.01$) reduction in the number and length of the haemorrhagic gastric lesions induced by ethanol (Table 1). The stomachs of 50 % of the rats treated with 500 mg/kg of E_1 and 33 % of the rats treated with 250 mg/kg of E_1 had neither lesions nor erythamatous patches. E_2, even at a dose of 500 mg/kg, had no significant effect on either the number or the length of the gastric lesions. The percentage reduction in the number of lesions by 125, 250 and 500 mg/kg of E_1 were 71, 88 and 88 respectively, while the length of the lesions were reduced by 76, 87 and 85% respectively. The number and length of lesions produced by ethanol had a negative correlation with the dose of E_1 ($r = - 0.97$). The EC_{50} of E_1 for the reduction in number of lesions and length of the lesions were 141.83 mg/kg and 146.26 mg/kg respectively.

Microscopic examination of the sections of stomachs of control rats revealed disruptions of the integrity of the mucosal surface, with deep necrotic penetrations in the mucosa and prominent congestions. In contrast, the E_1 treated rats the integrity of the epithelium was preserved, and neither necrotic damages nor congestions were observed, in both the control and E_1 treated rats, dilated capillaries filled with red blood cells were observed.

Effects of E_1 on indomethacin-induced gastric lesions

Administration of indomethacin to rats produced dot-like scattered lesions on the glandular portion of the stomach. E_1 at a dose of 500 mg / kg significantly ($P< 0.01$) reduced the number of lesions (by 52%) with respect to the control (control vs. $E_1 = 18.0 \pm 2.2$ vs. 8.6 ± 2.4).

Effect of E_1 on mucous content of the stomach

Administration of E_1 significantly increased (by 86%), the amount of mucous secreted by the rat stomach, as evident by the amount of alcian blue bound to the stomachs of these animals in comparison with the controls (control vs. treatment = 0.92 ± 0.18 vs. 1.71 ± 0.28 of alcian blue / stomach).

Effect of E_1 on volume and acidity of gastric juice secreted by the stomach

When compared with the controls, in the animals treated with E_1 there was a significant ($P< 0.01$) reduction (by 31 %) in the volume of gastric juice production. (Control vs. $E_1 = 5.30 \pm 0.19$ vs. 3.64 ± 0.22 ml / stomach). However, administration of E_1 did not result in a significant alteration of the free acidity, bound acidity or the total acidity of the gastric juice (free , bound and total acidity of controls vs. E_1 were 0.045 ± 0.005 vs. 0.043 ± 0.005 mol l^{-1} ; 0.030 ± 0.002 vs. 0.036 ± 0.002 mol l^{-1} and 0.075 ± 0.005 vs. 0.079 ± 0.004 mol l^{-1} respectively).

DISCUSSION

The results of the present investigation demonstrated that E_1, but not E_2 possesses powerful gastroprotective properties. (in terms of number and length of lesions, and histopathology.) Further, E1 also

Table 1: Effect of orally administered Anisomeles indica extracts on ethanol-induced gastric lesions in rats.

Treatment	Number of lesions (Mean ± SEM)	Length of lesion (mm) (Mean ± SEM)
Control (1 ml DW)	26.58 ± 2.71	60.16 ± 8.97
125 mg/ kg E_1	7.75 ± 1.40*	17.80 ± 4.57*
250 mg/kg E_1	3.17 ± 0.74*	7.80 ±2.39*
500 mg/kg E_1	3.08 ± 1.35*	8.95 ± 2.36*
500 mg/kg E_2	29.08 ± 3.38	69.70 ± 10.3

*As compared with controls *P<0.01, E_1= pre flowering stage; E_2= flowering stage; DW=distilled water*

exerted significant protection against indomethacin-induced gastric lesions, with a relatively low EC_{50} value. Lack of activity of E_2 is likely to be due chemical differences between $E_1$and E_2 [5]. Enhancement of mucous secretion, reduction of gastric juice secretion and a reduction in the acidity of gastric juice are some of the mechanisms by which other plants such as *Momordica charantia* [8], *Murraya koenigii* [10], *Teucrium buxifolium* [7] Turnera *ulmifolia* [12] and *Evolvulus alsinoides* [13] have been shown to exert gastroprotective activity. In the present investigation, it has been demonstrated that E_1 can significantly enhance gastric mucous secretion while reducing the gastric juice secretion in rats. In a previous investigation, we have shown that E_1 also has powerful anti-histamine activity [6]. The triterpenoids present in E_1 [5] could be responsible for the mucous enhancing and antihistamine activities as triterpenoids have been reported to mediate such actions [14]. The enhanced mucous secreted after administration of E_1 may help to protect against the ethanol- and indomethacin-induced damage by preventing the action of acid and pepsin on the stomach mucous epithelium [15]. H_2-antagonists are recommended for the prevention of gastric lesions induced by NSAID's [16]. Therefore, the antihistamine activity in E_1 may also contribute to the protective activity against indomethacin –induced gastric lesions.

The reduction of gastric juice secretion mediated by E_1 could be attributed to its antihistamine effect, because it is well established that antihistamine drugs like cimetidine which block H_2 receptors in the stomach, reduces secretion of gastric juice [15]. Although H_2 receptor blockers are also expected to reduce the acidity of gastric juice [15], such an effect was not observed in the present investigation. This may possibly be due to the prostaglandin (PG) synthesis inhibitory activity of E_1 [17] mediating an increase in the acid secretion, thereby compensating for the reduced acid secretion mediated by H_2 receptor antagonism. Impaired PG synthesis in the gastric mucosa has been reported to increase gastric acid secretion [18] H_2 antaginosts such as cimetidine reduce the activity of pepsin by decreasing gastric juice acidity, leading to an impairment of protein digestion [19]. Thus, the non-alteration of the gastric juice acidity by E_1 demonstrates its ability to counter gastritis without affecting protein digestion. Free radicals are involved in the pathogenesis of gastric mucosal injuries [20] and herbal antioxidants are known to have gastroprotective properties [13, 21]. E_1 possesses potent free radical scavenging effects [6]. Therefore, it is possible that free radical scavenging mechanisms also play a role in gastroprotective effects of E_1. From the overall results obtained in the present investigation together with our previous studies [5,6] showing absence of overt signs of general toxicity, hepatotoxicity and renotoxicity following chronic administration of E_1 it may be concluded that *A. indica* at the pre-flowering stage is therapeutically useful for the alleviation of gastric ulcers induced by NSAID's and alcohol. Further support for this view is provided by the recent observations of [22] that a methanolic extract of *A.indica* (leaves and stems) at the pre-flowering stage, can significantly inhibit *in vitro,* the growth of 13 strains of *Helicobacter pylori*, a gram negative bacterium, that is recognized as being the primary etiological agent responsible for the development of gastritis, dyspepsia and peptic ulcer disease, in developing countries.

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