Original Article

Anti-ulcer potential of *Oxystelma esculentum*

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Oxystelma esculentum is a perennial twiner growing near water-logged areas in the Indian subcontinent. It is used traditionally in stomach ulcers. The present work deals with the investigation of anti-ulcer potential of *O. esculentum.* The plant was successively extracted with solvents of varying polarities, which served as the test extracts. Anti-ulcer effect was checked in Wistar rats using aspirin- and ethanol-induced acute ulcer models. The petroleum ether extract was found to possess the most effective anti-ulcer activity. This proves the traditional claim of the plant as an anti-ulcer drug. Phytochemical screening of this extract revealed the presence of important classes of compounds like cardenolides, flavonoids, phenolics, sterols and triterpenoids. This bioactivity-guided phytochemical screening can guide further therapeutic investigations and isolation of pharmacologically active compounds from *Oxystelma esculentum*.

Key words: Anti-ulcer, *oxystelma esculentum, oxystelma secamone, periploca esculenta*

INTRODUCTION

Oxystelma esculentum R. Br. syn. *Oxystelma secamone* Linn., *Periploca esculenta* Roxb., *Periploca secamone* Linn., *Sarcostemma secamone* Bennet, *Sarcostemma esculentum* Linn., *Asclepias rosea* R. Br., is a perennial twiner found throughout the plains of the Indian subcontinent near water-logged areas.[1] The plant is used as anti-ulcer, laxative, diuretic, antiseptic, depurative, anthelmintic, aphrodisiac, hepatoprotective and useful in leucoderma and bronchitis. Decoction of plant is used in ulcer, sore-throat and itches. Milky juice is used as galactogogue, anti-periodic, anti-ulcer and as a vulnerary. Leaves are used as antiperiodic. Its root is prescribed in jaundice. Fruit is bitter, tonic, expectorant and anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent.[2,3] The present work deals with not only investigating the anti-ulcer activity of the plant, but also finding the most potent extract and performing its phytochemical screening, so as to guide further fractionation of pharmacologically important constituents from this plant.

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MATERIALS AND METHODS

Collection and Authentication

Oxystelma esculentum in flowering and fruiting stage was collected from Barda Hills near Porbandar, Gujarat, India, in October 2008. Herbarium of the collected sample was prepared and deposited in Department of Pharmacognosy, RK College of Pharmacy (No. RKCP/COG/01/2008). Authentication was done by Dr. N. R. Sheth, Head of Department of Pharmaceutical Sciences, Saurashtra University.

Preparation of Extracts

Successive extraction of 1 kg powder of the entire plant was carried out using four solvents in the decreasing order of their polarity index: petroleum ether, chloroform, methanol and distilled water. Complete extraction of the powder with each solvent was carried out in round-bottom flask at a temperature <50°C. Their concentrations were adjusted in the solvents according to their dose.

Pharmacological Study

The pharmacological study was approved by the Institutional Animal Ethics Committee (RKCP/COG/RP/10/06) and carried out according to CPCSEA guidelines. All animals were maintained under environmentally controlled conditions of 24±1°C and 12 h-light and -dark cycle. The animals were acclimatized to laboratory conditions for 1 month before starting the pre-clinical trials. All studies were performed under standard conditions of temperature, light, humidity and noise.

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The experimental animals were divided into seven groups, with six animals in each group: Normal control, Disease control, Standard and Test extracts (Petroleum Ether extract, Chloroform extract, Methanol extract and Aqueous extract).

Male Wistar rats weighing 250–300 g were deprived of food 36 h prior to the experiment but were allowed free access to water. During this time they were kept in cages with mesh at the bottom to prevent coprophagy. Three animals per group were placed in one cage. Normal control group received normal saline (25 ml/kg) orally. Disease control group received aspirin 20 mg/kg in 1% CMC (carboxymethyl cellulose) in water orally. Standard control group received 300 mg/kg Ranitidine (Pharma Pvt. Ltd.) orally. Two groups of three animals were used for each dose of the test extract. Three animals of the test extract groups received orally a dose of 200 mg/kg and the remaining three animals from each of these groups received dose of 400 mg/kg body weight.[4] The rats were administered the standard and test extracts orally, 30 min prior to administration of aspirin 20 mg/kg in 1% CMC. Six hours later, the animals were euthanized with chloroform; the stomachs were excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs were stretched on a piece of foam core mat, mucosal side up.[5]

The same procedure was applied to ethanol-induced acute ulcer model, except that 1 ml 80% ethanol was kept as the disease control and the animals were excised after 1 h.[6] The length and breadth of the lesions were measured using Vernier caliper and the Ulcer index and Ulcer protection percent was calculated as follows[7] [Tables 1 and 2, Figures 1 and 2].

Calculation of area

Area of circular lesion=$\pi D^2/4$
Area of linear lesion=length×breadth
Area of stomach mucosa=$\pi D^2/8$
where D=diameter of stomach mucosa
Every five petechiae were counted as 1 mm^2 area.

Ulcer index

Ulcer index (UI) = $10/X$,
where X = Total area of stomach mucosa/total ulcerated area.

Ulcer protection%

Ulcer protection (UP) = [(Disease control mean ulcer index – test mean ulcer index)/disease control mean ulcer index]100

Results were calculated as mean±standard deviation (SD). Statistical analysis of control and test data was performed by one-way ANOVA followed by Dunnett's test (Sigma-stat software). A probability value of P<0.001 was considered statistically significant.

Phytochemical Screening

Petroleum ether extract was found to have the most potent and statistically significant anti-ulcer activity. This extract was subjected to phytochemical screening involving established methods for detecting various classes of phytoconsituents [Table 3].[8-13]

Table 1: Anti-ulcer activity of various extracts in aspirin-induced acute ulcers

Groups	Ulcer index (UI)	Ulcer protection (UP) %
Normal control	0.0	–
Disease control (Aspirin)	12.8±0.2	–
Standard (Ranitidine)	3.2±0.3	75.00
Pet Ether ext 200 mg/kg	4.2±0.3	67.19
Pet ether ext 400 mg/kg	3.3±0.2	74.22
Chloroform ext 200 mg/kg	5.1±0.4	60.16
Chloroform ext 400 mg/kg	4.7±0.5	63.28
Methanol ext 200 mg/kg	4.4±0.4	65.63
Methanol ext 400 mg/kg	4.1±0.3	67.97
Aqueous ext 200 mg/kg	4.6±0.3	64.06
Aqueous ext 400 mg/kg	4.3±0.3	66.41

Values are expressed as mean±SD; Number of animals (*n*)=6

Table 2: Anti-ulcer activity of various extracts in ethanol-induced acute ulcers

Groups	Ulcer index (UI)	Ulcer protection (UP) %
Normal control	0.0	–
Disease control (Aspirin)	10.8±0.3	–
Standard (Ranitidine)	1.8±0.3	83.33
Pet Ether ext 200 mg/kg	2.2±0.3	79.63
Pet Ether ext 400 mg/kg	1.9±0.2	82.41
Chloroform ext 200 mg/kg	3.5±0.4	67.59
Chloroform ext 400 mg/kg	2.9±0.5	73.15
Methanol ext 200 mg/kg	3.2±0.3	70.37
Methanol ext 400 mg/kg	2.7±0.3	75.00
Aqueous ext 200 mg/kg	3.3±0.3	69.44
Aqueous ext 400 mg/kg	3.1±0.3	71.30

Values are expressed as mean±SD; Number of animals (*n*)=6

Table 3: Phytochemical screening of petroleum ether extract

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's	-ve
	Wagner's	-ve
	Hager's	-ve
	Mayer's	-ve
Flavonoids	Shinoda	+ve
	Fluorescence	+ve
Phenolics	Ferric chloride	+ve
	Folin ciocalteu	+ve
Sterols and triterpenoids	Libermann Burchardt	+ve
	Salkowski	+ve
Carotenoids	Antimony trichloride	-ve
Cardenolides	Kedde's	+ve
	Baljet's	+ve
	Legal's	+ve

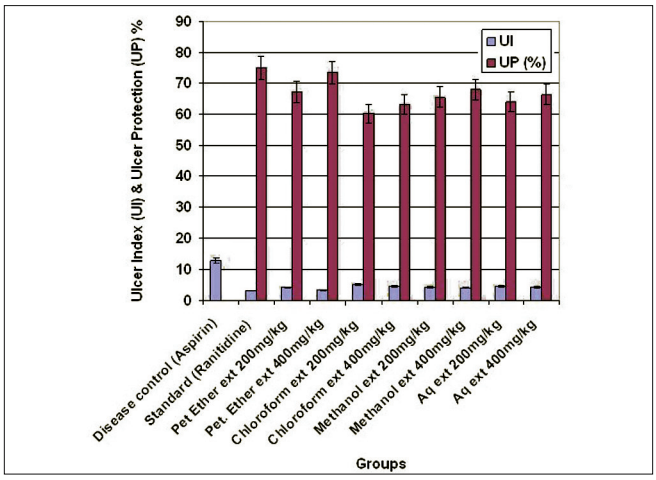

Figure 1: Comparison of anti-ulcer potential in aspirin-induced ulcers

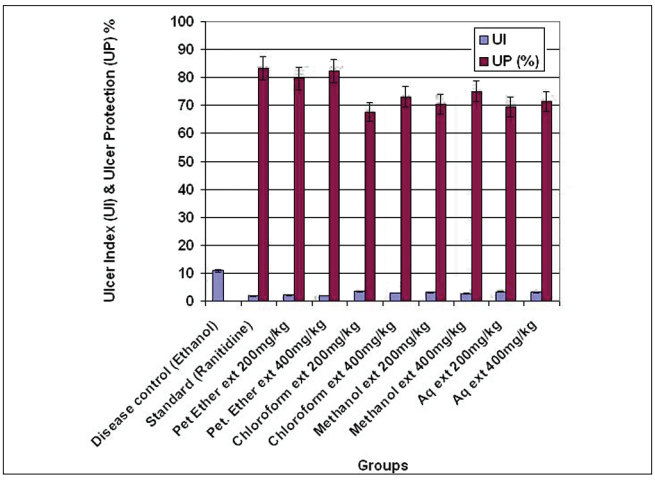

Figure 2: Comparison of anti-ulcer potential in ethanol-induced ulcers

RESULTS

In the aspirin-induced ulcer model, the ulcer index of standard group was 3.2±0.3 whereas that of Pet. ether ext. (400 mg/kg) was 3.3±0.2. In the ethanol-induced ulcer model, the ulcer index of standard group was 1.8±0.3 whereas that of Pet. ether ext. (400 mg/kg) was 1.9±0.2. In the aspirin-induced ulcer model, the ulcer protection of standard group was 75% whereas that of Pet. ether ext. (400 mg/kg) was 74.22%. In the ethanol-induced ulcer model, the ulcer index of standard group was 83.33% whereas that of Pet. ether ext. (400 mg/kg) was 82.41%.

DISCUSSION

The present study shows that the petroleum ether extract of *Oxystelma esculentum* has the most potent, statistically significant (P<0.001) and dose-dependent anti-ulcer activity amongst all extracts, comparable with Ranitidine (Standard) at the dose of 400 mg/kg. This proves the traditional claims of this plant as a potent anti-ulcer drug.

The anti-ulcer activity shown by the petroleum ether extract in aspirin-induced ulcers suggests that the extract prevents change in permeability of gastric mucosa, prevents back diffusion of gastric acid, inhibits histamine release,[14] inhibits prostaglandin synthesis, inhibits lipid peroxidation and activates cyclo-oxygenase.[15]

The anti-ulcer activity shown by the petroleum ether extract in ethanol-induced ulcers suggests that the extract has a cytoprotective effect, i.e. it protects the gastric mucosa by mechanisms other than gastric acid secretion.[16] Such mechanisms include inhibition of leukotrienes,[17] pepsinogen[18] and substance P,[19] free radical scavenging,[20] increasing gastric mucosal blood flow,[21] increasing the protective glycoprotein content and thereby strengthens the gastric mucosa[22] and prevention of oxidation of the mucosal xanthine dehydrogenase.[23]

The bioactivity-guided phytochemical screening of petroleum ether extract revealed the presence of cardenolides, flavonoids, phenolics, sterols and triterpenoids, which may be responsible for the anti-ulcer effect and can be further fractionated and investigated for their role and utility in any of the anti-ulcer mechanisms.

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