Research Article

Investigations on *Ipomoea reniformis* seeds for antiulcer potential in rats

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Abstract

Objective: Aim of present study was to study pharmacological screening of *Ipomoea reniformis* seeds extract for antiulcer effect on Pyloric ligation and ethanol-induced ulcer model. **Material and methods**: The antiulcer effect of standard drug ranitidine and plant extracts (Petroleum ether, Ethanol extract and Aqueous extract) were studied on the ulcer Index and extent of mucosal damage in the stomach. In present study, Ranitidine drug was used as reference for comparison of antiulcer effect. **Results and conclusion**: The present study showed that *Ipomoea reniformis* seeds extracts possess anti-ulcer activity in both animal models. The intensity of heamorrhage and lesions was significantly reduced with pretreatment and helpful for the protective effect of all extracts. The submucosal edema of animal treated with ethanolic plant extract of 500 mg/Kg dose showed reduced mucosal edema with wounds infiltration and the muscularis propria appears normal. *Ipomoea reniformis* seeds extract showed a significant decrease in the ulcer development in both the animal models pyloric ligation and ethanol-induced ulcer model. In pylorus ligation, both the doses showed significant anti-ulcer activity by reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. The intensity of heamorrhage and lesions was significantly reduced upon pretreatment with the extract, revealing the protective effect of ethanol extract.

Keywords*: Ipomoea reniformis,* antiulcer, pyloric ligation and ethanol

Introduction

Ulcers are characterized histologically as a break in the mucosa of the alimentary tract that reaches out through the muscularis mucosae into the submucosa or more profound. They can happen in any segment of the gastrointestinal plot presented to the over the top measure of acid- peptic juices (Kumar et. al., 2006). The gastric ulcer are arranged Aphthous ulcers, Esophageal ulcers and Peptic ulcer based on their event in gastrointestinal tract in mouth, throat and stomach or the duodenum separately (Kumar et. al., 2012).

Peptic ulcers are persistent, consistently solitary, wounds that occur in any piece of the gastrointestinal parcel introduced to the strong action of corrosive peptic juices. In any occasion 90 % of peptic ulcers are either in the principal part of the duodenum or in the stomach. The infection was achieved by the microorganisms H. pylori and more proportion of corrosive pepsin release liable for age of peptic ulcer. The usage of nonsteroidal calming drugs (NSAIDs) makes shock, outrageous injury, septicaemia, intracranial wounds. The using of neighborhood aggravation like alcohol, smoking and spiced food by human as food supplement is in like manner liable for production of peptic ulcers (Mohan, 2013).

Ipomoea reniformis chois (Convolvulaceae) is an interminable, much fanned flavor (creeper). It is found comprehensively appropriated wherever all through the India, extraordinarily in soaked spots in upper gangetic plain, Gujarat, Bihar, West Bengal, Western- Ghats, moving up to 900 m in the inclines, Goa, Karnataka in India, Ceylon and Tropical Africa. The major/huge constituents of the plant are Caffeic, Sinapic corrosive esters, Ferulic and Pcoumaric.

Ipomoea reniformis decoction is said to go about as deobstruent, diuretic; important in affliction, neuralgia, cerebral agony, anthelmintic; diseases of the kidney, the lungs, the uterus; incredible in tortures, fevers urethral deliveries, iron insufficiency and *lucoderma* Leaf juice is given in rat eats and snake snack. In epilepsy powder of

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leaves is sniffed up. Paste of the root used in developing. Root is moreover having diuretic and purgative property. Decoction of whole plant is taken inside to treat stomach issues (Chattertee, 2003; Nadkarni, 1954).

Various parts of *Ipomoea reniformis* plant tested for various activities. It is accounted for to have numerous significant restorative properties. In the Indigenous arrangement of Medicine, Ipomoea reniformis has been professed to be helpful for hack, migraine, neuralgia, ailment, diuretic, irritation, inconveniences of nose, fever because of development of liver and furthermore in kidney illnesses. Powder of leaves is utilized as a snuff during epileptic seizures, Juice goes about as laxative and the root is having diuretic, purgative, and applied in the illness of the eyes and gums. The entire plant decoction is basically liable for its therapeutic uses (Agarwal, 1947; Warden and Hooper, 1890). The literature study showed that *Ipomoea reniformis* has numerous activities but antiulcer activity study on seeds of *Ipomoea reniformis* is lacking. Therefore we have selected this plant for present study.

Material and methods

Collection of plant materials

The seeds of *Ipomoea reniformis* were purchased from local market of Bhopal region of India. The plant seeds were verified by botanist. The seeds were washed with fresh running tap water and shade-dried. Dried seeds were powdered uniformly using a mechanical grinder. The seeds were powdered and stored in containers (airtight) for further studies and evaluation.

Phytochemical Screening

Successive solvent extraction of selected plant i.e. *Ipomoea reniformis* seeds was performed using soxhlet extractor. Air-dried powdered of *Ipomoea reniformis* seeds was successively extracted with solvents of increasing polarity (Petroleum ether; Ethanol as aextraction solvent) using soxhlet apparatus. The seeds of plant were also extracted by maceration method with water to get aqueous extract.

Qualitative Phytochemical Tests

The extracts were then used for the various qualitative test to identified the presence of various phytoconstituents i.e. alkaloids; glycosides; flavonoids; carbohydrates; aminoacids; saponins; sterols and terpenoids; cardiac glycosides; coumarins; carotenoids; tannins; phenolic compounds; fixed oils and fats etc (Kokate, 1994).

Anti-ulcer activity

Animals

Wistar rodents of one or the other sex (150-200gms) were housed in independent enclosures at controlled room temperature (24 ±2oC; relative stickiness 60-70%) in a light dim of 24 h. They were taken care of with standard pellet diet and water not indispensable.

The temperature in the exploratory animal room was kept 22±30C. Counterfeit lighting was given. The creatures were adjusted to standard research center states of temperature (25 ± 30°C) and kept up on 12:12 h light dim cycle. The creatures were housed in purified polypropylene confines containing sterile paddy husk as bedding. They were furnished with standard rodent chow diet and refined water not indispensable.

The animals were haphazardly chosen and kept in their confines for at any rate 7 days preceding dosing to consider acclimatization to the lab conditions.

Plant Extracts

Petroleum ether, ethanol and water extracts of *Ipomoea reniformis* seeds were utilized independently for study. The test chemicals (Petroleum ether, ethanol and water extracts *Ipomoea reniformis* seeds and standard medication (Ranitidine) were set up as a suspension in distilled water utilizing mortar and pestle.

Animals Grouping

In all the trial models, male albino rats were chosen and separated into four gatherings of six animals each. Creatures were abstained for 24 hour before the examination, however had free admittance to water. Group I treated as vehicle control, gotten just refined water; group II standard group, gotten ranitidine 50 mg/kg (P.O.), III and IV treated as treatment group, gotten the reviewed portion of *Ipomoea reniformis* seeds extract at 500, 250 mg/kg, (P.O.) for 7 days (once in a day) respectively.

Pylorus Ligation Induced gastric ulcer

The method includes, albino rats were abstained in individual enclosures for 24 hour of *Ipomoea reniformis* seeds Petroleum ether, Ethanol extract and Aqueous extract), reference medication and control vehicle was administered 1 hour before pyloric ligation. At that point the pre-treated animals were anesthetized by sedative ether; the mid-region was opened by a little midline cut beneath the xiphoid process. The pyloric segment of the stomach was ligated without making any harm its veins. The stomach was isolated cautiously and the abdominal wall was sealed by interrupted sutures. The animals were denied of water during the postoperative period. Four hours after ligation, the stomach was analyzed out and contents was gathered into clean cylinders. The volume, pH and total acid content of gastric juice were resolved. The substance were centrifuged, sifted and exposed to titration for estimation of total acidity. From the supernatant, aliquots (1 ml each)

were taken for the assurance of pH, total or free acidity and pepsin activity.. Each stomach was inspected for injuries in the fore stomach portion and indexed according to severity (Sahoo et. al., 2016).

Albino rats of Wistar strain, (150-200 g) was divided randomly into four groups (n=6) and received the following medications orally for five days. Dose of ranitidine was 20 mg/kg.

Group I – Distilled water 5 ml/kg;

Group II - Ranitidine 20 mg / kg body wt;

Group III – *Ipomoea reniformis* seeds extract 500 mg / kg body wt

Group IV – *Ipomoea reniformis* seeds extract 250 mg / kg body wt

All treatments were administered orally for 5 days and on the fifth day, food was withdrawn though water was allowed ad libitum. On sixth day, all rats received aspirin (200 mg/kg) to induce gastric ulcer. After one hour, pylorus was ligated under light ether anaesthesia (Shay et al., 1945).

Ulcer index

Stomach was cut along the greater curvature, washed and placed on a card board and ulcer index was counted from the glandular portion. Each lesion was measured along the greatest length and evaluated singly according to their dimensions and severity (area of glandular portion of stomach / area of ulceration in mm^2 scale).

The ulcers were scored according to the following scale (Thirunavukkarasu et al., 2009):

Normal coloration – 0; Red coloration – 0.5; Spot ulcer – 1; Haemorragic streaks – 1.5; Ulcer – 2; Perforation - 3

Mean ulcer score for each animal will be expressed as ulcer index. The ulcer index was determined as follows:

Ulcer index = 10/x; (x = area of glandular portion of stomach / area of ulceration in mm^2 scale)

Determination of anti-secretory effect

The anti-secretory activity was examined by the supernatant fluid analysis as titratable acidity against 0.01 N NaOH at pH 7 and the total acid used was calculated.

Free and total acidity

Amount of 0.01 N NaOH required to titrate to the methyl yellow end point is the measure of the free acid present. The amount of 0.01N NaOH required to titrate from the beginning to the phenolphthalein end point, is a measure of the total acid present in the sample.10 Acidity (mEq/L/100mg.

Ethanol induced mucosal damage

The rats were fasted for 24 hours before the experiment. After 1 hour of administration of *Ipomoea reniformis* seeds extract Petroleum ether, Ethanol extract and Aqueous extract), ranitidine and vehicle control treatment, 1ml of absolute ethanol (0.5 ml/100g) was orally administered to each rat of every group. After 1 hour, the animals were sacrificed with excess of anaesthetic ether and stomach was opened along the greater curvature, cleared of residual matter with saline and the inner surface was examined for severity of ulceration. Ulcer index and % ulcer protection were calculated.

Histopathological study

The gastric tissue tests were fixed in unbiased supported formalin for 24 h. The tissues were handled by the standard technique and segments were cut stained with haematoxylin and eosin Bancroft (Banccroft and Stevens, 1996). The slides were inspected minutely for morphological changes, for example, clog, drain, oedema and disintegrations utilizing a self-assertive scale for the evaluation of seriousness of these changes.

Statistical Analysis

The statistical investigation was completed utilizing Graph Pad programming variant (Graph Pad crystal programming Inc.) The qualities were communicated as mean ± SEM. The measurable examination was done by one path investigation of change (ANOVA) trailed by Dunnet's t-test. P values< 0.05 were viewed as significant.

Results and discussion

Phytochemical studies

The presence of different chemical constituents in *Ipomoea reniformis* seeds was detected by subjecting them to successive extraction using solvents in the order of increasing polarity. The extracts obtained were then dried completely and kept in vacuum dessicator. The colour; consistency and extractive value in different solvent for *Ipomoea reniformis* seeds.

Results exposed that %w/w yield in successive extraction of *Ipomoea reniformis* seeds in petroleum ether; ethanol and aqueous were 3.5; 8.1; and 8.9 respectively indicating higher in water and low in petroleum ether. Colour of extract of *Ipomoea reniformis* seeds was Amber coloured in petroleum ether; dark brown in ethanol and dark brown in water. Consistencies of the seeds extracts were sticky and semisolid in petroleum ether while non sticky and solid in ethanol and water extracts. Dissimilar result of %w/w yield; colour and consistency of extracts of *Ipomoea reniformis* seeds helps in identification of plant.

Qualitative analysis

Qualitative phytochemical examination of successive

extracts in petroleum ether; ethanol and aqueous extract of *Ipomoea reniformis* seeds powder was carried out and outcomes are shown in Table 3. The results indicated that the alkaloids flavonoid were present in ethanol and aqueous extracts of *Ipomoea reniformis* seeds.

Ethanol and aqueous extracts of *Ipomoea reniformis* seeds; contained carbohydrates. Phytosterols were found in petroleum ether extracts of *Ipomoea reniformis* seeds. Glycosides and proteins are absent in petroleum ether; extract while present in ethanol and aqueous of *Ipomoea reniformis* seeds. Anthraquinone glycosides were absent in all extracts of *Ipomoea reniformis* seeds. Result of Qualitative phytochemical test in successive extracts of *Ipomoea reniformis* seeds helps in identification of plant.

Antiulcer activity

The visible effect of standard drug Ranitidine and *Ipomoea reniformis* seeds extract (Petroleum ether, Ethanol extract and Aqueous extract) were studied on the ulcer Index and extent of mucosal damage in the stomach.

The animals divided in to four different groups Group I i.e. control group, in which animals treated with oral administration of Distilled water. The characteristic lesions produced in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red

Table 1. Biochemical Parameters study of petroleum ether extract of *Ipomoea reniformis* seeds

Animal groups	Biochemical Parameters		
	Vol. of gastric juice (ml/100g)	Free acidity mEq/l/100mg	Total acidity mEq/100mg
Group I, vehicle control, received only distilled water	3.1±0.14	29.2±0.62	68.4±1.42
Group II - Ranitidine 20 mg / kg body wt	1.4±0.13*	14.6±0.28*	42.6±0.65*
Group III – *Ipomoea reniformis* seeds extract 500 mg / kg body wt	2.8±0.11*	31.2±0.19*	53.2±0.14*
Group IV – *Ipomoea reniformis* seeds extract 250 mg / kg body wt	4.1 ±0.11	42.2±0.15	69.1±0.21

Values are mean ± SEM; n=6 (ANOVA),*p<0.05

Table 2. Biochemical Parameters study of ethanol extract of *Ipomoea reniformis* seeds

Animal groups	Biochemical Parameters		
	Vol. of gastric juice (ml/100g)	Free acidity mEq/l/100mg	Total acidity mEq/100mg
Group I - vehicle control, received only distilled water	3.1±0.14	29.2±0.62	68.4±1.42
Group II - Ranitidine 20 mg / kg body wt	1.4±0.13*	14.6±0.28*	42.6±0.65*
Group III – *Ipomoea reniformis* seeds extract 500 mg / kg body wt	1.6±0.14*	15.8±0.39*	43.7±0.44*
Group IV – *Ipomoea reniformis* seeds extract 250 mg / kg body wt	2.6 ±0.21	21.9±0.35*	49.4±0.71*

Values are mean ± SEM; n=6 (ANOVA),*p<0.05

Table 3. Biochemical Parameters study of aqueous extract of *Ipomoea reniformis* seeds

Animal groups	Biochemical Parameters		
	Vol. of gastric juice (ml/100g)	Free acidity mEq/l/100mg	Total acidity mEq/100mg
Group I - vehicle control, received only distilled water	3.1±0.14	29.2±0.62	68.4±1.42
Group II - Ranitidine 20 mg / kg body wt	1.4±0.13*	14.6±0.28*	42.6±0.65*
Group III – *Ipomoea reniformis* seeds extract 500 mg / kg body wt	1.9±0.17*	21.1±0.29*	48.1±0.14*
Group IV – *Ipomoea reniformis* seeds extract 250 mg / kg body wt	3.4 ±0.11	28.1±0.15***	59.1±0.31*

Values are mean ± SEM; n=6 (ANOVA),*p<0.05

Table 4. Percent ulcer protection effect of petroleum ether extract of *Ipomoea reniformis* seeds

Animal Groups	Pyloric ligation		Ethanol induced	
	Ulcer Index	Percent Protection	Ulcer Index	Percent Protection
Group I- Control	1.1 ±0.02	82.18%	1.1 ±0.02	82.18%
GroupII- Standard (Ranitidine)	0.04±0.05*	94.28%	0.04±0.05*	94.28%
Group III – Plant Extract 500mg	3.52±0.01*	61.21%	4.26±0.13*	59.12%
Group IV – Plant Extract 250 mg	4.88±0.05*	51.36%	5.61±0.03*	42.78%

All values represent Mean ± SEM, n=6 in each group. *P < 0.05. Control group (Group I) is compared with standard and extract doses

Table 5. Percent ulcer protection effect of ethanol extract of *Ipomoea reniformis* seeds

Animal Groups	Pyloric ligation		Ethanol induced	
	Ulcer Index	Percent Protection	Ulcer Index	Percent Protection
Group I- Control	1.1 ±0.02	82.18%	1.1 ±0.02	82.18%
Group II- Standard (Ranitidine)	0.04±0.05*	94.28%	0.04±0.05*	94.28%
Group III – Plant Extract 500mg	1.12±0.03*	82.16%	3.21±0.14*	72.07%
Group IV – Plant Extract 250 mg	3.28±0.01*	68.06%	4.78±0.11*	55.25%

All values represent Mean ± SEM, n=6 in each group. *P < 0.05. Control group (Group I) is compared with standard and extract doses

Table 6. Percent ulcer protection effect of aqueous extract of *Ipomoea reniformis* seeds

Animal Groups	Pyloric ligation		Ethanol induced	
	Ulcer Index	Percent Protection	Ulcer Index	Percent Protection
Group I- Control	1.1 ±0.02	82.18%	1.1 ±0.02	82.18%
GroupII- Standard (Ranitidine)	0.04±0.05*	94.28%	0.04±0.05*	94.28%
Group III – Plant Extract 500mg	2.92±0.01*	72.11%	4.28±0.21*	57.34%
Group IV – Plant Extract 250 mg	4.18±0.05*	58.16%	5.11±0.02*	51.35%

All values represent Mean ± SEM, n=6 in each group. *P<0.05. Control group (Group I) is compared with standard and extract doses

lesions. Group II have animals were pretreated with the standard drug as Ranitidine, showed considerable protection from ulcer in gastric mucosa. The Group III were treated with plant extract of petroleum ether significantly reduced the ulcer index at 500 mg/kg. The Group IV were treated with plant extract of petroleum ether significantly reduced the ulcer index at 250 mg/kg doses. Ranitidine drug act as reference material for identify the valuable effect of the ulcer protection. The intensity of heamorrhage and lesions was significantly reduced with pretreatment and helpful for the protective effect of plant extract.

The histopathological observation of ethanol-induced ulcer models showed perforated ulcer, deep ulceration of granular epithelium and loss of the histological structure, almost reducing the submucosa. Histopathological evaluation of ethanol extract of *Ipomoea reniformis* seeds showed healing of ulcer with few inflammatory cells and at dose dependent manner.

In conclusion, ethanol extract of *Ipomoea reniformis* seeds showed a significant ulcer healing in both the animal models (Pyloric ligation and ethanol-induced ulcer model) used in the study. In pylorus ligation, both the doses showed significant anti-ulcer activity by reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the

control group. The intensity of heamorrhage and lesions was significantly reduced upon pretreatment with the extract, revealing the protective effect of plant extract in dose dependent manner.

Conflict of interest: None

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