

Evaluation of antiulcer and antioxidant potential of *Ipomoea Reniformis* leaf extract against experimentally induced ulcer in rats

Shree Lakshmi Devi S.^{1*}, Abilash S. C.², Sengottuvelu S.³

¹Department of Pharmacology,

²Department of Pathology,
DM Wayanad Institute of
Medical Sciences, Wayanad,
Kerala, India

³Department of Pharmacology,
Nandha College of Pharmacy,
Erode, Tamilnadu, India

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***Correspondence to:**

Dr. Shree Lakshmi Devi S.,
Email: drshree20@gmail.com

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ABSTRACT

Background: Ulcer is a major disease of gastrointestinal system which affects about 10% of the world's population. As conventional drugs can produce undesirable side effects on long term use it can be replaced by herbal medicines more safely. The study was designed to evaluate the antiulcer activity of ethanolic leaf extract of *Ipomoea reniformis* against pyloric ligated ulcer and cold restraint stress induced ulcer models in rats.

Methods: Male Wistar albino rats were used. 0.1% Carboxy Methyl Cellulose (CMC) was used as vehicle. Ethanolic leaf extract of *Ipomoea reniformis* was employed at two dose levels (200 and 400mg/kg). Omeprazole (10mg/kg) was used as reference control in both the models. The test drugs were administered by suspending in CMC, through oral route. In pyloric ligated ulcer model, gastric volume, ulcer index and percentage protection were measured. In cold restraint stress induced ulcer model, ulcer index and percentage protection was measured. The stomach homogenate was subjected to antioxidant profiles.

Results: Ethanolic leaf extract of *Ipomoea reniformis* dose dependently decreased the gastric volume in pyloric ligated ulcer model and significantly reduced the ulcer index in both the models. *Ipomoea reniformis* in both the doses significantly protected the animals against stress-induced free radical damage due to decrease in LPO and reversal of changes induced by stress on SOD and CAT.

Conclusions: From the result, it was concluded that the *Ipomoea reniformis* leaf extract exhibited antiulcer activity in both the tested models were due to its antisecretory and antioxidant property.

Keywords: Antioxidant, *Ipomoea reniformis*, Ulcer index and antisecretory

INTRODUCTION

Peptic ulcer disease (PUD) results from an imbalance between stomach acid-pepsin and mucosal defense barriers. It affects 4 million people worldwide annually.¹ The incidence of PUD has been estimated at around 1.5% to 3%.² Although potent anti-ulcer drugs are available, most of them produce several toxicities, thus emphasizing the need to search for new alternatives.³ As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care, reinforcing the theory that plant extracts can be good sources of new drugs. *Ipomoea reniformis* belonging to

the family Convolvulaceae, is a perennial, much branched herb (creeper). It is widely distributed all over the India, especially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa.⁴ In the Indigenous system of medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums.⁵ *Ipomoea reniformis* reported to possess antioxidant,

antihypertensive, analgesic, anti-inflammatory, antipyretic, antidiabetic, antibacterial, anticancer and nephroprotective activities.⁶⁻¹² The traditionally important, *Ipomoea reniformis* was reported to possess various pharmacological activities. However, there is no scientific evidence on prevention of peptic ulcers of *Ipomoea reniformis* against above traditional claims so far. Hence, the present study was conducted with the aim to investigate the antiulcer and antioxidant activity of ethanolic extract of *Ipomoea reniformis* leaves in albino rats.

METHODS

Plant material

The leaves of *Ipomoea reniformis* were collected from outskirts of Tirunelveli district, in the month of December. It was identified and authenticated as *Ipomoea reniformis* by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The voucher specimen (BSI/SRC/12/42/2015-16/Sci/1555) has been deposited in department for further references.

Preparation of extract

The leaves of *Ipomoea reniformis* were, shade dried and then ground into coarse powder. The powder was then subjected to exhaustive extraction by a maceration process using 70% ethanol as a solvent at room temperature for 7 days. The ethanolic extract was concentrated by vacuum distillation to dry. The collected extract was stored in desiccators and used for further pharmacological study.

Animals

Male Wistar Albino rats weighing between 150-160g were used for the study. The animals were obtained from animal house of Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light: dark cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were in accordance with the guidelines of the IAEC.

Screening of antiulcer activity

Pyloric ligation induced gastric ulceration

Male Wistar albino rats were used in the study. The animals were divided into 4 groups of six animals each. Animals were fasted for 24 hour before the study, but had free access to water. Group I treated as control, received

0.1% Carboxy Methyl Cellulose (CMC); group II and III treated as treatment groups, received 200 and 400 mg/kg of ethanolic extract of *Ipomoea reniformis* for 7 days and group IV as standard group, received Omeprazole (10 mg/kg). All the test drugs were administered by suspending in CMC, once daily for 7 days, through oral route using gastric intubation tubes.

On day 7 after the last dose of test drugs, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. Then the pre-treated animals were anaesthetized by pentobarbitone sodium (45mg/kg). The abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was ligated without causing any damage to its blood vessels. The stomach was isolated carefully and the abdominal wall was sealed by interrupted sutures. The animals were deprived of water during the postoperative period. 4hrs after pyloric ligation, the animals were sacrificed with excess pentobarbitone sodium and the stomach was dissected out. The gastric contents were collected in tubes and volume was measured. The stomach was then incised along the greater curvature and examined for lesions in the fore stomach portion then indexed according to severity.^{13,14} The percentage inhibition of ulceration was calculated and compared with control.

Cold restraint stress induced ulcers

The animals were divided into five groups of six rats in each group. Group 1 represented normal control (non-ulcerated) animals received 0.1% CMC. Group 2 represented as stress control animals received 0.1% CMC. Group III and IV served as treatment groups, received 200 and 400 mg/kg of ethanolic extract of *Ipomoea reniformis* and group V as reference control, received Omeprazole (10mg/kg). The drugs were administered for three days, orally by suspending in 0.1% CMC solution. On day 3, for 18 h fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 h at 4-6°C.¹⁵ The animals were then sacrificed by cervical dislocation. The stomach was taken out and cut open along the greater curvature and observed for ulcer index as mentioned earlier.¹³ The fundus of stomach was subjected to antioxidant activities.

Antioxidant activity

The fundus of stomach was used for estimation of free radical scavenging activity of *Ipomoea reniformis*. The fundic part of the stomach was homogenized (5%) in ice cold 0.9% saline with a glass homogenizer. The homogenate was centrifuged at 800 rpm for 10 minutes followed by centrifugation of the supernatant at 2000 rpm for 5minutes and the fraction was used for the antioxidant activity.

Lipid peroxidase (LPO)

LPO product malondialdehyde (MDA) was estimated using 1,1,3,3-tetraethoxypropane as the standard and is expressed as nmol/mg protein.¹⁶

Superoxide dismutase (SOD)

The inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored formazan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit (U) of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in 1 min under the defined assay conditions and the results have been expressed as U/mg protein.¹⁷

Catalase (CAT)

Decomposition of H₂O₂ in presence of catalase was followed at 240 nm. One unit of (U) CAT was defined as the amount of enzyme required to decompose 1 mmol of H₂O₂/min, at 25°C and pH 7.0 and the results were expressed as U/mg protein.¹⁸

Statistical analysis

The results were expressed as the Mean±SEM for each group. Statistical differences were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's 't' test GraphPad InStat 3. Results were considered to be statistically significant at P<0.05.

RESULTS

Pyloric ligation induced gastric ulceration

Ipomoea reniformis leaf extract was studied for its antiulcer activity against pyloric ligation induced ulcer in rats and the results were shown in Table 1. The gastric volume accumulated in the control groups was 10.65±0.90 ml of pyloric ligated rats. The reference control Omeprazole significantly (P<0.001) decreased the gastric volume to 3.48±0.16 ml compared to control groups. The ethanolic leaf extract of *Ipomoea reniformis* 200mg/kg (P<0.05) and 400mg/kg (P<0.01) significantly decreased the gastric volume to 5.88±0.45 and 4.95±0.26 ml respectively, compared to control. *Ipomoea reniformis* leaf extract showed dose dependent reduction in the gastric volume.

Table 1: The effect of *Ipomoea reniformis* leaf extract on gastric volume and ulcer index of pyloric ligation induced ulcer in rats.

Groups	Drug treatment	Gastric volume (ml)	Ulcer index	% Protection
I	Control- 0.1% CMC (1ml/kg)	10.65±0.90	18.22±1.06	-
II	<i>Ipomoea reniformis</i> (200mg/kg)	5.88±0.45*	8.73±0.74**	52.08
III	<i>Ipomoea reniformis</i> (400mg/kg)	4.95±0.26**	4.93±0.19***	72.94
IV	Reference control omeprazole (10mg/kg)	3.48±0.16***	3.52±0.01***	80.68

Values are presented as mean ± SEM (n = 6)

*P<0.05, **P<0.01 and ***P<0.001 Vs Control

Table 2: The effect of ethanolic leaf extract of *Ipomoea reniformis* on ulcer index and antioxidant parameters (LPO, SOD and CAT) in cold restraint stress induced ulcer in rats.

Groups	Drug treatment	Ulcer index	%Protection	LPO (nmols of MDA/mg of protein)	SOD (U/mg of protein)	CAT (U/mg of protein)
I	Normal Control	-	-	133.25±6.72***	93.22±5.65***	54.03±4.80***
II	Stress control 0.1% CMC (1ml/kg)	18.66±5.82	-	239.64±6.55	146.33±5.42	21.38±1.56
III	<i>Ipomoea reniformis</i> (200mg/kg)	9.62±2.48**	48.45	184.92±7.95**	120.88±5.20**	31.72±2.05*
IV	<i>Ipomoea reniformis</i> (400mg/kg)	6.74±0.64***	63.87	162.30±6.74***	105.77±4.66***	38.54±2.92**
V	Reference control Omeprazole (10mg/kg)	3.86±0.45***	79.31	144.72±5.58***	114.34±5.09***	47.32±3.42***

Values are presented as mean ± SEM (n = 6)

*P<0.05, **P<0.01 and ***P<0.001 Vs Stress Control

The rats treated with reference standard Omeprazole showed significant decrease in ulcer index and enhanced the percentage of ulcer protection. The rats treated with

Ipomoea reniformis leaf extract (200mg and 400mg/kg) also significantly (P<0.001 and P<0.01, respectively) decreased the intensity of gastric mucosal damage. The

ulcer index and % protection of gastric lesion was 8.73 ± 0.74 and 52.08% respectively, in the groups of animals received *Ipomoea reniformis* leaf extract (200mg/kg) as compare control. The ulcer index and % protection of gastric lesion was 4.93 ± 0.19 and 72.94% respectively, in the groups of animals received *Ipomoea reniformis* leaf extract (400mg/kg) as compare control and the effect was equipotent with reference control Omeprazole. The ulcer index and % protection of gastric lesion was 3.52 ± 0.01 and 80.68% respectively, in the groups of animals received Omeprazole.

Cold restraint stress induced ulcers

The effect of ethanolic leaf extract of *Ipomoea reniformis* on ulcer index and Antioxidant parameters (LPO, SOD and CAT) in cold restraint stress induced ulcer in rats were shown on Table 2. Hypothermic and Immobilization stress produced considerable ulcerogenicity in rats. The ulcers were in the form of haemorrhagic mucosal lesions in the stomach, which were confined to the rugae of glandular segment. *Ipomoea reniformis* at the doses of 200 and 400 mg/kg resulted in a significant ($P < 0.01$ and $P < 0.001$) reduction in ulcer index and % protection of ulcer was 48.45 and 63.87, respectively when compared with the control group. The reference control Omeprazole showed significant ($P < 0.001$) decrease in ulcer index and the % protection of ulcer was 79.31 in stress induced ulcer model.

Antioxidant activity

Stress significantly increased lipid per oxidation in the gastric mucosa with concomitant increase in SOD and decrease in CAT. *Ipomoea reniformis* in both the doses of 200 and 400mg/kg significantly protected the animals against stress-induced free radical damage as seen from the decrease in LPO and reversal of changes induced by stress on SOD and CAT.

DISCUSSION

This study was carried out to evaluate the antiulcer and antioxidant effect of ethanolic leaf extract of *Ipomoea reniformis* on pylorus ligation-induced and cold and restraint stress induced ulcer models. Ethanolic leaf extract of *Ipomoea reniformis* reported to have tannins, flavonoids, saponins, phenolic compounds, terpenoids, steroids and carbohydrates.¹⁹ Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, as well as act as free radical scavengers, and inhibit $H^+/K^+-ATPase$.²⁰ Saponins may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation.²¹ In addition, terpenoids are also reported to have potent activity against gastric ulcers.²² In our study, the antiulcer activity exhibited by the ethanolic leaf extract of *Ipomoea reniformis* against pyloric ligation may be due to the

presence of the phytoconstituents such as, flavonoids, tannins, saponins and terpenoids. In pyloric ligated ulcer model, *Ipomoea reniformis* significantly decreased the gastric volume at both the dose levels supports the antisecretory action through which it may exhibits antiulcer activity.

Stress increases the formation of reactive oxygen species, decreases the cell proliferation rate, increases gastric juice secretion, and promotes inhibition of prostaglandin synthesis, leading to alterations in the circulating nitric oxide and the gastric mucosa.²³ In the present study, in cold and restraint ulcer model, ethanolic leaf extract of *Ipomoea reniformis* significantly reduced the ulcer index there by confirms the gastroprotective effect against stress-related injuries.

The role of free radicals in gastric ulcerations is well documented.²⁴ *Ipomoea reniformis* significantly reduced lipid per oxidation in rat gastric mucosa. SOD scavenges the super oxide radical O_2^- , one of the reactive oxygen species (ROS) responsible for lipid peroxidation.²⁵ This reaction leads to increase in generation of peroxy radical $H_2O_2^-$, which is also capable of producing more oxidative damage.²⁶

CAT and other peroxidases further reduce $H_2O_2^-$. Hence, the anti-oxidant activity in gastric mucosal homogenates observed from decrease in LPO may be due to increase in SOD and CAT levels. Stress-induced ulceration involves damage by ROS apart from acid and pepsin related factors.²⁷

During stress LPO and SOD were significantly increased and CAT level was significantly decreased. The increase in SOD was due to increased ROS generation during mucosal damage. This led to increased generation of $H_2O_2^-$ and its accumulation due to decreased CAT level. Inactivation of gastric peroximes during stress may also aggravate the mucosal damage.²⁸

This evidently caused increased lipid per oxidation and mucosal damage as seen from the increase in ulcer index in comparison to the control group. *Ipomoea reniformis* effectively alleviated stress-induced ulcers with marked decrease in LPO, suggesting decrease in oxidative damage. This may be due to restoration of balance between free radical scavenging enzymes SOD and CAT in the gastric mucosa, effectively counteracting the free radicals generated by cascade of reactions as described earlier. Thus, the anti-ulcerogenic activity of *Ipomoea reniformis* may also be due its anti-oxidant effects.

CONCLUSION

Antiulcer activity of ethanolic leaf extract of *Ipomoea reniformis* was studied by employing pyloric ligation induced gastric ulcer and cold restraint stress induced ulcer in rats. From the result, it was concluded that the *Ipomoea reniformis* leaf extract exhibited antiulcer

activity in both the tested models. The above said activity showed by *Ipomoea reniformis* may be due to its antisecretory and antioxidant property. Further study may be conducted to isolate the active constituent may bring a potential and safe herbal based antiulcer agent to mankind.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee (932/a/06/CPCSEA)

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