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R E S E A R C H A R T I C L E

Evaluation for Anti Ulcer Effects of Methanolic Extract of *Ipomoea Batatas* Plant Leaves Using *In-vivo* Models

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A B S T R A C T

In the presant work the Methanolic extract of *Ipomoea batatas* treated groups shows a significant effect when compared to control group animals which indicating that the plant having the anti ulcer activity. And also the results showed that the Methanolic extract of the *Ipomoea batatas* having the antioxidant activity. The acute toxicity study conducted for Methanolic extract of *ipomoea batatas* indicates that safe up to 2000mg/kg body weight. Ulcer can minimize by some life style changes like, avoid eating at least two hours before bed time and whatever foods might cause discomfort, such as ethanol, caffeine beverages (coffee and pop), fatty foods, and highly seasoned foods. It is important to try to stop smoking, since smoking has been linked to ulcer formation, reduced healing, and ulcer recurrences. Also, try to minimize stress in life. Stress may worsen ulcer symptoms.

Keywords: *Ipomoea batatas*, ulcer, Methanolic extract.

A R T I C L E I N F O

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CONTENTS

1. Introduction

Over the past decades, herbal medicine has become a thing of global significance with medicinal and economic implications. Wide spread use of herbs throughout the globe has raised serious concerns over its quality, safety,

and efficacy. Our investigation showed that these investigated medicinal plants could prevent ulcer in a dose-dependent manner. Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins. A peptic ulcer in the stomach is called a gastric ulcer. One that is in the duodenum is called a duodenal ulcer. Peptic ulcers happen when the acids that help you digest food damage the walls of the stomach or duodenum.

The most common cause is infection with a bacterium called Helicobacter pylori. Another cause is the long-term use of non-steroidal anti-inflammatory medicines (NSAIDs) such as aspirin and ibuprofen. Stress and spicy foods do not cause ulcers, but can make them worse. As many as 70-90% of ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach. Ulcers can also be caused or worsened by drugs such as aspirin and other NSAIDs. There has been considerable pharmacological investigation in to the antiulcer activity of some compounds.

In a gastric ulcer, generally, the acid secretion is normal or low. In a duodenal ulcer, acid secretion is high in half of the patients but normal in the rest. Gastric and duodenal ulcers are common pathologies that may be induced by a variety of factors such as stress, smoking, nutritional deficiencies and noxious agents including non-steroidal anti-inflammatory drugs (NSAID). Currently available treatments for peptic ulcers include antacids (systemic and nonsystemic) and drugs which reduce acid secretion such as H2 anti-histaminics, proton pump inhibitors, anticholinergics, prostaglandin analogues, ulcer protectives, ulcer healing drugs and anti-H. pylori drugs. These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease, and are often expensive for the poor.

In light of the above, it is pertinent to study natural products from food/plants as potential anti-ulcer compounds. Due to less side effects compared to synthetic drugs, currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care. Sweet potato (Ipomoea batatas (L.) Lam.) from the family Convolvulaceae, is widely grown in tropical, subtropical & warm temperate regions and in Asian countries, particularly China. The tubers of Ipomoea batatas are commonly known as sweet potato. Sweet potato has been reported to possess anti oxidant, anti-diabetic, wound healing, anti-bacterial, and anti-mutagenic properties.

The aim of the presant work is to investigate the anti ulcer activity of Methanol extracts in ethanol induced ulcer in rats. Sweet potato is a member of the morning glory family (*Convolvulaceae*) and is therefore not closely related to potato (*Solanum tuberosum*), which is a member of *Solanaceae*. Sometimes known by the common name yam, sweet potato should not be confused with Dioscorea species, which are also known as yams but belong to a different plant family (*Dioscoreaceae*).

Sweet potato is a member of the morning glory family (Convolvulaceae) and is therefore not closely related to potato (*Solanum tuberosum*), which is a member of Solanaceae. Sometimes known by the common name yam, sweet potato should not be confused with *Dioscorea* species, which are also known as yams but belong to a different plant family (Dioscoreaceae).

Long cultivated for its edible root tubers, sweet potato is an important carbohydrate source in the tropics, especially in Central America and New Guinea.

Materials

Trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazinemethosulfate (PMS), ferrozine, glutathione reduced, batho phenanthroline sulfonate disodium salt, Thiobarbituric acid (TBA), and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, ammonium iron (II) sulfatehexahydrate $[(NH_4)_2Fe(SO_4)_26H_2O]$, 1-chloro-2,4-dinitrobenzene (CDNB), chloramine-T, hydroxylamine hydrochloride, Dimethyl-4-aminobenzaldehyde, and 2,4-dinitro phenylhydrazine (DNPH) were obtained from Merck, Mumbai, India. Ferritin was purchased from MP Biomedicals, USA. Streptomycin sulphate was obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. The standard oral iron-chelating drug, desirox, was obtained from Cipla Ltd., Kolkata, India.

2. Materials and Methods

Collection and Authentification of Plant Material

The turbs of *ipomoea batatas* are collected and authenticated by Dr. K Madhava Chetty, department of botany, Sri Venkateswara University, Tirupathi.

Extraction of Plant Material

The dried turbs are grinded in to a coarse powder with the help of suitable grinder

Hot Continuous Extraction (Soxhlet)

In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

Evaporation of Solvent

The filtrates (Methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *ipomoea batatas* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids. As per the standard methods.

Material

Drugs: Standard drug ranitidine.

Chemicals:

0.01N NaOH, phenolphthalein indicator, Topfer's reagent, 80% ethanol, Formalin, gum acacia, Anaesthetic ether obtained from Zeal chemicals, wargal.

Reagents: Benedict's reagent, barfoed's reagent, million's regent, wager's regent, Hager's regent. Mayer's regent.

Animals

The animals used in the present study were adult male wistar rats (10–12 weeks old with body weight 150-200 g), obtained from the animal house of the sanzyme bio labs pvt ltd, hyderabad. The animals were housed in colony cages, under standard laboratory conditions (12 h light, 12 h dark cycle), with free access to standard commercial diet and water. All experimental procedures used in the present study were approved by the Ethics Committee

Acute toxicity studies

The Wister rats of single sex, weighing between 150 to 200 g were selected and divided in to 5 groups each consisting of 5 animals. They were maintained under standard conditions (room temperature at 22±3˚C, 12 hr light/dark) and allowed free access to water along with standard pelleted diet for one week before the experiment. The animals were subjected for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 5 groups and observed at regular intervals of 1, 2, 4, 8, 12 and 24 hours for skin changes, morbidity, aggressiveness, increase oral secretion, sensitivity to the sound and pain as well as respiratory movements and mortality. $1/10^{th}$ of the highest dose is taken for the studies.

Method of Induction and Experimental Animal Protocol

Ethanol-induced gastric ulcer: The albino rats were randomly divided into four groups of six animals each. And the animals are given the plant extract for 19 days. On 20^{th} day the animals were given 20% ethanol (ethanol and normal saline 20:80) orally. Animals were fasted for 24 h before experiment but with free access to water.

Experimental procedure

First group treated with 1ml of 80% ethanol orally on the day of experiment at about 10 AM with the help of an oral feeding tube. 2^{nd}, 3^{rd}, 4^{th} groups of animals were treated with ranitidine, low and high doses of beet root extracts respectively one hour before ethanol administration. One hour after drug treatment of 2^{nd}, 3^{rd}, 4^{th} groups of animals were treated with 1 ml of 80% ethanol by p.o, to induce ulcers. The animals were sacrificed after 1hr of ethanol administration. The stomach was opened and calculates the ulcer index and percentage inhibition of ulcer [97].

Table no- IV.1. Experimental design of Ethanol-induced gastric ulcer

Groups	Treatment
I	control (1ml of 80% ethanol)
II	Ranitidine (20mg/kg)
III	***Ipomoea batatas*** 200 mg/kg
IV	***Ipomoea batatas*** 400 mg/kg

Estimation of gastric volume, pH:

The gastric content that was transferred into centrifuge tubes was used for estimation of gastric volume, pH. The tubes were centrifuged at 1000 rpm for 10 min and the gastric volume was directly read from the graduation on the tubes. The supernatant was then collected and pH was determined by using a digital pH meter [100].

Determination of total acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted [100].

Determination of free acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity [100].

Acidity was expressed as

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality x 100}}{} \text{ mEq/1it/100gm}$$

Determination of Ulcer Index (UI)

The ulcerative index was calculated by severity of gastric mucosal lesions and graded as follows; [100]

0=no ulcer
1=superficial ulcer
2=deep ulcer
3=perforation

$$UI = UN + US + UP \times 10^{-1}$$

UN=average of number of ulcers per animal
US=average of severity score
UP=Percentage of animals with ulcers

% gastro protection was calculated according to;

% gastro protection = (UIC-UIT)/UIC*100

Where, UIC-ulcer index of control.

UIT-ulcer index of test

Histopathological evaluation

The gastric tissue was fixed in 10% ethanol buffer formalin and processed though graded ethanol, xylene and impregnated with paraffin wax; sections were made by microtome. After staining with haemotoxylin and eosin stain (Culling, 1974), the sections were examined under a research microscope by a person who was not aware of experimental protocols. The different histopathological indices screened were: congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulcerations

Statistical analysis: The values are expressed as mean value ± standard deviation (SD). The data were evaluated by using the SPSS (version 12.0) and one-way ANOVA, followed by Bonferroni t-test. Statistical significance was considered when value of $P < 0.05$.

3. Results and Discussion

Phytochemical analysis:

Tab: 1. Phytochemical Analysis.

Phytoconstituents	Present or Absent
Carbohydrates	Absent
Glycosides	Present
Fats	Absent
Gums & mucilages	Absent
Proteins & amino acids	Present
Saponins	Present
Tannins & Phenolic compounds	Absent
Phytosterols	Absent
Flavonoids	Absent
Alkaloids	Absent

Pharmacological studies:

Acute toxicity studies

The Methanolic extract of *ipomoea batatas* was subjected for the acute toxicity study to determine the therapeutic dose using wistar rats in controlled environment. Acute oral toxicity study was performed as per OECD-423 guidelines. Acute toxicity study carried out on EECA up to the dose of 2000 mg/kg demonstrated that the extract did not show any sign of toxicity and mortality. Hence 200 and 400 mg/kg dose of the extract selected for evaluation of anti-ulcer activity.

Effect of Methanolic extract of *Ipomoea batatas* in Ethanol induced gastric ulcer:

In ethanol induced gastric ulcer model, the ulcer index of control group is 11.083±0.4282. The animals treated with Methanolic extract of *ipomoea batatas* at 400 mg/kg dose showed significant (*P<0.01*) reduction in the number of ulcer and ulcer index is 8.516±0.42816. Ranitidine at 20mg/kg showed significant (*P<0.01*) reduction in the number of ulcer and ulcer index is 8.516±0.42816. Extract at 200mg/kg shows the protection against the ethanol induced gastric ulcer, ulcer index 10.35±0.5627. Administration of beet root 1 h before the induction of gastric lesions by ethanol showed significant activity, and inhibited the ulcer index in dose dependent manner. The Methanolic extract of *ipomoea batatas* was found to possess remarkable ulcer-protective properties at 200 mg/kg and 400 mg/kg when compare to toxic control group.

Table 2: Effect of Methanolic extract of *Ipomoea batatas* on ulcer index and %ulcer protection in ethanol induced gastric ulcer.

Groups (n=5)	Treatment	UI	% ulcer protection
I	Control	12.083±0.4282	0.00
II	Standard	9.562±0.4216**	22.74
III	***Ipomoea batatas*** 200 mg/kg	11.35±0.5627ns	6.613
IV	***Ipomoea batatas*** 400mg/kg	9.516±0.42816**	23.16

Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at $P<0.01^{**}$ compared to control group, P>0.05 ns-non significant

Volume of gastric content:

Gastric content volume high (3.2±0.1291) in control group. Gastric content volume siginificantly decreases in Methanolic extract of *ipomoea batatas* at 200 (1.179±0.213) and 400mg/kg (1.184±0.195) doses. Gastric content volume in standard group (1.184±0.195).

Table 3: Effect of Methanolic extract of *Ipomoea batatas* on gastric content volume

Groups	Treatment	Volume of gastric content
I	Control	1.211±0.121
II	Standard	1.183±0.103**
III	***Ipomoea batatas*** 200 mg/kg	1.179±0.213**
IV	***Ipomoea batatas*** 400mg/kg	1.184±0.195**

Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at $P<0.01^{**}$ compared to control group,

PH:

Table 4: Effect of Methanolic extract of *Ipomoea batatas* on gastric juice PH

Groups	Treatment	PH
I	Control	2.831±0.118
II	Standard	2.691±0.138**
III	***Ipomoea batatas*** 200 mg/kg	2.758±0.192**
IV	***Ipomoea batatas*** 400mg/kg	2.868±0.185**

Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at $P<0.01^{**}$ compared to control group.

Total acidity (mEq/lit):

Table 5: Effect of Methanolic extract of *Ipomoea batatas* on total acidity

Groups	Treatment	Total acidity(mEq/lit)
II	Control	55.4±1.482
III	Standard	54.6±1.216
IV	***Ipomoea batatas*** 200 mg/kg	52.2±1.324
V	***Ipomoea***	53.4±0.233

	batatas 400mg/kg	

Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at $P<0.01^{**}$ compared to toxic control group.

e. Free acidity (mEq/lit):

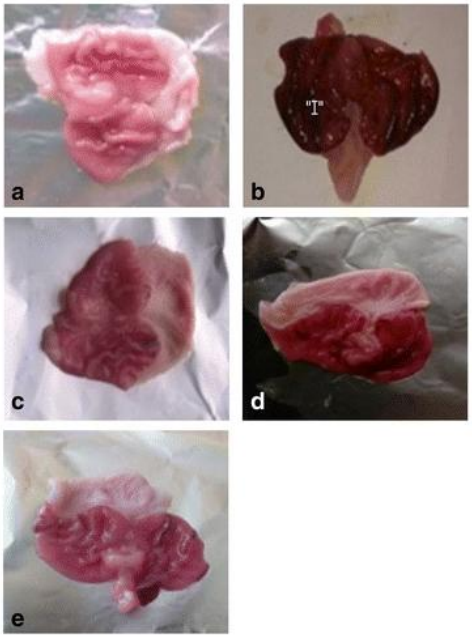
In pylorus ligation induced gastric ulcer model the free acidity high (283±2.171) in control group. Free acidity significantly decreases in Methanolic extract of *ipomoea batatas* at 200 (66.3±0.4447) and 400mg/kg (21.76±0.2088) doses. Free acidity significantly decreases in standard group (24±0.1880) compared toxic control group**.**

Table 6: Effect of Methanolic extract of *Ipomoea batatas* on free acidity

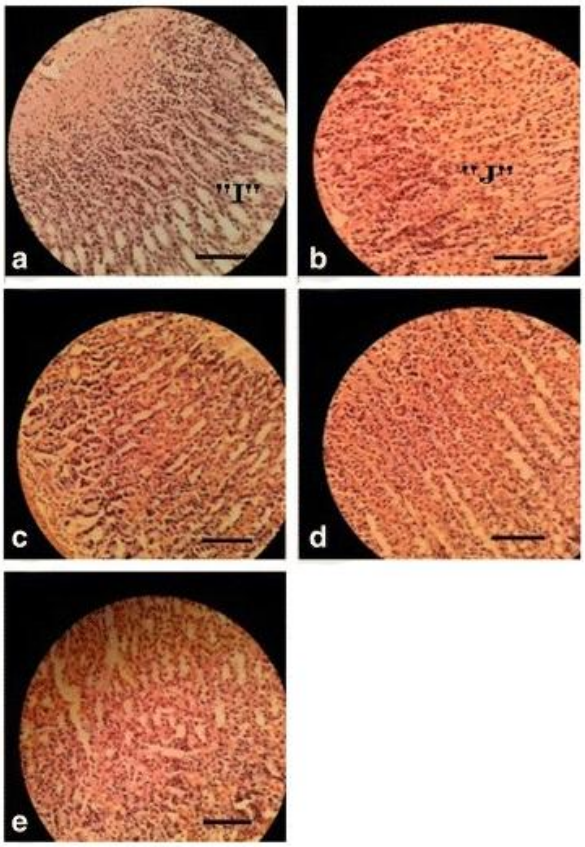
Groups	Treatment	Free acidity(mEq/lit)
II	Control	23±0.101
III	Standard	23.4±0.180
IV	***Ipomoea batatas*** 200 mg/kg	26.2±0.447
V	***Ipomoea batatas*** 400mg/kg	24.76±0.288

Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at $P<0.01^{**}$ compared to toxic control group

f. Histopathological evaluation:



Gross appearance of the gastric mucosa. **a** Normal control: no mucosal damage was observed, **b** Ethanol control: marked ulcers along with hemorrhagic streaks and mucosal damage were observed, **c** 20 mg/kg ranitidine, mild injuries were observed in the gastric mucosa as compared to the ethanol control group, **d** 200 mg/kg Methanolic extract of *Ipomoea batatas* : moderately reduced gastric mucosal damage and ulcers were observed, **e** 400 mg/kg Methanolic extract of *Ipomoea batatas*: significantly reduced gastric mucosal damage and ulcers were observed, "I" indicates gastric mucosal damage and hemorrhagic streaks.



a Normal control: no mucosal damage was observed, **b** Ethanol control: marked ulcers along with hemorrhagic streaks and mucosal damage were observed, **c** 20 mg/kg ranitidine, mild injuries were observed in the gastric mucosa as compared to the ethanol control group, **d** 200 mg/kg Methanolic extract of *Ipomoea batatas* : moderately reduced gastric mucosal damage and ulcers were observed, **e** 400 mg/kg Methanolic extract of *Ipomoea batatas*: significantly reduced gastric mucosal damage and ulcers were observed, "I" indicates gastric mucosal damage and hemorrhagic streaks,"J" indicates degeneration of gastric mucosa and infiltration of inflammatory cells frequently observed in the specimen from the stomach, "K" indicates almost complete regeneration of gastric mucosa

Ethanol causes mucosal damage by decreasing cytoprotective prostaglandin levels through inhibition of PG synthesis and also results in back diffusion of H+ ions into the gastric mucosa and inhibits the release of mucus. In this model Methanolic extract of *ipomoea batatas* was produced its ulcer protective effect by counteracting the inhibition of PG synthesis and enhancing the mucus release. *ipomoea batatas* extract was significantly reducing the ulcer index compare to control group.

4. Conclusion

In conclusion, the Methanolic extract of ***Ipomoea batatas*** treated groups shows a significant effect when compared to control group animals which indicating that the plant having the anti ulcer activity. And also the results showed that the Methanolic extract of the ***Ipomoea batatas*** having the antioxidant activity. The acute toxicity study conducted for Methanolic extract of *ipomoea batatas* indicates that safe up to 2000mg/kg body weight. Ulcer can minimize by some life style changes like, avoid eating at least two hours before bed time and whatever foods might cause discomfort, such as ethanol, caffeine beverages (coffee and pop), fatty foods, and highly seasoned foods. It is important to try to stop smoking, since smoking has been linked to ulcer formation, reduced healing, and ulcer recurrences. Also try to minimize stress in life. Stress may worsen ulcer symptoms.

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