Evaluation of anti-ulcer activity of the leaf extract of *Solanum pubescens* wild. (Solanaceae) in Wistar albino Rats

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**Abstract**

The present study showed that the methanolic extract of *Solanum pubescens* wild leaves possess Anti- ulcer in animal model. The anti-ulcer activity was evaluated against Pylorus ligated ulcers. Gastric ulcer disease is an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin). Oral administration of methanol extract of *solanum pubescens* wild at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 36.28% and 52.3% respectively compared to the ulcer control, proving the anti-ulcer activity. The standard drug omeprazole (20mg/kg) exhibited percentage inhibition of 70% when compared with ulcer control. Extract treated and ulcer control group was compared with normal control group. Result showed a significant decrease in ulcer development in the animal model (pylorus ligated model) used in the study. By pylorization, both the doses showed significant ulcer activity by reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. The intensity of haemorrhage and lesions was significantly reduced upon pretreatment with the extract, revealing the protective effect of MESP. Flavonoids and tannins are the major constituents that are present in the leaves of *Solanum pubescens* wild which may be responsible for its Ulcer protective and Anti-ulcer activity.

**Keywords:** Peptic ulcer, *Solanum pubescens*, Wistar rats, Acute Oral toxicity, ANOVA

**INTRODUCTION**

Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources. Although potent anti-ulcer drugs are available, most of them produce several toxicities, thus emphasizing the need to search for new alternatives. 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 medicines for the first line of primary health care, reinforcing the theory that plant extracts can be good sources of new medicines for the first line of primary health care, reinforcing the theory that plant extracts can be good sources of new medicines. Ethiopia is a country characterized by a wide range of climatic and ecological conditions possessing enormous diversity of flora and fauna, including a wide range of potentially useful medicinal plants.

In the Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. In this modern era also 75–80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects. 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 which are the active principles of antiulcer activity.

The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors). Peptic ulcers are once believed to be caused by spicy food and stress; these have been found merely to be aggravating factors and the real causes have been found by research to include bacterial infection (Helicobacter pylori) or reaction to various medications, particularly NSAIDS (non steroidal anti-inflammatory drugs). Helicobacter pylori, NSAIDS drugs, emotional stress, alcohol abuse, and smoking are the principal etiological factors associated with peptic ulcer. The Gram-negative bacterium Helicobacter pylori remains present between the mucous layer and the gastric epithelium and is strategically designed to live within the aggressive environment of the stomach. Initially, Helicobacter pylori resides in the antrum but over time migrates toward the more proximal segments of the stomach.

Many synthetic anti-ulcer drugs are available in the market but most of them are associated with many adverse and unwanted effects like gastro-intestinal irritation, ulceration and fluid retention. In addition to aggravating the ulcers, NSAIDS also produce hepato-toxicity and nephro-toxicity. Since ancient times people have been relying on plants either as prophylactic of therapeutic agents to restore and maintain the health. Medicinal plants have been used in the development of new drugs which may have an invaluable role.
in the progress of drug discovery. Therefore a need for the development of anti-ulcer agents from natural source with more powerful activity and lesser side effects.

**MATERIAL AND METHODS**

**Plant collection:**
The leaves of *Solanum pubescens* were collected from Satupra MP. It was identified and authenticated by Department of Botany, Safiya College, and Bhopal M.P.

**Preparation of extract:**
The leaves of *Solanum pubescens* were shade dried and then ground till they became coarse powder in a mortar-pestle. The powdered material thus obtained was subjected to extraction using Methanol. The extracts obtained were distilled to remove excess of the solvent and then evaporated at 40°C to get a semi-solid mass. These extracts were subjected to phytochemical tests which have been described below.

**Animals:**
Wistar rats of either sex (150-200gms) were housed in separate cages at controlled room temperature (24±2°C; relative humidity 60-70%) in a 12hr light-dark cycle. They were fed with standard pellet diet and water ad libitum.

**Qualitative Phytochemical analysis:**

**Determination of Acute Oral toxicity (LD50) of Solanum pubescens willd**

<table>
<thead>
<tr>
<th>Name of the study</th>
<th>Acute toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guideline followed</td>
<td>OECD425 method-acute toxic class Method</td>
</tr>
<tr>
<td>Animals</td>
<td>Healthy young adult non-pregnant Swiss albino mice.</td>
</tr>
<tr>
<td>Bodyweight</td>
<td>25-30 g</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Administration of dose and volume</td>
<td>2000 mg/kg body weight, single dose in 0.2 ml</td>
</tr>
<tr>
<td>Number of groups and animals</td>
<td>2 groups and 6 animals</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Oral by using mice oral feeding needle</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Carboxy Methyl Cellulose (CMC)</td>
</tr>
</tbody>
</table>

**Housing and feeding conditions:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>22°C±3°C</td>
</tr>
<tr>
<td>Humidity</td>
<td>40-60%</td>
</tr>
<tr>
<td>Light</td>
<td>12 h: 12h (light:dark cycle)</td>
</tr>
<tr>
<td>Feed</td>
<td>Standard laboratory animal food pellets with water ad libitum</td>
</tr>
</tbody>
</table>

**Study period and observation parameters:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial observation</td>
<td>First 30 minutes</td>
</tr>
<tr>
<td>Special attention</td>
<td>First 1-4hrs after drug administration</td>
</tr>
<tr>
<td>Long term observation</td>
<td>Upto 14days</td>
</tr>
<tr>
<td>Direct observation parameters</td>
<td>Diarrhea, sitting in the corners, sniffing excessively, standing on hind limbs</td>
</tr>
<tr>
<td>Additional observation parameters</td>
<td>Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.</td>
</tr>
</tbody>
</table>

**Study procedure:**

Acute oral toxicity was performed as per Organization for Economic Cooperation for Development (OECD) guideline 425 methods. The extract was administered in a single dose by gavages using specially designed mice oral needle. Animals are fasted 24h prior to dosing (food was withheld, but not water). (OECD Guideline for testing of chemicals 425).

The powdered material thus obtained was subjected to extraction using Methanol. The extracts obtained were distilled to remove excess of the solvent and then evaporated at 40°C to get a semi-solid mass. These extracts were subjected to phytochemical tests which have been described below.

**Pharmacological evaluation:**

**Animal selection:**
Healthy adult male Wistar albino rats weighing between 150 and 200gms were selected for the anti-Ulcer studies.

**Housing and feeding condition:**
The temperature in the experimental animal room was kept

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22±30°C. Artificial lighting was provided. The animals were acclimatized to standard laboratory conditions of temperature (22±30°C) and maintained on 12:12 h light: dark cycle19. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They were provided with regular rat chow diet and distilled water ad libitum.

**Preparation of animals:**

The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

**Extracts & Standards drug used:**

**Extract used:** Methanolic extract of *Solanum pubescens* wild leaves. Standard drug used: Omeprazole: 20mg/kg b.w. Drugs, Omeprazole and the test extract of *Solanum pubescens* wild were suspended in 0.5% CMC and used for anti-ulcer studies. Each drug suspension was freshly prepared just before administration20.

**Preparation and administration of doses**

The extract was solubilised in 0.5% Carboxy Methyl Cellulose prior to experimental use to obtain the desired concentrations (200 and 400mg/kg body weight) in 1ml. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 hrs.

**Anti-ulcer activity:**

*Pylorus ligation* induced Gastric Ulcers:

Rats were divided into four groups of six animals each21. All the animals selected for the study were of weight between 200-250 gms.

- **Group I** (control), received, 0.5% CMC.
- **Group II** (reference standard) was treated with 20mg/kg omeprazole.
- **Group III** treated with 400 mg/kg methanolic extract of *Solanum pubescens* wild.
- **Group IV** was treated with 200 mg/kg methanolic extract of *Solanum pubescens* wild.

Animals in all the groups were fasted for 36 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and *pylorus spolition* of stomach was lifted out and ligated22. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Four hours after the pyloric ligation, the animals were sacrificed by an excess dose of ether. The stomach was carefully removed and the gastric contents were collected. The gastric juice was centrifuged at 1000 rpm and gastric volume was measured23. Free and total acidities of the supernatant were determined by titration with 0.1N NaOH and expressed as mEq/L/100 gms. The stomach was cut open along the greater curvature and pinned onto a soft board for evaluating the gastric ulcers and t calculate ulcer index. Ulcer scoring is done according to the scale mentioned below.

**Ulcer Index:**

After the incision of the stomach at the greater curvature the ulcers were observed. And the number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using venire calipers24. The following arbitrary scoring system was used to grade the incidence and severity of lesions.

- Normal coloration - 0
- Red coloration - 0.5
- Spot ulcer - 1
- Hemorrhagic streaks - 1.5
- Ulcer - 2
- Perforation - 3

**Determination of Free Acidity and Total Acidity:**

The gastric contents were centrifuged at 1000 rpm for 10mins. 1ml of supernatant was diluted with 9ml distilled water. A volume of 2ml diluted gastric juice was treated with 0.1N sodium hydroxide run from a micro burette using 3-4 drops of Töpler’s reagent as indicator until a canary yellow colour was observed. The volume of NaOH run down was noted25. This corresponds to free acidity. Further, 2-3 drops of phenolphthalein was added and titrated with NaOH until pinkcolour was restored. This gives total acidity. Free acidity and Total acidity are expressed in terms of ml of 0.1N HCl per 100 gms of gastric contents. This is the same as mEq/lit. Acidity may be calculated by using the following formula:

\[
\text{Acidity} = \text{volume of NaOH} \times \text{Normality of NaOH} \times 1000 \text{ mEq/lit}
\]

**Histopathological Evaluation:**

The gastric tissue samples were fixed in neutral buffered formalin solution for duration of 24 hrs. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/or anti-ulcerogenic activity of methanolic extract of *Solanum pubescens*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor26,27. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes (P. Thirunavukkarasu et al.,2009).

**Statistical Analysis:**

Statistical analysis was carried out using Graph Pad Prism 5 software version 5.04 (Graph Pad prism software Inc.) The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet’s t-test. P values< 0.05 were considered significant28.

**RESULTS AND DISCUSSION**

The methanolic extract of *Solanum pubescens* wild was subjected to Phytochemical and pharmacological screening. Pharmacological screening involved the evaluation of anti-ulcer activity29. The results of the methanolic extract of *Solanum pubescens* has been documented below.

**Preliminary Phytochemical**

The colour of the Alcoholic test extract (methanolic extract of *Solanum pubescens* willd.) was found to be dark green in colour and the consistency was found to be sticky. The extracts were subjected to phytochemical screening for the presence of type of phyto-constituents30. The phytochemical screening revealed that methanolic extract contains resins, alkaloids, Phytosterols, Phenols and Flavonoids of the phytosterols, the ones identified were tri-terpenes. The results have been tabulated in the table 2 showing various phyto constituents in the extract.
Table 2: Preliminary phytochemical analysis of Extract

<table>
<thead>
<tr>
<th>Phyto constituents</th>
<th>Extracts</th>
<th>Petroleum ether</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phytosteroids</td>
<td></td>
<td>-</td>
<td>_</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein and amino acids</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td></td>
<td>-</td>
<td>_</td>
</tr>
<tr>
<td>Phenols &amp; tannins</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthaquinone Glycosides</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) represents Absence; (+) represents Presence

Acute toxicity Study:

Methanolic Extract of *Solanum pubescens* willd did not produce any toxic symptoms or mortality up to the dose level of 2000 mg/kg body weight. There was neither change in behavioral pattern or any sign of toxicity during the observations up to 24 hrs for mortality. Thus the extract was considered to be safe for pharmacological evaluation. Biological evaluation was carried out at doses of 200 and 400mg/kg.

Result: From acute toxicity study it was observed that the administration of Methanolic extract of *Solanum pubescens willd* to mice did not induce any toxicity of extract and mortality in the animal’s up to 2000mg/kg orally.

Pharmacological evaluation:

Evaluation of Anti-ulcer activity was carried out for the methanolic extract of the plant. Anti-ulcer was performed by Pylorus ligation induced ulcer.

Anti-Ulcer Evaluation:

Pylorus Ligation method:

In pyloric ligation induced ulcer model, Oral administration of MESP in two different doses (200 mg/kg and 400 mg/kg) showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. MESP was showing protection index of 52.3% and 36.28% at the dose of 400mg/kg and 200mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug a protection percentage of 70% has been observed. Since the ulcer protective percentage of MESP at 200mg/kg is 36.28% it can be considered to be less significant in the context of the study.

Table 3: Ulcer index and % protection

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer Index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I-Control</td>
<td>11.3±0.11</td>
<td>--</td>
</tr>
<tr>
<td>Group II Standard (omeprazole)</td>
<td>3.39±0.07***</td>
<td>70%</td>
</tr>
<tr>
<td>Group III - <em>S.pubescens willd</em> Extract 400mg</td>
<td>5.48±0.07***</td>
<td>52.3%</td>
</tr>
<tr>
<td>Group IV - <em>S.pubescens willd</em> Extract 200mg</td>
<td>7.2±0.06*</td>
<td>36.28%</td>
</tr>
</tbody>
</table>

All values represent Mean ± SEM, n=6 in each group. P <0.001. Control group is compared with standard and extract doses.
Gastric volume, Free Acidity and Total Acidity:
The results of various acid secretory parameters such as Gastric volume, pH, free acidity and Total acidity of methanolic extract of Solanum pubescens wildd on pylorus ligation induced gastric ulcer in rats are summarized in Table 4. Estimation of acid secretory parameters was increased significantly in the control group. Administration of MESP exhibited a significant (p < 0.001) reduction in all the parameters and the results were comparable with the standard drug Omeprazole 20mg/kg. In control group the mean gastric juice was 3.52ml. Omeprazole, the standard drug decreased the mean gastric volume (1.28ml), which is statistically significant. Apart from the standard, ethnologic extract also showed decrease in the mean gastric juice at both the doses of 400 and 200 mg/kg. The extracts reduced them gastric juice volume to 1.59ml and 1.86 ml respectively. The test extracts showed the decreased in gastric juice volume on comparison to control group and thus indicate their anti-secretary mechanism. This demonstrates the dose dependent effect of MESP.

Table 4: Gastric volume, pH, free acidity and Total acidity

<table>
<thead>
<tr>
<th>Group</th>
<th>Gastric Volume</th>
<th>pH</th>
<th>Free Acidity</th>
<th>Total Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.52±0.02</td>
<td>1.41±0.12</td>
<td>55.01±2.28</td>
<td>67.79±1.31</td>
</tr>
<tr>
<td>Std(Omeprazole)</td>
<td>1.28±0.05***</td>
<td>5.21±0.13***</td>
<td>20.90±0.76***</td>
<td>32.06±3.316***</td>
</tr>
<tr>
<td>Extract 400mg</td>
<td>1.598±0.03**</td>
<td>3.5±0.08***</td>
<td>29.86±0.77**</td>
<td>41.96±0.715***</td>
</tr>
<tr>
<td>Extract 200mg</td>
<td>6±0.04**</td>
<td>4.63±0.14***</td>
<td>39.66±1.14*</td>
<td>55.56±0.99***</td>
</tr>
</tbody>
</table>

All values represent Mean ± SEM, n=6 in each group. P <0.001. Control group is compared with standard and extract doses.
Graph 4 Comparison of Free Acidity of the Control, Standard and the Extract (400 and 200mg/kg) groups.

Graph 5: Comparison of Total Acidity of the Control, Standard and the Extract (400 and 200mg/kg) groups.

ULCER IMAGES (Pylorus Ligation):

Figure 1: Ulcer Images (Pylorus Ligation)
Histopathology examination:
The histopathological examination of stomachs of the rats showed a better picture of the gastric lesions and the damage occurred to the stomach mucosa\(^{36,37}\). Acute ulceration of stomach was observed in group-I, Group (A) (control group rats) show mucosal ulceration consisting of necrosis, cellular debris, neutrophils and degenerated epithelial cells Group-II (B) shows gastric mucosa with intact epithelium, lamina propria and muscularis mucosa\(^38\). Group (C) shows intact mucosa with plenty of regenerative epithelial cells Group (D) shows focal mucosal ulceration consisting of degenerated cells and also contains few regenerative epithelial cells.

**DISCUSSION**
In this study, the anti-ulcer activity of methanolic extract of *Solanum pubescens* willd has been studied. The anti-ulcer activity was evaluated against Pylorus ligated ulcers. Gastric ulcer disease is an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin). Ulcer caused by pylorus ligation is due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa and break down of the gastric mucosal barrier. The activation of the vagus- vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion.

Antiucler effect is supported by the decrease in the aggressive factors like gastric volume, decrease in free and total acidity and an increase in the resistance factors like pH showing the anti-secretary mechanism. It is significant to note when the pH was nearing 5.2 (Std) and 3.5 (Ext 400mg/kg), the ulcer score appeared less. The antulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandins. Prostaglandins have a vital protective role. The mucosal defense mechanism maybe due to the epithelial cells of the gastric mucosa, which are impermeable to H\(^+\) ions thereby forming a physical barrier.

The methonolic extract of *solanum pubescens* willd was evaluated by using pylorus ligation method, Oral administration of methanol extract of *solanum pubescens* willd at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 36.28% and 52.3% (p<0.001) respectively compared to the ulcer control, proving the anti ulcer activity. The standard drug omeprazole (20mg/kg) exhibited percentage inhibition of 70% when compared with ulcer control. Extract treated and ulcer control group was compared with normal control group.

**CONCLUSION**
The present study showed that the methonolic extract of *Solanum pubescens* willd leaves possess Anti ulcer in animal model. *Solanum pubescens* willd showed asignificant decrease in the ulcer development in the animal models (pylorus ligated model) used in the study. In pylorusligation, both the doses showed significant 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 MESP. Flavonoids and tannins are the major constituents that are present in the leaves of Solanum pubescens wild which may be responsible for its ulcer-protective and Anti-ulcer activity.

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**Author’s contribution:** CK Tyagi, Satendra Saha and Sunil Kumar Shah. Contributed equally to manuscript drafting, writing, data collection, conceptualization and observation. All authors read and approved the final version of the manuscript.

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**Conflicts of Interest:** The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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