Research Article

GASTRO PROTECTIVE AND ANTIOXIDANT EFFECT OF EUPHORBIA PROSTRATA AGAINST INDOMETHACIN INDUCED GASTRIC ULCERS IN HEALTHY ADULT MALE ALBINO RABBITS

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ABSTRACT

Euphorbia prostrata belongs to family Euphorbiaceae locally known as Doodhi is small annual herb. Current study was conducted to evaluate the gastro protective and antioxidant effectiveness of the aerial parts of E. prostrata in indomethacin induced ulcer in adult male albino rabbits. A total 36 mice were taken and divided into 6 equal groups. At day 0 and 14 of experiment the blood sample was collected from each animal and serum was separated from blood which was used for the determination of Total oxidant status (TOS), Total antioxidant capacity (TAC), catalase (CAT) and Malondialdehyde (MDA). At the end of the experiment, all of the rabbits were slaughtered. The gastric contents were collected to total acid output, gastric pH and gastric volume. To confirm the data results was subjected to one way analysis of variance (ANOVA). Results when compared with the extensive mucosal damage in the indomethacin trated group, gross evaluation revealed a marked protection of the gastric mucosa in the experimental groups, with significantly reduction in gastric pH, gastric volume and acid output. In these groups, CAT and TAC levels were significantly increased (P<0.05) which indicate reduction in gastric damage. In conclusion to these results E. prostrata has potent antioxidant properties at dose rate of 240 mg/kg and it is also very effective for the treatment of gastric ulcer at the same dose.

Keywords: Euphorbia prostrata, gastroprotective, Ranitidine, indomethacin

INTRODUCTION

Stomach is one of the essential parts of gastrointestinal tract that helps in digestion of different foods. Gastric mucosa is the layer that covers the stomach protecting it from various agents such as acid, pepsin and different drugs. Gastric mucosal layer can be damaged by the alteration between various offensive and defensive factors in the stomach. The offensive factors include the secretion of acid and pepsin, Helicobacter pylori infection and bile salts whereas the defensive factors includes the prostaglandins, mucus secretion, blood flow and cellular regeneration. Damaging of mucosa by these factors will lead towards the gastric ulcer. Gastric ulcer is a common gastrointestinal problem affecting the wide variety of people all over the world causing both morbidity and mortality. Other factors causing gastric ulcer includes stress, smoking, alcohol and improper or excessive use of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are the most widely used drugs in the treatment of inflammation reducing pain, fever, swelling and redness. These act by inhibiting the cyclo-oxygenase (COX) enzyme depressing both COX-1 and COX-2. COX enzyme is involved in the production of various types of prostaglandins that performs different functions of body such as regulating body temperature, causing inflammation, maintaining mucosal layer of stomach thus maintaining the proper blood flow and increasing the production of mucus and regulating kidney functions. COX-1 inhibition accounts for gastrointestinal and renal side effects whereas COX-2 inhibition produces therapeutic effects. Due to the inhibition of the COX enzyme specifically COX-1, prostaglandins production is decreased that leads towards the damaging of gastric mucosal layer producing gastric ulcer. Indomethacin belongs to the class of NSAIDs. It is an indole derivative that also acts by inhibiting the COX enzyme thus inhibiting the production of prostaglandins. It is used for the treatment of arthritis. It has both analgesic and anti-pyretic effects. It has ability to cause the gastric ulcer by the production of free radicals, inducing lipid peroxidation, inhibition of prostaglandins and leukocytic infiltration. E. prostrata is a small annual herb present all over the world. It belongs to the family Euphorbiaceae and is locally known as Doodhi. It contains flavonoids, saponins and tannins as phytochemical constituents. Many plants possessing saponins have been shown to contain gastroprotective properties in various experimental models. The property of these saponins is due to inhibition of gastric acid secretion and initiation of mucous membrane protective factors. Flavonoids are naturally occurring compounds possessing gastroprotective activity. Many plants containing flavonoids have been found to show antulcer properties. Due to low toxicity, flavonoids have a therapeutic value ideal for treating gastrointestinal diseases related to Helicobacter pylori infection. Keeping in view the phytochemical constituents of this plant it can be used to treat the gastrointestinal diseases.

MATERIALS AND METHODS

Drugs
Indomethacin (Indocin®) was given at the dose of 20 mg/kg to cause ulceration. The synthetic antulcer drug Ranitidine (zantac®) was purchased from GlaxoSmithKline Laboratories Limited Karachi, Pakistan and drug was given at the dose rate of 10 mg/kg body weight to the adult male albino rabbits once in a day for 14 days of experiment.

Plant Material
Plant of E. prostrata was collected from the premises of University of Agriculture Faisalabad and identified for authentication by plant Taxonomist, Department of Botany, University of Agriculture Faisalabad. The shade-dried aerial parts of plant were powdered by the use of mechanical grinder, passed through mesh sieve no. 200 and stored in airtight container in the pharmacology laboratory of
Experimental Animals and Feed
Thirty six male adult albino rabbits weighing 1.63 ± 0.25 kg were selected for the current study and divided into six equal groups. Animals were housed in experimental animal room, Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. Animals were housed at temperature 22 ± 2°C, humidity 65-70 % and a 12 hours light/dark cycle for a week before start of experiment and during experiment. Animals were provided with standard feed and water ad libidum. Further, the institutional ethical committee of University of Agriculture Faisalabad approved all procedures adopted in this study. Ethical Clearance No. for the manuscript is DGS / 36901-904 dated 21.12.2013

Experimental Design
All rabbits divided into groups and given different treatments as shown in Table 1.

Surgical Procedures
The animals were fasted for at least 6 hours before the surgical procedure. On the 14 day of experiment these animals were sacrificed, blood samples were collected and then the stomach of the animal was isolated with the help of sharp scissors. The stomach was cut along the greater curvature and the contents were collected into small tubes. These gastric contents were then centrifuged at 2000 rpm for 5 minutes. The supernatant was separated and its volume was expressed as ml/100 g body weight. This supernatant was used for the estimation of various biochemical parameters.

Blood Sampling
Blood samples were collected at 0 and 14 days. The samples were allowed to clot for 20 minutes at refrigeration temperature and then centrifuged for 5 minutes at 4000 rpm to separate serum. Serum was stored at -4 °C till the estimation of different antioxidant parameters.

Acid Output
The acid output was calculated by titrating the supernatant fluid with 0.05N NaOH. Acidity was expressed as mEq/L per 100 gram of body weight. The pH was measured with glass electrode. 

\[
\text{Acidity} = \left(\frac{\text{Volume of NaOH} \times \text{Normality}}{0.1}\right) \times 100
\]

Ulcer Scores
The number of ulcers was noted and the severity was determined by the following scores. Normal coloration (0.0), Red coloration (0.5), Spot ulcer (1.0), Hemorrhagic stress (1.5), Deep ulcers (2.0), Perforation (3.0).

Ulcer Index
The number of ulcers was noted and the severity was determined with the following scores: Ulcer index (UI) was calculated using the formula:

\[
\text{UI} = \frac{\text{US} + \text{UN} + \text{UP}}{10}
\]

Where, US = Mean severity of ulcer score. U
N = Average number of ulcers per animal.
UP = Percentage of animals with ulcer incidence

Curative Ratio
The curative ratio from the ulcer was calculated for the treated groups by using the following equation.

\[
\text{Percentage (\%)} = \frac{\text{CUI} - \text{TUI}}{\text{CUI}} \times 100
\]

Where, CUI = Ulcer index of control groups.
TUI = Ulcer index of treated groups

Biochemical Examination
Malondialdehyde (MDA) was determined according to the method developed by Ohkawa. Enzymatic activity of enzyme catalase (CAT) was measured by method of Goth. The total oxidant status (TOS) and total antioxidant capacity (TAC) in serum was measured by methods developed by Erel using spectrophotometer.

Statistical Analysis
The values was expressed as Mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) and statistical differences among different treatment groups was determined by Duncan’s Multiple Range test at 5 % level of significance.

RESULTS
Antulcer Evaluation Parameters
Results showed that mean values of ulcer scores were increased significantly (p < 0.05) in indomethacin treated group as 2.85 ± 0.11 while for Ranitidine treated group ulcer scores was 0.43 ± 0.14. Compared with ulcer control, highest dose of E. prostrate (240 mg/kg) showed significant reduction in ulcer score as 0.75 ± 0.11. The mean values of ulcer index for indomethacin treated group was highest as 3.78 ± 0.1 while it was significantly lower in Ranitidine treated group and experimental groups when compared with control. It has been noted from the result that the curative ratio was calculated for treated groups at 60, 120 and 240 mg/kg showed gastric protection of 44.8 %, 61.64 % and 67.43 % respectively shown in Table 2. It was also observed that pH was decreased significantly in indomethacin treated rabbit as 1.58 ± 0.05 while Ranitidine enhanced pH of gastric mucosa as 4.37 ± 0.80. Our test plant also increased the pH of gastric mucosa at the dose of 240 mg/kg as 4.07 ± 0.11. It was observed from the result that gastric volume also increased after the administration of indomethacin as 23.03 ± 0.38 while administration of Ranitidine and E. prostratea significantly (P < 0.05) decreased gastric volume 16.40 ± 0.33 and 17.00 ± 0.21 respectively. Total acid output in indomethacin treated rabbit was significantly increased as 27.19 ± 0.36 while Ranitidine and E. prostratea valuably decreased total acid output as 18.00 ± 0.04 and 18.44 ± 0.15 respectively presented in Table 2.

Biochemical Examination
Biochemical parameters were also measured in the current study. Results suggested that indomethacin significantly decreased the TAC and CAT levels while it caused a significant increased TOS and MDA activity. However administration of ranitidine significantly enhanced the TAC and CAT activity and caused a significant reduction in TOS and MDA levels as that of E. prostratea presented in Table 3.
The present study was carried out to investigate the gastroprotective effect of *E. prostrata* in healthy adult male albino rabbits where gastric ulcer was induced by oral administration of indomethacin that belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) and is used in the treatment of ankylosing spondilitis, osteoarthritis, rheumatoid arthritis, gout arthritis, bursitis, tendonitis, sinovitis, and other inflammatory diseases because of its effective suppression of pain, fever, color, and edema but it has highest potential to cause gastric damage. The mechanism of non-steroidal anti-inflammatory drugs (NSAIDs) in causing the gastric ulcer includes the non-selective inhibition of cyclo-oxygenase 1 (COX-1) and cyclo-oxygenase 2 (COX-2) enzymes which are responsible for the formation of prostaglandins. Inhibition of these two enzymes leads to the inhibition of formation of prostaglandins that ultimately leads to the damaging of gastric mucosa and thus produces gastric ulcer\(^23,24\). Prostaglandins protect the gastric epithelial lining by increasing the production of bicarbonate ions and balancing the blood flow towards gastric mucosa. Furthermore, prostaglandins are also responsible for increasing the secretion of gastric mucus. Secretion of gastric mucus can also be increased by nitric oxide and cholinergic mediators\(^25\). The imbalance in the defensive factor due to offensive factor leads towards the formation of ulcer in gastric lining. Different types of synthetic drugs or agents are used for the treatment of gastric ulcer now-a-days. These agents include proton pump inhibitors (omeprazole and lansoprazole), histamine receptor blocking agents (ranitidine, famotidine) and cimetidine, antacids and parasympathetic blocking agents such as pirenzipine and telzepine\(^26,27\). Among all these agents, proton pump inhibitors and H\(_2\) receptor blockers are most commonly used agent to cure gastric ulcer. Although all of these agents are effective but they may be associated with different types of side-effects\(^28\). Owing to the various adverse effects associated with almost all of these drugs, it is therefore necessary to establish more effective and safe therapy for managing gastric ulcer. So, focus is now diverted towards the use of medicinal plants in curing gastric ulcer as these have less toxic effects as compared to synthetic drugs\(^29\). Phytochemical screening of *E. prostrata* revealed that it contained flavonoids, saponins and tannins and these constituents have gastroprotective activity. This phytochemical conformation of *E. prostrata* could elucidate the gastroprotective activity of the aerial parts of plant which was identified in our study. In order to evaluate the gastroprotective effect various ulcer parameters were determined that are gastric pH, total acid output, gastric volume, ulcer scores, curative ratio and ulcer index. Normally stomach pH is 2.5-3 but after the administration of indomethacin it significantly reduced to 1.58 shown in Table 1. Enhanced reduction in pH leads towards ulcer. Over production of histamine contribute the increased secretion of gastric juice and concentration of hydrogen ions in the juice decreased that reflect to the decreased pH that is also an

### DISCUSSION

Table 1: Feeding and drug Administration schedule in rabbits during the experimental period of 0-14 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Control</td>
<td>Routine diet only (0-14 days)</td>
</tr>
<tr>
<td>Group 2: Untreated control on indomethacin 20 mg/kg</td>
<td>Routine diet + indomethacin for 0-14 days</td>
</tr>
<tr>
<td>Group 3: Treated control on synthetic antulcer drug tablet Zantac® (Ranitidine) 10 mg/kg orally</td>
<td>Routine diet + indomethacin + Zantac® (Ranitidine) (0-14 days)</td>
</tr>
<tr>
<td>Group 4: Treated on <em>E. prostrata</em> powder 60 mg/kg orally</td>
<td>Routine diet + indomethacin + <em>E. prostrata</em> powder (0-14 days)</td>
</tr>
<tr>
<td>Group 5: Treated on <em>E. prostrata</em> powder 120 mg/kg orally</td>
<td>Routine diet + indomethacin + <em>E. prostrata</em> powder (0-14 days)</td>
</tr>
<tr>
<td>Group 6: Treated on <em>E. prostrata</em> powder 240 mg/kg orally</td>
<td>Routine diet + indomethacin + <em>E. prostrata</em> powder (0-14 days)</td>
</tr>
</tbody>
</table>

Table 2: Mean ± SE values of ulcer score, pH, gastric volume, acid output, ulcer index and curative ratio after the 14 days of treatment with per oral drugs and *E. prostrata* powder in rabbits

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>indomethacin</th>
<th>Ranitidine</th>
<th><em>E. prostrata</em></th>
<th><em>E. prostrata</em></th>
<th><em>E. prostrata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Routine diet</td>
<td>20 mg/kg</td>
<td>10 mg/kg</td>
<td>60 mg/kg</td>
<td>120 mg/kg</td>
<td>240 mg/kg</td>
</tr>
<tr>
<td>Ulcer score</td>
<td>0.16 ± 0.10</td>
<td>2.85 ± 0.11*</td>
<td>0.43 ± 0.14</td>
<td>2.16 ± 0.16*</td>
<td>1.5 ± 0.12*</td>
<td>0.75 ± 0.11</td>
</tr>
<tr>
<td>Ulcer index</td>
<td>0</td>
<td>14.07 ± 0.1</td>
<td>7.38 ± 0.1</td>
<td>7.35 ± 0.23</td>
<td>5.40 ± 0.1</td>
<td>4.27 ± 0.08</td>
</tr>
<tr>
<td>% Curative ratio</td>
<td>0</td>
<td>0</td>
<td>73.24</td>
<td>44.8</td>
<td>61.64</td>
<td>67.43</td>
</tr>
<tr>
<td>Gastric volume (ml/100 g)</td>
<td>16.83 ± 0.0</td>
<td>23.03 ± 0.38*</td>
<td>16.40 ± 0.33</td>
<td>21.72 ± 0.42*</td>
<td>20.34 ± 0.12*</td>
<td>17.00 ± 0.21</td>
</tr>
<tr>
<td>Gastric pH</td>
<td>2.97 ± 0.06</td>
<td>1.58 ± 0.05*</td>
<td>4.37 ± 0.11*</td>
<td>2.47 ± 0.13</td>
<td>3.04 ± 0.09*</td>
<td>4.07 ± 0.11*</td>
</tr>
<tr>
<td>Acid output (mEq/L/100 g)</td>
<td>18.73 ± 0.21</td>
<td>27.19 ± 0.36*</td>
<td>18.00 ± 0.04</td>
<td>24.33 ± 0.27*</td>
<td>21.55 ± 0.27*</td>
<td>18.44 ± 0.15</td>
</tr>
</tbody>
</table>

Values are mean ± SE; (n = 6) *p < 0.05 when compared with control

Table 3: Mean ± SE values of TAC, TOS, MDA and CAT after the 14 days of treatment with per oral drugs and *E. prostrata* powder in rabbits

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>indomethacin</th>
<th>Ranitidine</th>
<th><em>E. prostrata</em></th>
<th><em>E. prostrata</em></th>
<th><em>E. prostrata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Routine diet</td>
<td>20 mg/kg</td>
<td>10 mg/kg</td>
<td>60 mg/kg</td>
<td>120 mg/kg</td>
<td>240 mg/kg</td>
</tr>
<tr>
<td>TAC (nmol/L)</td>
<td>1.62 ± 0.00</td>
<td>0.97 ± 0.2*</td>
<td>1.58 ± 0.00*</td>
<td>1.18 ± 0.02*</td>
<td>1.34 ± 0.01*</td>
<td>1.52 ± 0.01*</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td>4.08 ± 0.07</td>
<td>10.45 ± 0.01*</td>
<td>5 ± 0.06*</td>
<td>9.23 ± 0.04*</td>
<td>8.12 ± 0.05*</td>
<td>5.32 ± 0.08*</td>
</tr>
<tr>
<td>MDA (nmol/L)</td>
<td>3.27 ± 0.04</td>
<td>10.36 ± 0.04*</td>
<td>4.82 ± 0.38*</td>
<td>9.04 ± 0.26*</td>
<td>7.91 ± 0.08*</td>
<td>5.30 ± 0.22*</td>
</tr>
<tr>
<td>CAT (KU/L)</td>
<td>7.47 ± 0.12</td>
<td>3.58 ± 0.13*</td>
<td>7.86 ± 0.09*</td>
<td>4.93 ± 0.06*</td>
<td>6.03 ± 0.10*</td>
<td>7.41 ± 0.13*</td>
</tr>
</tbody>
</table>

Values are mean ± SE; (n = 6) *p < 0.05 when compared with control
offensive factor. Similarly indomethacin caused remarkably increased in ulcer total acid output, gastric volume, ulcer scores, and ulcer index. The gastric ulcer induced by indomethacin through different processes comprising enhancement of leukocyte infiltration in gastric mucosa that damages gastric epithelial lining. Enhancement of leukocyte infiltration increases peroxidation of lipids and cause the formation of reactive oxygen species that leads towards rupturing of gastric epithelial lining\(^{30}\) by inducing \(H^+ / K^+\) ATPase in gastric mucosal cells. It also reduces the PGE2 level due to which gastric lesion are produced in gastric mucosa furthermore due to reduction in level of PGE2 neutrophil infiltration occurs tumor necrosis factor (TNF-\(\alpha\)) is also increased which further lead to the gastric damage\(^{31,32}\). After the administration of synthetic drug ranitidine cause considerably increased in \(\text{pH}\), and decreased gastric volume, total acid output, ulcer scores and ulcer index. This reduction in the ulcer parameters occurs due to the ability of ranitidine to inhibit the binding of histamine on the parietal cell\(^{32}\). Highest dose of plant \(E.\ prostrata\) (240 mg/kg) showed similar result to that of ranitidine. It also causes significant reduction in all above parameters. This result showed that \(E.\ prostrata\) has gastroprotective effect. Our results coinside with may other studies\(^{33,35}\). In addition to ulcer parameters different types of biochemical parameters or oxidative health biomarkers were also determined in the present study including total oxidant status, malondialdehyde, total antioxidant capacity and CAT were also determined in the current study. It has been concluded from the results that when indomethacin was given at a dose rate of 20 mg/kg, total oxidant status and malondialdehyde were enhanced significantly while total antioxidant capacity and CAT activity were reduced significantly. Indomethacin encourages the reactive oxygen metabolites that may play a role in gastric damage. These reactive species cause damage to the biochemical markers e.g. lipid and increased the production of free radicals that increased MDA production and decreased CAT and these free radicals production also because of impairment of cellular enzyme that involve in defensive mechanism of gastric ulcer such as total antioxidant capacity and CAT activity\(^{36}\). On the other hand, administration of ranitidine along with indomethacin significantly reduced total oxidant status and malondialdehyde and increased the catalase activity. Mean \(\pm\) SEM values of total oxidant status, malondialdehyde and catalase. It has also been revealed from the results that on administration of powder of aerial parts of \(E.\ prostrata\) at various doses of 60, 120 and 240 mg/kg significantly reduced total oxidant status and malondialdehyde while it increased the CAT activity. Moreover, highest dose of \(E.\ prostrata\) at 240 mg/kg in rabbits have similar effects to that of ranitidine. \(E.\ prostrata\) has cytoprotective effect due to the presence of phenolic substances such as ellagic acid and gallic acid that are responsible for its anti-inflammatory activity and the decreased the release of histamine\(^{37}\). These phenolic compounds also have property of mucoprotection. Phytochemical screening, further investigate that phenolic and flavonoids substances have highest free radical scavenging and antioxidant property\(^{38,39}\). From various results of the present study, it can be concluded that \(Euphorbia prostrata\) is having the gastroprotective activity due to presence of different mechanisms. Results also suggested that \(E.\ prostrata\) can be as effective as synthetic antiulcer drug ranitidine regarding its gastroprotective activity as similar results have been observed. Indomethacin possesses different mechanisms that accounts for its ulcerogenic activity.

**CONCLUSION**

\(E.\ prostrata\) extract significantly enhanced the TAC and CAT activity comparable to synthetic antiulcer drug ranitidine while it caused a significant reduction in TOS and MDA levels. Results of the study revealed that extract of \(E.\ prostrata\) at 60, 120 and 240 mg/kg showed gastric protection of 44.8 %, 61.64 % and 67.43 % respectively.

**REFERENCES**


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