Protective Effect of Solanum indicum Var. Distichum Extract on Experimentally Induced Gastric Ulcers in Rat

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Abstract: The anti-ulcerogenic effects of Solanum indicum var. distichum alcoholic extract on aspirin and ethanol induced ulceration in rats with respect to antioxidant status in the gastric mucosa have been investigated. Animals were divided into two main classes, alcohol injury group and aspirin injury group and then each of them was classified into seven subgroups including controls administered saline or extract only without injury as well as injury groups with pretreatment with extract at 10% LD₅₀ or 25% LD₅₀. The inhibition of lipid peroxides, which were highly elevated in rats with acute gastric mucosal injury, was taken as an index of oxidative stress. The activities of antioxidant defense enzymes were decreased considerably by oral gastric administration of aspirin and ethanol. The decreased levels of antioxidant biomarkers were altered to be near normal status upon pretreatment with S. indicum extract (SIE) when compared to the ulcer induced rats. S. indicum extract may exert its gastroprotective effect by a free radical scavenging action. In histopathological study, aspirin or alcohol caused a marked damaging effect on the stomach in the form of gastric mucosal ulceration with deformation of the gastric glands structure, hemorrhage and dilatation of blood vessels, while plant extract as a protecting agent caused restoration of normal structure of gastric mucosa occurred, being more observed with the larger concentration which showing complete regeneration of mucous secreting cells with quite normal distribution of mucopolysaccharides in the tissue. Our observations suggest that the current extract may have considerable prophylactic effect in the treatment of gastric ulcer.

Key words: Antioxidants • Alcohol ulcer • Aspirin • Gastric ulcer • Solanum indicum

INTRODUCTION

Ulcers are caused due to imbalance between aggressive and defensive factors of the gastric mucosa. Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defense mechanism [1]. Ethanol induces ulcers by the reduction of gastric mucosal blood flow and mucus production in the gastric lumen, this leads to a decrease in endogenous glutathione and prostaglandins levels and increase of ischemia, gastric vascular permeability, generation of free radicals and production of leukotrienes. It had been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration [2] and scavenging these free radicals can play an appreciable role in healing these ulcers. Ethanol reduces the cysteine, which is required for glutathione synthesis, thereby depleting glutathione level which is important for the maintenance of mucosal integrity and depletion of glutathione from the gastric mucosa induces macroscopic mucosal ulceration [1]. Aspirin injures gastrointestinal mucosa and oxygen-derived free radicals mediate injury of this mucosa therefore oxy-radicals may play a pathogenetic role in
the evolution of aspirin – induced erosive gastritis [3]. The present study was conducted to evaluate the protective effect of *S. indicum var. distichum* in terms of its antioxidant status on aspirin and ethanol induced gastric mucosal damage.

**MATERIALS AND METHODS**

**Plant Material:** *Solanum indicum* was identified by herbarium of Orman Botanical Garden. *Solanum* fruits were collected from the aerial parts of the plant growing in wild and then were air dried under shade at ambient temperature, ground to small granules and subjected to sequential soxhlet extraction with petroleum ether and aqueous methanol (70%). The methanol extract thus obtained was dried under reduced pressure by rotary evaporator to be free from any alcoholic residues. The concentrated aqueous methanolic extract was triturated in normal saline for rats' oral administration.

**Animals, Housing and Experimental Design:** The acute toxicity test for the plant extract was carried out to evaluate any possible toxicity. Male albino mice (n=8) were tested by administering different doses of the extract by increasing or decreasing the dose, according to the response of animal [4]. The dosing patron was 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 mg/kg body weight, while the control group received only the normal saline. All groups were observed for any gross effect or mortality during 48h. Death of half of examined animals was observed at 3000 mg/kg b.wt.

**Anti-ulcer Effect of *Solanum indicum* Extract on Alcohol Induced Gastric Mucosal Injury:** Adult male albino rats weighing 150-180g were obtained from animal house of National Research Centre. Each kedge was contained six male albino rats and they were feed on standard diet and maintained under standard laboratory condition, temperature through the housing was controlled at 24°C; relative humidity was 65± 5% with light/ dark cycles (12/12 h). The dose was selected on basis of acute toxicity. Plant extract was employed at oral dose as aqueous suspension using distilled water. Rats were divided into seven groups each of six rats; group I was normal control was given 1ml normal saline orally (NC), group II received plant extract at 300 mg/kg b.wt (SIE1 at 10% LD₉₀), group III received plant extract at 750 mg/kg (SIE2 at 25% LD₉₀), group IV received 1ml absolute alcohol/100g b.wt orally to induce gastric ulcer [5], group V received anti ulcer drug in a recommended dose, (famotin at 20 mg/kg) [6], group VI received 1ml alcohol orally with SIE1treatment one hour before and after administration [7], group VII received 1ml alcohol orally with treatment with SIE2, one hour before and after administration. Rats were sacrificed; their stomach were excised and opened along the greater curvature. Gastric mucosal tissue was taken from the antral portion of the stomach for biochemical estimations. The gastric mucosa was scrapped with a scraper, homogenized in ice cold phosphate buffer (pH 7.2) to prepare the mucosal homogenate. Homogenates were centrifuged at 3000 rpm for 10 min and the supernatants were used for further studies.

**Anti-ulcer Effect of *Solanum indicum* Extract on Aspirin Induced Gastric Mucosal Injury:** The animals were fasted for 48 hrs [8] prior to experiment but water was permitted. Group I received saline, group II received SIE1 for seven days, group III received SIE2 for seven days, group IV received aspirin at 400 mg/kg suspended in 0.5% carboxy methyl cellulose, group V received aspirin with pretreatment of SIE1 for seven days and group VI received aspirin with pretreatment of SIE2 for seven days. After the experimental period rats were scarified after anesthesia and their stomachs were opened along the greater curvature after four hours of aspirin administration.

The gastric tissue was fixed in buffered formalin for histopathological study while gastric mucosal tissues were taken and prepared as mentioned above for biochemical estimations. The reduced glutathione level in the stomach tissue was determined according to the method of Ellman [9]. Gastric superoxide dismutase (SOD) activity was estimated by the method of Kakkar et al. [10]. Catalase (CAT) activity was measured by following decomposition of H₂O₂ according to the method of Beers and Sizer [11]. Glutathione reductase activity was measured spectrophotometrically at 340nm [12] and the amount of the enzyme reducing 1µmol GSSG per min per mg protein was regarded one activity unit as elsewhere described. The TBARS level, an index of malondialdehyde (MDA), production was determined by the method of Ohkawa et al. [13]. The protein content was determined by the method of Bradford [14].

**Histopathological Study:** Specimens of stomach from all animals were dissected immediately after death, then opened along the greater curvature and washed thoroughly with distilled water to remove their contents to avoid digestion of upper layers of gastric mucosa by
digestive enzymes. All the specimens were fixed in 10% neutral-buffered formal saline for 72 hours at least, washed in distilled water and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 6µm thick were cut and stained with Haematoxylin and eosin [15] for histopathological investigation. Histochemical investigation using Periodic acid Schiff’s reagent [16] was performed to evaluate the mucopolysaccharide content in these tissues. Images were captured and processed using Adobe Photoshop version 8.0.

**RESULTS AND DISCUSSION**

**Protective Effect of *Solanum indicum* Extract on Gastric Antioxidant Parameters in Ethanol Induced Gastric Mucosal Injury Model:** Table 1 show that administration of *S. indicum* extract with tested doses significantly enhanced all assessed antioxidant enzyme activities, SOD, GR and CAT and glutathione concentration as compared to control group while ethanol significantly reduced all estimated parameters except lipid peroxidation which increased significantly by ethanol administration. Pretreatment with SIE1 significantly enhanced glutathione production in mucosa to reach 22.95 mg/g tissue after ethanol treatment which decreased it to be 9.9 mg/g tissue, while it was highly induced by SIE2 to reach the maximum recorded level (29.6 mg/g tissue) although famotin treatment enhanced glutathione production (15.2 mg/g) but is remained lower than SIE treatment. At the same time, famotin and SIE2 show the same level of enhancement for GR and SOD while SIE2 magnified CAT activity to reach the maximum recorded activity (20.95 U/mg protein). The ameliorative effect of SIE1 or SIE2 on all recorded antioxidant parameters occurred in decreasing level of lipid peroxides concentration and the SIE2 was the best one as compared to famotin or untreated groups.

Generally, administration of ethanol decreased GSH conc. and all determined enzyme activities, SOD, GR and CAT with elevation in lipid peroxides conc. in gastric mucosa while administration of famotin and pretreatment with SIE1caused significant increments in SOD, GR and CAT activities that caused significant reduction in lipid peroxides conc., but they remained lower than the pretreatment of SIE2 effect. Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals [17]. The process of lipid peroxidation is mediated by the interaction of hydroxyl radicals with the cell membrane; subsequently producing lipid-derived free radicals such as conjugated dienes and lipid hydroperoxides. These radicals are known to be extremely reactive products that cause oxidative damage [18]. Long-term stimulus with the low ethanol concentration may cause the impairment of the cell turnover function of the gastric mucosa and may be one of the mechanisms underlying the gastric pathology associated with alcohol abuse [19].

**Table 1:** Effect of *Solanum indicum* extract on antioxidant parameters in ethanol induced gastric mucosal injury model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutathione concentration</th>
<th>Superoxide dismutase</th>
<th>Glutathione reductase</th>
<th>Catalase U/min/mg protein</th>
<th>Lipid peroxides µmol/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>15.46±0.58</td>
<td>39.16±0.65</td>
<td>3.52±0.33</td>
<td>28.35±0.53</td>
<td>7.14±0.51</td>
</tr>
<tr>
<td>SIEI(300 mg/kg)</td>
<td>16.99±0.11</td>
<td>38.63±0.76</td>
<td>6.81±0.24</td>
<td>29.03±0.82</td>
<td>6.56±0.38</td>
</tr>
<tr>
<td>SIEII(750 mg/kg)</td>
<td>21.02±0.59</td>
<td>39.56±0.56</td>
<td>7.26±0.17</td>
<td>32.02±0.49</td>
<td>5.67±0.31</td>
</tr>
<tr>
<td>Ethanol vehicle</td>
<td>9.89±0.37</td>
<td>15.29±0.54</td>
<td>1.16±0.16</td>
<td>6.85±0.16</td>
<td>17.09±0.43</td>
</tr>
<tr>
<td>Ethanol+ famotin</td>
<td>15.18±0.39</td>
<td>24.19±0.64</td>
<td>2.73±0.38</td>
<td>11.89±0.15</td>
<td>9.79±0.30</td>
</tr>
<tr>
<td>Ethanol+ SIE1</td>
<td>22.95±0.14</td>
<td>21.48±0.57</td>
<td>1.95±0.002</td>
<td>15.07±0.56</td>
<td>7.99±0.01</td>
</tr>
<tr>
<td>Ethanol + SIE2</td>
<td>29.55±0.52</td>
<td>23.99±0.57</td>
<td>2.39±0.10</td>
<td>20.95±0.67</td>
<td>6.60±0.25</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D. a statistically significant p<0.05 & p<0.01

Groups have the same letter b or c in each parameter indicates no significant difference between them
Table 2: Effect of Solanum indicum extracts on the gastric antioxidant parameters in aspirin ulcer model

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutathione concentration mg/g tissue</th>
<th>Superoxide dismutase U/mg protein</th>
<th>Glutathione reductase µmol/min/mg protein</th>
<th>Catalase U/min/mg protein</th>
<th>Lipid peroxides µmol/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.95±3.83</td>
<td>38.61±0.437</td>
<td>5.55±0.38</td>
<td>28.35±0.53</td>
<td>6.87±0.23</td>
</tr>
<tr>
<td>SIE1</td>
<td>16.99±10.06</td>
<td>38.63±0.76</td>
<td>6.81±0.24</td>
<td>29.03±0.82</td>
<td>6.56±0.38</td>
</tr>
<tr>
<td>SIE2</td>
<td>21.02±0.59</td>
<td>39.56±0.56</td>
<td>7.26±0.17</td>
<td>32.02±0.49</td>
<td>5.67±0.31</td>
</tr>
<tr>
<td>Aspirin(400 mg/kg)</td>
<td>8.87±0.22</td>
<td>14.07±0.26</td>
<td>1.67±0.36</td>
<td>6.85±0.16</td>
<td>38.62±1.128</td>
</tr>
<tr>
<td>Aspirin + famotin</td>
<td>10.89±0.16</td>
<td>28.88±0.67</td>
<td>5.19±0.36</td>
<td>13.89±0.15</td>
<td>10.94±0.64</td>
</tr>
<tr>
<td>Aspirin + SIE1</td>
<td>15.77±0.21</td>
<td>29.96±0.34</td>
<td>3.54±0.32</td>
<td>17.07±0.56</td>
<td>11.57±0.97</td>
</tr>
<tr>
<td>Aspirin + SIE2</td>
<td>20.09±0.60</td>
<td>37.02±0.68</td>
<td>4.98±0.10</td>
<td>22.95±0.67</td>
<td>8.53±0.39</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D. a statistically significant p< 0.05 & p< 0.01
Groups have the same letter b or c in each parameter indicates no significant difference between them

Narcotizing agents such as ethanol, when given intragastrically to rats produce severe gastric hemorrhagic erosions. Oxygen free radicals are implicated in the pathogenesis of ethanol-induced gastric mucosal injury apart from other mechanisms such as mucosal leukotriene release, submucosal venular constriction. Ethanol-induced gastric injury is associated with the significant production of free radicals leading to increased lipid peroxidation [20] which causes damage to cells and their membranes. Accumulation of activated neutrophils in the gastric mucosa may be a source for free radicals. The ethanol-induced gastric mucosal damage was shown to be associated with the significant reduction in the non-protein sulphydryl concentration in cultured rat gastric mucosa cells [21].

**Effect of Solanum indicum Extracts on the Gastric Antioxidant Parameters in Aspirin Induced Gastric Ulcer Model:** Ulcer induction in aspirin model induced a marked reduction in the level of GSH and the activities of SOD, GR and CAT with elevation in TBARS level, while Solanum indicum extract treatment produced significant increments in GSH level and all determined enzyme activities with significant reduction in TBARS level (Table 2). Pretreatment with SIE1 and SIE2 aspirin induction considerably enhance SOD, GR and CAT activities with marked reduction of lipid peroxide concentration in mucosal tissue. SIE2 has the same data trend of famotin and they reach nearly the same values with GR and lipid peroxide conc., while SIE2 was superior than famotin with SOD (37&28 U/mg protein, respectively), CAT (22.9&13.9 U/min/mg protein, respectively) and GSH conc. (20 & 10 mg/g tissue, respectively).

Aspirin induces the reactive oxygen metabolites in animal models, which may contribute to mucosal injury [22]. The oxygen derived free radicals play a key role in the mechanism of aspirin induced acute gastric mucosal injury [23]. Administration of aspirin significantly decreased GSH concentration with significant decline in SOD, GR and CAT activities in gastric mucosa with significant increment in lipid peroxide. The potential antioxidant protective effect of (SIE) on gastric mucosal tissue is topic of high current interest. Many reports have demonstrated that most injury of gastric mucosa can be reduced by pretreatment with scavengers of reactive oxygen species [24]. Solanum extract protective effects were observed with two oral doses, SIE1 & SIE2. They offer gastroprotection against aspirin induced ulcer by significantly blocking lipid peroxidation. This effect may be due to scavenging activity of this extract.

Administration of famotin ameliorated all determined antioxidant parameters with significant reduction in lipid peroxides conc. to reach level lower than vehicle group. The same trend of data was recorded with SIE1 and SIE2 to reach values near to famotin and/or control groups, especially when animals were treated with high dose of Solanum extract. Oxygen handling cells have antioxidant enzymes such as CAT, SOD, GST and GPX decomposing O₂ and H₂O₂ production before they interact to form more reactive (OH) radicals [25]. SOD mainly act by quenching of superoxide (O₂⁻) and active oxygen free radical, produced in different aerobic metabolism [25]. Both SOD and CAT enzymes are highly specific in their catalytic mode of actions and it decreases the gastric mucosal damaging effect of aspirin [26]. Catalase is necessary for effective antioxidant activity [27]. The changes in CAT activity were significant with SIE1 and SIE2 may increases its protective effect through increases of antioxidant activity.
Fig. 1: (a) is a photomicrograph of a section of gastric mucosa of a control rat showing its normal structure. Notice the mucous secreting cells (arrow head) on the upper surface, lining the gastric pits and at the junction of the upper two thirds below the pits. (b & c) are two photomicrographs of sections of gastric mucosa of rats received (SIE1 & SIE2 respectively) showing quite normal architecture. (d) is a photomicrograph containing three sections of gastric mucosa from rat subjected to aspirin showing- in the left part- detachment of the upper mucous secreting cells with a portion of the fundic glands below (arrow) forming a deep notch (ulceration). The middle part shows marked hemorrhage (arrow head) with deformity in the tissue around in the middle of the gastric mucosa. The right part shows marked dilatation and congestion in the blood vessels (wavy arrow) with increased thickness of the muscularis mucosa layer. Diffuse cellular infiltration is observed at the bottom of the mucosa. (e) are two photomicrographs of gastric mucosa from rat subjected to aspirin and received SIE1, showing -in the left part- reformation of the architecture of the tissue, although, slight dilatation and congestion of blood vessels (arrow head) with mild cellular infiltration (arrow) are still present. The right part of the figure shows the same findings without blood vessels dilatation and congestion. (f) is a photomicrograph of gastric mucosa from a rat subjected to aspirin and received SIE2 showing complete regeneration of mucous secreting cells (arrow head) on the surface of the tissue. However, cellular infiltration (arrow) and a few vacuolar degenerated cells (v) are also noticed. (Hx. & E. x 100).

Fig. 2: (a) is a photomicrograph of a section of gastric mucosa from a control rat stained with Periodic acid Schiff’s reagent (PAS) to demonstrate the normal distribution of mucopolysaccharides in this tissue being concentrated on the upper surface and in the cells lining the gastric pits (arrow head). (b & c) are two photomicrographs of sections of gastric mucosa from rats received (SIE1 & SIE2 respectively) stained with (PAS) showing more or less normal distribution of mucopolysaccharides in the tissue. (d) is a photomicrograph of a section of gastric mucosa from a rat subjected to aspirin stained with (PAS) showing marked decrease of the intensity of the stain all over the upper surface of the tissue with complete negative result at the area showed detachment of the upper third of the mucosa (arrow) that was described in Hx. & E. section. (e) is a photomicrograph of gastric mucosa from rats subjected to aspirin and received SIE1, showing marked positive result of the stain specially in the gastric pits, but with discontinuous positive result on the upper surface of the tissue. (f) is a photomicrograph of gastric mucosa from rats subjected to aspirin and received SIE2, showing quite normal distribution of mucopolysaccharides in the tissue. (PAS x 100).
Fig. 3: (a) is a photomicrograph of two sections of gastric mucosa from rats subjected to ethanol showing- in the upper part- complete loss of upper mucous secreting cells (arrow). Marked deformity of the upper two thirds of the mucosa together with severe degeneration of many cells is observed. The lower half of the figure shows the same findings in addition to focal areas of hemorrhage in the bottom of the gastric mucosa (arrow head). (b) is a photomicrograph of two sections of gastric mucosa from rats subjected to ethanol and received famotin showing regeneration of the gastric mucosal tissue, although, some cells still show vacuolar degeneration (arrow). (c) is a photomicrograph of two sections of gastric mucosa from rats subjected to ethanol and received SIE1 showing amelioration of the ethanol-induced damage in the gastric mucosa. However, mild cellular infiltration at the bottom of the mucosa (arrow) and incomplete regeneration of the mucous secreting cells on the upper surface are seen. (d) is a photomicrograph of two sections of gastric mucosa from rats subjected to ethanol and received SIE2 showing restoration of the normal structure of the gastric mucosa specially in the mucous cells on the surface (arrow). (Hx. & E. x 100).

Fig. 4: (a) is a photomicrograph of a section of gastric mucosa from a rat subjected to ethanol stained with (PAS) showing complete negative result of the stain over a wide area of the upper surface of the mucosa (arrow head) with degeneration of many cells in the fundic glands (arrow). (b) is a photomicrograph of a section of gastric mucosa from rats subjected to ethanol and received famotin showing more or less normal distribution of mucopolysaccharides. (c) is a photomicrograph of gastric mucosa from rats subjected to ethanol and received SIE1, showing moderate positive result of PAS stain in the gastric pits and on the upper surface. (d) is a photomicrograph of gastric mucosa from rats subjected to ethanol and received SIE2, showing restoration of the normal distribution of mucopolysaccharides in gastric mucosa. (PAS x 100)
Hence the antioxidant activity of extract may be one of the important defensive factors involved in its ulceroprotective effect. The role of GSH as an endogenous gastric antioxidant in mucosal protection, however, remains controversial since recent evidence has indicated the positive correlation between gastric mucosal GSH levels and mucosal protection against ulcer in two models. Many studies have proved that antioxidants may play an important role not only by protecting against gastric mucosal injury, but also by inhibiting progression of gastric ulcer [28].

**Effect of Solanum indicum Extract on Stomach Histology in Aspirin and Ethanol Models:** Using Aspirin caused a marked damaging effect on the stomach in the form of ulceration of the gastric mucosa as noticed from shedding the upper surface epithelial layer with deformation of the structure of gastric glands, hemorrhage and dilatation of blood vessels. Using Solanum extract as a protecting agent gave very good results as restoration of normal structure of gastric mucosa occurred, being more observed with the higher concentration, SIE2. These results were confirmed by using PAS stain that showed aspirin caused loss of mucus secreting cells on the surface while using SIE, gastric mucosa regained its positivity to PAS stain denoting a protective and a stimulating effect of the extract on gastric mucosa. Using ethanol caused a dramatic damage to gastric mucosa, where complete deformation of the upper two thirds of the tissue, severe degeneration of functional cells and hemorrhage were noticed. Famotin as a protective agent caused regeneration of the gastric mucosal tissue, although, vacuolar degeneration of some cells was remained seen. On the other hand, Solanum extract as a protective agent, gave better results especially with the higher concentration. Histochemical examination for the mucopolysaccharides confirmed these results.

The results on histopathology of gastric mucosa showed ethanol induced congestion, hemorrhage, edema, erosions and necrosis also it induced depletion of protein gastric levels and increase content of MDA with depletion of GSH concentrations and increase in the contents of free radicals mediate tissue injury by stimulating lipid peroxidation and membrane damage by cross-linking proteins, lipids and nucleic acids, these results are inaccordance with those of Al-Shabana et al. [29]. Our results are in accordance with those reported by many scientists on Solanum genus [30] stated the hepatoprotective and antioxidant activities of S. trilobatum extract in the experimental rat model; it increased CAT, SOD and GPx activities which led to the recovery of these levels to near normal levels. Jainu and Devi [31] concluded that Solanum nigrum increased the same enzymes in aspirin induced gastric mucosal injury model. Al-Qirim et al. [32] reported that extract of Solanum nigrum can be used as prophylactic agent or curative agent in preventing/combating oxidative stress generated due to various diseases, it increased SOD, CAT and glutathione transferase activities with decreasing lipid peroxides.

**CONCLUSION**

In conclusion, it can be said that Solanum indicum var. distichum extracts exhibit a protective effect through free radical scavenging properties and reduces oxidative damage caused by aspirin or ethanol.

**REFERENCES**


