INVESTIGATION OF THE BIOCHEMICAL EVIDENCE FOR THE ANTIULCEROGENIC ACTIVITY OF SYNCLISIA SCABRIDA

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Objective: The antiulcerogenic activity of flavonoid (A) and alkaloid (B) fractions of the S. scabrida and their effect on alkaline phosphatase activity were studied.

Methods: The ethanol extract of S. scabrida was subjected to a column and preparative thin layer chromatography to obtain partially pure fractions of A and B which were subjected to antulcerogenic screening and effects on alkaline phosphatase activity using aspirin and 0.6N NaOH ulcer models.

Results: The results showed that fractions A and B significantly (P<0.05) reduced both the ulcer index and alkaline phosphatase activity when compared with aspirin or 0.6N NaOH only.

Conclusion: This study seems to implicate alkaline phosphatase as a biochemical evidence of the antiulcerogenic activity of S. scabrida.

SUMMARY

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INTRODUCTION

Alkaline phosphatase is an enzyme capable of catalyzing the hydrolysis of various phosphate esters at alkaline pH. Alkaline phosphatases from different sources exhibit three types of activity, hydrolytic, phosphotransferase and pyrophosphatase. This enzyme is produced in many cells of the body which includes: osteoblast, liver, intestinal mucosa, placenta, kidney and blood cells (erythrocytes, leucocytes, platelets). Alkaline phosphatase activity has been reported to be increased in bone disease, diseases of the liver and gastrointestinal lumen. The release of this enzyme has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration. Increased activity of this enzyme may be found in damaged tissues.

Our laboratory has shown that S. scabrida possesses anti-ulcer property. In the present study, we attempted to investigate the biochemical basis of the anti-ulcerogenicity of the active principles of S. scabrida. In view of the role of alkaline phosphatase in gastrointestinal tissue necrosis, alkaline phosphatase activity were studied in two ulcer models with a view of finding a possible correlation between the activity of this enzyme and the traditional ulcer index.

MATERIALS AND METHODS

Preparation of alkaloid and flavonoid fractions

The ethanol extract (% yield 2.7) of S. scabrida was subjected to column chromatography as described by Abbot and Andrew. From the column chromatography, three fractions were obtained after pooling the different fractions based on their similarities. Two of the fractions tested were positive for flavonoid and alkaloid. They were designated as fractions A and B respectively.

These two fractions (A and B) were subjected to preparative thin layer chromatography using butanol-acetic acid-water as an elution solvent as described by Stahl. This enabled us to obtain partially pure fractions of A and B.
Table 1. Effect of fractions A and B on alkaline phosphatase activity measured in gastric wall in aspirin-induced ulcer model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose/route</th>
<th>Ulcer index</th>
<th>Alkaline phosphatase activity (UI/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5ml/kg/po</td>
<td>-</td>
<td>22.40 ± 0.96</td>
</tr>
<tr>
<td>Aspirin only</td>
<td>200mg/kg/po</td>
<td>4.3 ± 0.62</td>
<td>48.75 ± 1.69a</td>
</tr>
<tr>
<td>Fraction A</td>
<td>440mg/kg/po</td>
<td>1.6 ± 0.17*</td>
<td>28.00 ± 1.27*</td>
</tr>
<tr>
<td>Fraction B</td>
<td>440mg/kg/po</td>
<td>2.0 ± 0.08*</td>
<td>27.38 ± 1.22*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for n=5.
*P < 0.05 when compared to aspirin group.
*aP < 0.05 when compared to distilled water.

Table 2. Effect of fractions A and B on alkaline phosphatase activity measured in gastric wall in 0.6N NaOH-induced ulcer model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose/route</th>
<th>Ulcer index</th>
<th>Alkaline phosphatase activity (UI/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>5ml/kg/po</td>
<td>-</td>
<td>20.50 ± 1.01</td>
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<tr>
<td>0.6N NaOH</td>
<td>1ml/po</td>
<td>4.5 ± 0.56</td>
<td>47.65 ± 1.69a</td>
</tr>
<tr>
<td>Fraction A</td>
<td>440mg/kg/po</td>
<td>1.8 ± 0.16*</td>
<td>25.00 ± 1.17*</td>
</tr>
<tr>
<td>Fraction B</td>
<td>440mg/kg/po</td>
<td>2.7 ± 0.07*</td>
<td>24.38 ± 1.02*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for n=5.
*Significant different from NaOH group. *P < 0.05 when compared to NaOH group. *aP < 0.05 when compared to distilled water.

Aspirin-induced ulcer

Male albino (Sprague-Dawley) rats weighing between 188-210 g were used. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided into four groups each containing five animals. They were fasted for 48 hours but water was allowed. The first group received distilled water only and the second group received aspirin (200 mg/kg, Merck, Germany) orally. The third and fourth groups were given 440 mg/kg of fractions A and B respectively orally thirty minutes prior to oral administration of 200 mg/kg aspirin. The control and test rats were anaesthetized 6 hour later with ether. The stomach was removed carefully from the body and an incision made along the greater curvature to expose the mucosal layer of the lumen and pinned flat on board with the lumen facing upwards. The stomachs were coded to prevent observer bias and studied with a magnifying glass (x10). Erosions formed on the glandular portion of the stomach were scored and each one was given a severity rating on a 1-3 scale. The overall total divided by a factor of 10 was designated as the ulcer index for that stomach. The glandular portion of the stomach for biochemical analysis was immersed in a buffer solution (sodium carbonate (anhydrous), sodium bicarbonate, distilled water), pH 10.

Sodium hydroxide induced ulcer

Female albino rats weighing between 175-205g were used in this study. After 24 hour fasting, the animals were divided into four groups of five each. The control group received 1.0 ml of distilled water orally 2 hours prior to administration of 1.0ml 0.6N sodium hydroxide (British Drug House, London, UK). The second group received only distilled water. The third and fourth groups were given 440 mg/kg of fraction A and B respectively orally 2 hours prior to oral administration of 1.0 ml 0.6 N sodium hydroxide. After two-hour post treatment, the animals were sacrificed under ether anesthesia. The stomach was excised carefully and opened along the greater curvature to expose the mucosal layer of the lumen. The exposed layer was flushed carefully with normal saline. The entire stomach was pinned into a board with the lumen facing upwards and then fixed with 10% formaldehyde in normal saline. The ulcer index was calculated using a modification of the Robert and Nezamis. The glandular portion of the stomach for biochemical analysis was immersed in a buffer solution (sodium carbonate (anhydrous), sodium bicarbonate, distilled water), pH 10.

Estimation of alkaline phosphatase

The excised wound tissue immersed in 4 ml of buffer solution was ground in a mortar and then centrifuged for 10 minutes. 3 ml of the supernatant solution were pipetted to a test tube to serve as the test solution. The alkaline phosphatase activity was measured using the method of Kind and King.

Statistical analysis: Data were shown as the mean ± standard error of mean and analysed by Student’s ‘t’ test. The level of significance for all experiments was <0.05.

RESULTS

Table 1 shows the effect of fractions A and B on ulcer index and alkaline phosphatase activity in...
aspirin-induced ulcer. The fractions A and B significantly (P<0.05) reduced the ulcer index and alkaline phosphatase activity when compared with that of aspirin only. Table 2 shows the effect of fractions A and B on ulcer index and alkaline phosphatase activity in 0.6N NaOH-induced ulcer. The fractions A and B significantly (P<0.05) reduced the ulcer index and alkaline phosphatase activity when compared with that of 0.6N NaOH only.

DISCUSSION

The anti-ulcerogenicity of alkaloid and flavonoid fractions of *S. scabrida* and their effects on alkaline phosphatase activity using aspirin and sodium hydroxide ulcer models were investigated. The alkaloid and flavanoid fractions had significant anti-ulcer properties. This is consistent with the work of Orisakwe and co-worker, which reported that alkaloid and flavonoid fractions of *S. scabrida* possess anti-ulcer property using albino rats. The results show that the fractions had significant effect on the alkaline phosphatase activity in the two ulcer models studied. The fractions significantly reduced the alkaline phosphatase activity when compared with the control. The release of this enzyme has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration. Increased activity of this enzyme may be found in damaged tissues. The increased activity of this enzyme found in the group treated with either aspirin or sodium hydroxide is in agreement with the above statement. When aspirin is in the lipid-soluble undissociated form it can damage the gastric mucosa. Aspirin causes a dose-dependent reduction in mucosal prostaglandin **E**<sub>2</sub> (PGE<sub>2</sub>) and PGI<sub>2</sub> biosynthesis accompanied by an increase in the mean area of gastric ulcerations. Aspirin is known to inactive irreversibly the PG synthetase systems, which mediates synthesis of prostaglandin in the mucosa. It is reasonable to assume that the observed gastric mucosal lesions induced by aspirin are due to a deficiency of mucosal prostaglandin. Sodium hydroxide induces ulceration by causing lesions on the surface of gastric mucosa when it comes in contact with it. Tengrup *et al.* identified the use of alkaline phosphatase activity as a marker for polymorph neutrophil infiltration to site of injury. The flavonoid and alkaloid fractions of this plant has been reported to decrease the number of neutrophils using smears from the erosions made on the gastrointestinal lumen. The reduction of both phosphatase alkaline activity and ulcer index by these fractions gives an indication that there is a correlation between this enzyme and ulcer index, thereby implicating alkaline phosphatase as a biochemical basis of antiulcerogenicity of *S. Scabrida.*

REFERENCES