EFFECT OF AQUEOUS EXTRACT OF AZOLLA FILICULOIDES IN GASTRIC MUCOSA OF ULCERATED RATS

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INTRODUCTION

The human population is more susceptible to gastrointestinal disorders owing to the changes in lifestyle and food habits. One of the gastrointestinal problems that commonly threaten the public is peptic ulcer. It happens mostly when the acids that helps to digest the food attacks the gastric mucosal lining of the stomach. The most common cause is the infection with a bacterium called Helicobacter pylori [1]. The other factor includes the long term consumption of nonsteroidal anti-inflammatory drugs (NSAIDs), increased stress, nutritional deficiencies, tobacco smoking, and imbalance in aggressive and defensive factors [2-3]. Lipid peroxidation-induced by the free radicals produced as a result of oxidative processes is the major rationale behind the aberration of gastric mucosa. The HCI-ethanol induced gastric mucosal lesions and other signs in the stomach appear more similar to the peptic ulcer caused by the long term consumption of NSAIDs. Allopathic drugs available in the market are capable of curing the ulcer symptoms, but are chosen diligently as it offers side effects like arrhythmia, nausea, abdominal pain, gynecomastia and impotence [4]. Due to the promising treatment results of herbal extracts, much interest is developing towards usage of the same in the treatment of Peptic ulcer [5-6].

Aquatic plants have become a potential source of beneficial substances like proteins, vitamins, fibers and medicinally active compounds. Azolla is an aquatic pteridophyte most commonly known for its agronomic usage due to its nitrogen-fixing ability. Recent studies in Azolla emphasized on the identification of its nutritional and pharmacological activities. From the previous research findings, it is evident that Azolla possesses antioxidant [7], antimicrobial [8], antifungal [9] and hepatoprotective activity [10], which can be attributed to the presence of secondary metabolites in significant quantities.

Based on these facts, an attempt has been made to investigate the effect of aqueous extract of Azolla filiculoides on gastric mucosa in experimentally induced ulcer in rats.

MATERIALS AND METHODS

Collection of plant and extract preparation

Whole plants of fresh Azolla filiculoides were collected from the Agricultural Microbiology Department of Tamil Nadu Agricultural University located at Coimbatore district, Tamil Nadu. The collected plant materials were washed thoroughly with tap water, rinsed with distilled water and air dried. The air dried plant materials were obtained by extracting 20 g of the plant powder with 20 ml of distilled water. The final concentration of the extract was made up to 1.5 ml. The gastric juice and mucosal scraps were collected from all the groups for biochemical analyses.

Results

In ulcer induced rats, there observed a significant (p<0.05) increase in the following parameters like ulcer index, gastric output, acid output, lipid peroxides and these levels were significantly (p<0.05) reduced to near normal values in AF pre-treated rats. A significant decrease was also observed in the levels of reduced glutathione, hexose, hexosamine, sialic acid and in the activities of antioxidant enzymes (glutathione-S-transferase and glutathione peroxidase) and antiperoxidative enzymes (catalase and superoxide dismutase) in ulcer induced rats. These values were restored back to near normalcy in AF pre-treated rats.

Conclusion

The results reinforce the antisecretory, acid neutralizing and the antioxidant potential of the whole plant extract of AF against experimentally induced gastric ulcer in rats. However, further studies are needed to identify the active principle involved in eliciting the antiulcer activity of the plant.

Keywords: Azolla filiculoides, Antisecretory, Acid neutralizing, Antioxidant.
Biochemical estimations

The filtrate obtained was used for the biochemical estimations. Bovine serum albumin was used as the internal standard for containing 0.15 N HCl in 70% v/v ethanol [11].

Excision of stomach and collection of gastric juice

After the treatment period the rats were undergone surgery, according to the procedures mentioned by Takeuchi et al. [12] and 4 h post-surgery the rats were sacrificed using an overdose of Chloroform and the stomachs were removed after clamping the esophagus. Along the greater curvature the stomach was cut open and the gastric juice was collected in a graduated tube. It was spun at 2000 rpm/10 min and the volume was noted. The acid output was determined in the gastric juice by volumetric titration using 0.2 N sodium hydroxide with phenolphthaline as indicator.

Lesions scoring

Using a dissecting microscope (10x) the lesions were scored independently according to the severity of ulcers observed [13]. Latter, the Ulcer Index (UI) was calculated as the mean ulcer score for each rat. The percentage protection offered by the sample was determined in the gastric juice by volumetric titration against 0.02 N HCl in 70% v/v ethanol [11].

Preparation of tissue homogenate

The mucosal scraps were gently collected using a thin glass slide and homogenized (600 rpm/5 min) with ice-cold 0.1 M Tris-HCl buffer (pH 7.4) using a Potter-Elvehjem homogenizer (Sigma-Aldrich). The filtrate obtained was used for the biochemical estimations.

Biochemical estimations

The total proteins were estimated using the method of Lowry et al. [14]. Bovine serum albumin was used as the internal standard for the determination of unknown concentration of proteins. The reduced glutathione content was determined based on the reaction of reduced glutathione with 5, 5'-dithiobis-2-nitrobenzoic acid to give a yellow compound, which was measured at a wavelength of 412 nm [15]. The reduced glutathione content was expressed as nmol/g of wet tissue. The extent of lipid peroxidation was assayed using the method of Ohkawa et al. [16], wherein the amount of malondialdehyde (MDA) liberated was estimated. 1, 1, 3, 3-tetraethoxypropane was used as a standard and the level of lipid peroxides formed was expressed as nmol of MDA formed/mg of protein. Sialic acids were analyzed by the addition of thiobarbituric acid and periodate solution to the sample and the change in absorbance was noted at 540 nm [17]. Sialic acid levels were expressed in mg/g of tissue. The total hexose and hexosamine were determined using the method of Wistler et al. [18] and their corresponding concentrations were expressed as mg/g of wet tissue. The glutathione S-transferase activities were determined using the standard substrate 1-chloro-2,4-dinitrobenzene (CDNB) as mentioned by Habig et al. [19]. The activities of GST enzyme were expressed as µmol of CDNB conjugate formed/min/mg of protein. The glutathione peroxidase (GPx) activity was estimated by the method of Paglia and Valentine [20]. GPx catalyzes the reduction of hydroperoxides using reduced glutathione as the substrate. Thus, the enzyme activity of GPx was expressed as nmol of GSH oxidized/min/mg of protein. According to the method of Takahara et al. [21], the activity of enzyme catalase (CAT) was estimated based on the reaction with dichromate-acetic acid reagent in the ratio (1:3). The catalase enzyme activity was expressed as nmol of hydrogen peroxide (H₂O₂ decomposed/min/mg of protein. The activities of the enzyme superoxide dismutase (SOD) were estimated using the method of Misra and Fridovich [22]. One unit of SOD was calculated as, the amount of protein required to inhibit half the percentage of autoxidation of epinephrine.

Table 1: Effect of aqueous extracts of A. filiculoides (whole plant) on the lesion index, volume of gastric output and total acidity in normal and experimental group of rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal Control)</th>
<th>Group II (AF pre-treated)</th>
<th>Group III (Ulcer induced)</th>
<th>Group IV (Group II+III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index</td>
<td>-</td>
<td>2.0±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5±0.14&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume of gastric juice (ml/4 h)</td>
<td>2.11±0.14</td>
<td>134.17±4.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.83±8.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Acid output (µEq/4 h)</td>
<td>139.33±5.13</td>
<td>256.5±6.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis

The data were expressed as mean±SD. Statistical significance was assessed using one way ANOVA followed by Tukey's test and p<0.05 was considered significant. The analyses were performed using statistical program SPSS 1.0.0 for Windows.

RESULTS

The effects of aqueous extract of Azolla filiculoides on gastric mucosa in experimentally induced ulcer were studied. There observed a significant (p<0.05) increase in the values of ulcer index, volume of gastric juice and the acid output in Group III rats as compared with that of Group I and Group II rats. In contrast, these values were significantly (p<0.05) reduced in the AF pre-treated Group IV rats. The results are shown in table 1.

Table 2: Effect of aqueous extracts of A. filiculoides (whole plant) on the biochemical parameters of normal and experimental group of rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal Control)</th>
<th>Group II (AF pre-treated)</th>
<th>Group III (Ulcer induced)</th>
<th>Group IV (Group II+III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14.08±0.22</td>
<td>7.56±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.55±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>GSH&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.79±0.14</td>
<td>2.08±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.95±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>GST&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5.2±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.97±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.48±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>GPx&lt;sup&gt;5&lt;/sup&gt;</td>
<td>19.03±2.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>107.8±5.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>172.67±12.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CAT&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3.3±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.3±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.0±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>SOD&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5.2±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.92±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>LPO&lt;sup&gt;7&lt;/sup&gt;</td>
<td>2.86±0.13</td>
<td>2.62±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.08±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Hexose&lt;sup&gt;8&lt;/sup&gt;</td>
<td>3.13±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.14±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.0±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Sialic acid&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9.09±0.15</td>
<td>4.4±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for 6 rats.

Values are mean±SD for 6 rats.

Statistical analysis
Group comparison

*a= significant (p<0.05) compared to group I & II rats
*b= significant (p<0.05) compared to group I & III rats
*abc= significant (p<0.05) compared to group III rats

No significant difference compared with group I rats

In HCl-ethanol plus pylorus-ligated Group III rats, a significant decrease in the concentration of total protein, reduced glutathione, hexose, hexosamine and sialic acid was observed. On the other hand, there observed a significant (p<0.05) elevation in the levels of lipid peroxides along with a concomitant reduction in the activities of glutathione dependent antioxidant enzymes (GST, GPx) and peroxidative enzyme (SOD, CAT). Oral pre-treatment with aqueous extract of AF markedly reduced the elevated levels of lipid peroxides, while the levels of reduced glutathione, hexose, hexosamine and sialic acid were augmented to near normalcy. The activities of antioxidant and antiperoxidative enzymes were also restored back to near normal values as shown in table 2.

**DISCUSSION**

In general, herbal medicines are considered to be less toxic, efficient and affordable to people of all age groups. Hence, this way of treatment will be most appropriate for the treatment of gastric ulcer and other gastrointestinal disorders. The oral pre-treatment with aqueous extract of AF (200 mg/kg body weight) ameliorated the deleterious effects caused by the administration of HCl-ethanol in the gastric mucosa of ulcer induced rats.

The ulcer is an outcome of an imbalance between the endogenous gastric mucosal defensive and offensive factors. In the present study, there observed a remarkable increase in the offensive factors like gastric juice and total acidity, whereas a decrease in defensive factors like mucopolysaccharides, reduced glutathione, antioxidant and peroxidative enzyme activities in ulcer induced rats. The ligation of pylorus results in the physiological accumulation of gastric juice, along with large amounts of pepsin that apparently leads to an auto-digestion of the mucosal barrier in the gastric mucosa [29]. An increase in gastric volume is encountered as a result of increased vascular permeability caused by the aberrations in the gastric mucosal barrier. The perturbation of this barrier is known to elicit excessive secretion of histamine that acts through the histamine receptors on the parietal cell and initiates increased acid secretion in the stomach. In general, the secretion of gastric acid plays a meticulous role in aggravating gastric mucosal injury and the antiulcer drugs accelerate the healing process by reducing this acid secretion. Oral administration of aqueous extract of AF significantly reduced the volume of gastric juice and total acidity in pre-treated rats. These results are in concordance with the reports of Oluwabummi et al. [23], who reported that the methanolic extract of *Gomphrena celosioides* significantly reduced the volume of gastric juice and acidity in pre-treated rats. The anti-secretory effect observed in the present study, may be attributable to the presence of compounds having the ability to antagonize histamine receptors in the parietal cells. The reduction in the total acidity clearly indicates the acid neutralizing ability of the plant extracts.

The stomachs of ulcer induced rats in the present study were displayed with morphological aberrations, lesions and a reduction in total protein content. This loss of total gastric mucosal proteins may be due to its leakage into the gastric juice. In the present study, the pre-treatment of AF extracts not only reduced the ulcer indices, but also replenished the levels of total protein in group IV rats. These findings are in agreement with the results obtained by Mahendran et al. [24], who reported that the increased losses of protein are associated with HCl-ethanol induced damage to gastric mucosa in rats and their levels were returned to normalcy by the treatment with *Garcinia cambogia* extract. One of the major defensive factors in the gastric mucosa is the mucus that are mainly composed of glycoproteins and are responsible for the protection of gastric mucosa against physical, chemical and microbial disturbances. The ratio of carbohydrate to protein represents mucus activity in the mucosa against physical, chemical and microbial disturbances. The percentage ulcer protection offered by the plant extract against HCl-ethanol induced ulcerogenesis was 67.85. The ulcer protective index for sulphasalazine is 90.75, which is much higher than that of the plant extract [24]. The ulcer indices are reduced in ulcer induced rats. These results are in concordance with the reports of Deoda et al. [29].

**CONCLUSION**

The results of the present study indicate the overall antiulcerogenic effect of aqueous extract of *Azolla filiculoides* in rats. The ameliorating effects elicited by the plant extract may be due to its


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acid neutralizing, antioxidant potential and also by sustaining a balance between the concentrations of offensive and defensive factors. However, further studies must emphasis on the identification of the exact mechanism of action involved in ulcer healing and also the active principle involved in the plant.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES


