ULCER-HEALING ACTIVITY OF ARCHACHATINA MARGINATA MUCIN ON INDOMETHACIN-INDUCED GASTRIC ULCERATION IN ALBINO RATS

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The objective of this study was to evaluate the ulcer healing activity of Archachatina marginata mucin on indomethacin-induced gastric ulceration in albino rats. Methods: Thirty (30) adult male albino rats were divided into six groups of five rats each. Group I was the normal control and was administered with 2ml/kg b.w distilled water. Group II was induced with 120mg/kg b.w indomethacin only. Groups III, IV and V were induced and treated with 200, 400 and 800mg/kg b.w mucin respectively while group VI was induced and treated with 20mg/kg b.w omeprazole. Treatment lasted for 10 days after which animals were sacrificed by cervical dislocation. Harvested stomach tissue and gastric juice were processed and analyzed using standard methods. Result: Oral administration of indomethacin caused ulceration at the glandular region of rat stomach indicated by a high ulcer index (6.28±0.33). However, this was low in group V administered with 800mg/kg mucin (1.52±0.29). A similar value was recorded for the omeprazole treated group (1.24±0.25). Gastric mucus was reduced in the stomach tissue of rats in group II (0.22±0.05 abs/g of tissue). However, contrary values were recorded for other groups. This was obvious in groups V and VI (0.5±0.08 abs/g of tissue) and (0.53±0.07 abs/g of tissue) respectively. Free acidity and total acidity were significantly higher in group II (63.40±7.66mEq/L) and (91.40±6.34mEq/L) respectively. However, the reduction in free acidity and total acidity was observed in the group treated with 800mg/kg b.w mucin (47.40±7.19mEq/L) and (71.00±7.00mEq/L) respectively. A similar observation was made on the omeprazole treated group.
(34.00±5.40mEq/L) and (54.80±5.35mEq/L) respectively. **Conclusion:**

By the outcome of this study, It can be deduced that mucin from *A. marginata* possesses appreciable ulcer healing potential

1. Introduction

Gastric ulcer is one of the two most common types of peptic ulcer (Kaur *et al.*, 2012). It is a deep lesion penetrating through the entire thickness of the gastrointestinal (GI) mucosa and muscularis mucosa presenting symptoms such as abdominal discomfort, pain or nausea (Tarnawski *et al.*, 2001). It is a common global problem with increasing incidence and prevalence. Although, the etiology of gastric ulcer is not clearly defined, it is generally believed that it occurs due to an imbalance between the gastric mucosal protective factors and the aggressive factors (El-far *et al.*, 2012). One of the major main factors implicated in the pathogenesis of gastric ulcer is the abusive use of non-steroidal anti-inflammatory drugs (Kaur *et al.*, 2012). Treatment of ulcers depends on the use of some synthetic drugs that reduce the rate of stomach acid secretion notably, the proton pump inhibitors. Unfortunately, cases of relapses and adverse reactions have been reported about these therapies (Amani *et al.*, 2012). Thus, interest in the use of natural products in the treatment of gastric ulcer becomes imperative.

Human beings have been using animal resources for therapeutic purposes since ancient times. Animals and products derived from different organs of their bodies have constituted part of the inventory of medicinal substances used in various cultures since ancient times (Oladapo *et al.*, 2012). Snails are rich in a wide array of bioactive substances which have made them valuable in the treatment of several human ailments such as arteriosclerosis, whooping cough, anaemia, asthma, age-related problems, hypertension and rheumatism (Abere and Lameed, 2008).

Mucins belong to a family of large extracellular, high molecular weight O-glycosylated proteins formed by tandem repeats of amino acid chain rich in cysteine, serine and threonine coated with oligosaccharide side chain. Mucins are important components of innate defence at the body’s mucosal surfaces (Linden *et al.*, 2008). Mucin from the mucilage of *Archachatina marginata* has demonstrated wound healing activities (Adikwu and Ikejiuba, 2005). Thus, the objective of the present study is to evaluate the ulcer healing activity of *A.marginata* mucin.

2. Materials and Methods

**Snails**

Two hundred and fifty (250) fresh mature African giant land snails (*Archachatina marginata*) weighing 100-450g were purchased from a snail farm in Benin City, Edo State Nigeria. They were carried in the basket to avoid suffocation and cracking.

**Experimental Animals**

Thirty (30) adult male albino rats (150-200g) were obtained from the Animal House of the Department of Pharmacology Ahmadu Bello University Zaria. They were housed in plastic cages and placed on standard pellet (Niger feed). Animals were kept in a well-ventilated room with a 12/12hr light/dark cycles for three weeks to acclimatize.

**Mucin Extraction**

The fleshy bodies of the snails were removed from their shells. They were placed in 250ml of cold water and washed until the mucin was thoroughly washed off. The washing was pooled together in a plastic container and was subsequently precipitated using chilled acetone to yield mucin which was air dried and pulverized into a fine powder with mortar and pestle before being stored in an air tight container (Adikwu, 2005).
Median Lethal Dose 50% Test (LD_{50})
Acute toxicity test was carried out on snail mucin. Three groups of three rats each were administered 10mg, 100mg, and 1000mg/kg of mucin orally. The rats were observed for 24hrs for effects of toxicity. Following the absence of mortality in any of the groups, another three groups of one rat each were administered with 1600, 2900 and 5000mg/kg of extract respectively. The animals were observed for 48 hrs for effects of toxicity (Lorke, 1983).

Experimental Design
Thirty (30) adult male albino rats were randomly divided into six groups of five rats each. The rats in all groups were starved for 48hrs after which 120mg/kg b.w indomethacin was administered orally to animals in all groups except the standard control. Group I (standard control) received 2ml/kg b.w distilled water only. Group II was left untreated. Groups III, IV and V received 200mg/kg, 400mg/kg and 800mg/kg body weight mucin respectively while Group VI received the standard drug (20mg/kg omeprazole). After treatment, animals were anesthesized by intramuscular injection of ketamine hydrochloride. The abdomen was opened by midline incision. The pylorus was located and ligated. The stomach was sutured. After 4hrs, animals were sacrificed by cervical dislocation. The stomach content was collected in a tube and centrifuged for use.

Ulcer Index
The length (mm) of each lesion was measured, and the lesion index was calculated by adding the length of all lesions in the fundic region of the stomach (Jiang et al., 2008).

Free acidity and Total acidity
Free acidity and total acidity were measured by the method of Kulkarni 2010. Two drops of methyl orange indicator were added in a diluted supernatant of gastric juice in a conical flask. 0.01N NaOH was taken in a burette and was allowed to titrate till the contents of the flask changed turned yellow. Then two drops of phenolphthalein were added, and the titration continued until the solution turned orange to determine the total acidity.

Gastric Mucus Evaluation
Gastric wall mucus was determined according to the modified procedure of Corne et al. (1994) the glandular segments of the stomach was excised and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v alcian blue solution (in 0.16 M sucrose solution buffered with 0.05 ml of sodium acetate at pH 5). Tissue was stained for 2 hrs in alcian blue, and excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 min. Dye complexes with the gastric wall mucus were extracted with 10 ml of 0.5 M MgCl₂ which was shaken intermittently for 1 min at 30 min intervals for 2 hrs. 4ml of the blue extract was then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 mins, and the absorbance of the aqueous layer was recorded at 580 nm.

Histological Examination
The stomach tissues were fixed in 10% buffered formalin overnight and then processed in an automated tissue processor. Stomach tissues were embedded, sectioned by a microtome and stained with haematoxylin and Eosin stain. Each section was examined with the aid of a light microscope with a magnification of ×100

Statistical Analysis
Data were expressed as Means ± SD. The data were analysed using the analysis of variance (ANOVA). The differences in mean were compared using Duncan Multiple Range Test. Mean values were
considered significantly different at P < 0.05.

3. Result

Table 1: Ulcer index of ulcerated treated rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>2ml/kg distilled water</td>
<td>0</td>
</tr>
<tr>
<td>Group II (Negative control)</td>
<td>120mg/kg indo</td>
<td>6.28 ± 0.33d</td>
</tr>
<tr>
<td>Group III (Mucin200)</td>
<td>Indo+ Mucin200mg/kg</td>
<td>3.40 ± 0.31c</td>
</tr>
<tr>
<td>Group IV (Mucin400)</td>
<td>Indo+ Mucin400mg/kg</td>
<td>2.40 ± 0.35b</td>
</tr>
<tr>
<td>Group V (Mucin800)</td>
<td>Indo Mucin800mg/kg</td>
<td>1.52 ± 0.29a</td>
</tr>
<tr>
<td>Group VI (Standard20)</td>
<td>Indo+Omeprazole20mg/kg</td>
<td>1.24 ± 0.24a</td>
</tr>
</tbody>
</table>

Values are means ± SD of five determinations.
Values with different superscript in a column are significantly different (P<0.05).

Table 2: Gastric secretions and mucus of ulcerated rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose</th>
<th>Free acidity (mEq/L)</th>
<th>Total acidity (mEq/L)</th>
<th>Gastric Mucus Absorbance/g of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>2ml/kg distilled water</td>
<td>48.40 ± 7.95b</td>
<td>76.80 ± 9.78b</td>
<td>0.55±0.08c</td>
</tr>
<tr>
<td>Group II (Negative control)</td>
<td>120mg/kg indo</td>
<td>63.40 ± 7.66c</td>
<td>91.40 ± 6.34c</td>
<td>0.22±0.05a</td>
</tr>
<tr>
<td>Group III (Mucin200)</td>
<td>Indo+ Mucin200mg/kg</td>
<td>60.20 ± 9.80c</td>
<td>86.80 ± 7.66c</td>
<td>0.29±0.07a</td>
</tr>
<tr>
<td>Group IV (Mucin400)</td>
<td>Indo+ Mucin400mg/kg</td>
<td>45.40 ± 7.79b</td>
<td>72.00 ± 6.36b</td>
<td>0.41±0.03b</td>
</tr>
<tr>
<td>Group V (Mucin800)</td>
<td>Indo Mucin800mg/kg</td>
<td>47.40 ± 7.19b</td>
<td>71.00 ± 7.00b</td>
<td>0.51±0.08bc</td>
</tr>
<tr>
<td>Group VI (Standard20)</td>
<td>Indo+Omeprazole20mg/kg</td>
<td>34.00 ± 5.40a</td>
<td>54.80 ±5.35a</td>
<td>0.53±0.07c</td>
</tr>
</tbody>
</table>

Values are means ± SD of five determinations.
Values with different superscript in a column are significantly different (P<0.05).
Histopathological findings

Plate A represents the photomicrograph of an intact mucosa of rats administered 2ml/kg distilled water. Plate B: shows the stomach tissue of the negative control indicating that oral administration of indomethacin caused a severe focal erosion of the mucosal layer of the stomach. Plate C: is the photomicrograph of stomach tissues of ulcerated rats treated with 200mg/kg mucin showing a sizable surface of injury. Plate D: shows the photomicrograph of stomach tissue of ulcerated rats treated with 400 mg/kg mucin showing a highly reduced ulcer area. Treatment with 800mg/kg mucin caused a reduction in the ulcer area while the standard drug (omeprazole) brought about the regeneration of the disrupted tissue integrity.
4. Discussion

Table 1 shows that the ulcer index of ulcerated rats treated with A. marginata mucin significantly reduced in a dose-dependent manner. However, there was no significant difference in the ulcer index of treatment groups administered with 800mg/kg of mucin (1.52±0.29) and 20mg/kg omeprazole (1.24±0.24). This may be attributed to the presence of the glycoconjugates such as glycosaminoglycans and proteoglycans (mucin) which have been reported to have wound healing properties (Adikwu and Ikejiuba, 2005). This report is consistent with the finding of Adikwu and Alozie (2007) which shows that snail mucin in detarium gum gel in wound played important roles in wound healing.

Table 2 shows that groups administered with 800mg/kg mucin and the standard drug (omeprazole) significantly decreased free acidity and total acidity (47.40±7.19), (7100±7.00) and (34.00±5.40), (54.80±5.35) respectively compared to the negative control (63.40±7.66) and (91.40±6.34) respectively. This may be as a result of the presence of zinc in mucin. Thus, this result is consistent with the finding of Kirchhoff et al. (2010) which revealed that zinc salt inhibited acid secretion in the isolated rat and human gastric glands. There was no significant difference in the gastric mucus of the group administered 800mg/kg mucin compared to the omeprazole treated group and the normal control. This may be due to the gastroprotective potential of zinc and the immune body stimulatory effect of mucin (Opaka et al., 2010) and consequently improved delivery of prostaglandin and increased mucus secretion. This finding is in tandem with the finding of Cho et al. (1976) which showed that pretreatment with mucin increased gastric mucus.

5. Conclusion

The fact that synthetic anti-ulcer therapies are characterized by some adverse effects in addition to being expensive has necessitated the need for dependable alternatives. The huge demand on the plant biodiversity as a source of medication, has however called for the need to explore novel bioactive products from medicinal animal such as the African giant snail not only provide mankind with useful anti-ulcer therapy that will be affordable and having little or no side effects, but will also reduce the burden of huge demand imposed on the plant biodiversity as source of therapy for the ever increasing human population.

References


