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(RESEARCH ARTICLE)

Effect of *Archachatina marginata* mucin on the aggressive factors of gastric ulcer challenged wistar rat stomach tissue

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Abstract

The aim of this study was to investigate the effect of *Archachatina marginata* mucin on the aggressive factors of gastric ulcer challenged rat stomach tissue. Thirty adult male wistar rats were divided into six groups of five rats each. Group I (normal control) was administered with 2 ml/kg b.w distilled water; Group II was administered with 120 mg/kg b.w indomethacin only. However, Groups III, IV and V were administered with 200, 400 and 800 mg/kg b.w mucin respectively, while Group VI was administered with the standard drug (mistoprostol) daily for 10 days. After the 10th day of pretreatment, Groups III-VI were administered with 120 mg/kg b.w indomethacin. Gastric juice was collected, after animals had been anaesthetized. The rats were sacrificed by cervical dislocation seven hours after indomethacin administration. Free acidity, total acidity, pepsin activity, gastric juice volume and acid output were determined using standard methods. There was a significant increase in the level of free and total acidy as well as pepsin activity with a concomitant increase in gastric juice volume as well as acid output (59.00±5.43 mEq/L), (85.20±7.49mEq/L), (1.39±0.62µg/ml), (4.40±0.77ml) and (0.26±0.04 µEq/L/4hrs) in Group II compared to Group I (normal control) (40.60±6.39mEq/L), (71.00±9.30mEq/L), (1.18±0.41µg/ml), (2.58±0.87 ml) and (0.11±0.03µEq/L/4hrs) respectively. However, there was a significant reduction in the level of free and total acidity as well as pepsin activity with a concomitant decrease in gastric juice volume and acid output in Groups III, IV and V administered with 200, 400 and 800 mg/kg b.w. While values recorded with 800 mg/kg b.w mucin was not significantly different from those obtained with the 20 µg/kg b.w standard drug (mistoprostol). In conclusion, It can be deduced from this work that mucin from *A. marginata* can be considered suitable candidate for the development of a gastric ulcer drug.

Keywords: *Archachatina marginata*; Mucin; Aggressive factors; Free acidity and Total acidity

1. Introduction

The stomach, a vital component of the gastrointestinal tract (GIT) is a sack-like organ known for its crucial role in nutrient utilization both in humans and animals. The balance existing between the protective and the aggressive factors of the gastric mucosa accounts for its structural and functional integrity [1]. The pre-epithelial barrier enhanced by prostaglandins remains cardinal to mucosal protection [2]. While this could be considered the first line of gastric mucosal defense, next to it, being the surface epithelial cells characterised by the uninterrupted presence of mucins, buffer, phospholipids, prostaglandins, trefoil peptides and peptide growth factors with the ultimate gastro-defense formation being the functional presence of the endothelium which releases angiogenic growth factors to

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produce potent vasodilators such as nitric oxide and prostacylins [2]. These defense lines are in a delicate balance with the aggressive factors of the stomach which are pepsin, reflux biles and most importantly hydrochloric acid.

The Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) could be considered the most commonly prescribed class of medication [3]. It is a member of a wide class of therapeutic agents which wield both analgesic and anti-inflammatory potentials [4] however with the ability to induce gastric ulceration by inhibiting prostaglandins endoperoxide synthase-1 (PTGS-1) [5]. In order to ameliorate the numeous shortcomings that characterise the use of NSAIDs, cytoprotective agents such as mistoprostol and sucralfate have been developed and recommended for use. These drugs which work by stimulating mucus throughout the lining of the GIT unfortunately, in addition to being expensive, have resulted to adverse effects such as diarrhea, abdominal discomfort, headache and constipation [6].

Mucin is a member of the family of large extracellular, high molecular weight O-glycosylated proteins that are formed by tanden repeats of amino acid chain rich in cysteine, serine and threonine coated with oligosaccharide side chain [7]. Mucin derived from *Archachatina marginata* has demonstrated wound healing and bactericidal activities [8].Therefore, there's need to ascertain the effect of snail mucin on the aggressive components of the stomach, an assessment vital to its consideration as a candidate for possible development of a potential anti-ulcer therapy.

2. Material and methods

2.1. Snails

Mature African giant land snails (*Acharchatina marginata*) weighing 100-450g were procured from a snail farm in Benin City, Edo State Nigeria. The snails were conveyed in a plastic basket.

2.2. Experimental animals

Thirty (30) adult male wistar rats (150-200g) were used for the study. The animals were housed in plastic cages in a well-ventilated room with a 12/12hr light/dark cycles for three weeks to acclimatize.

2.3. Mucin extraction

Snail shells were cracked with a rod and the fresh fleshy bodies dislodged after which 250ml of cold water was used to thoroughly wash off the mucin. The recovered mucin was collected before being precipitated with chilled acetone and after wards air dried and pulverized into a fine powder [9].

2.4. Median lethal dose 50% (LD50)

The determination of the acute toxicity test on mucin involved three groups of three wistar rats each. The various groups were separately administered with 10, 100 and 1000mg/kg of mucin orally. The rats were observed for 24hrs for effects of toxicity. Being that mortality was not observed in any of the groups, another three groups of one rat each was each administered with 1600, 2900 and 5000mg/kg of mucin separately. The animals were observed for 48 hrs for signs of toxicity [10].

2.5. Induction of gastric ulcer

Animals were pretreated with mucin for 10 day, after which they were starved for 48 hours in a metal cage. 120mg/kg body weight single dose of indomethacin was administered orally to rats in all groups except the normal control group [11].

2.6. Experimental design

Group 1: (Normal control) 2ml/kg b.w distilled water p.o.
Group 2: (Negative control) administered 1 20mg/kg only p.o.
Group 3: Pretreated with 200mg/kg b.w of mucin p.o.
Group 4: pretreated with 400mg/kg b.w of mucin p.o.
Group 5: Pretreated with 800mg/kg b.w of mucin p.o
Group 6 Pretreated with 20µg/kg b.w of mistoprostol p.o

2.7. Collection of gastric juice

Each rat was anaesthetized by intravenous administration of 50mg/kg of ketamine hydrochloride. Three minutes after which midline incision was carried out on the rats. Pylorus was located and ligated with chromic cat gut. The skin was sutured by interlocking pattern. The rats were transferred to starvation cages and allowed to recover. Animals were sacrificed by cervical dislocation seven hours after indomethacin administration.

The stomach tissues which were excised carefully by keeping the esophagus closed before being opened along the greater curvature to facilitate collection of gastric juice into a suitable container [12].

2.8. Estimation of total and free acidity

Exactly 10ml of gastric juice sample was introduced into a conical flask prior to the addition of 2-3 drops of the methyl orange. This was titrated against 0.1M NaOH till the red color of the content disappeared (pH 3.5) giving a pale orange colour. End-point value was noted and considered the free acidity. To the content, 2-3 drops of phenolphthalein was added and titrated further against 0.1M NaOH till a faint pink colour appeared again, the end-point value noted was considered the total acidity [13].

2.9. Acid out put

This was calculated by the method of Ishizuka *et al* [14] thus: Acid output = Acidity × volume of gastric juice.

2.10. Determination of pepsin activity

The enzymatic activity of pepsin in undiluted gastric juice was determined according to the method described by Prino *et al* [15].

2.11. Percentage Inhibition

This was calculated according to the method of Hano *et al*. [16] using the formulae below

$$P.I(\%) = \frac{mean\ ulcer\ index(negative\ ctrl) - mean\ ulcer\ index\ (testgroup) \times 100}{mean\ ulcer\ index(negative\ ctrl)}$$

2.12. Histological examination

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

2.13. 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. $P < 0.05$ was considered statistically significant.

3. Result and discussion

Gastric secretion is a colourless, watery digestive fluid. It is the source of aggressive factors of the stomach which are hydrochloric acid and pepsin [17]. Table 1.0 shows the aggressive components of gastric juice harvested from the stomach tissue of rats pretreated with *A. marginata* mucin prior to oral administration of indomethacin. Oral administration of 120mg/kg indomethacin distorted the delicate balance between the aggressive and protective factors of the stomach in the negative control (group II). This was evident by the observed increase in level of free and total acidities as well as pepsin activity alongside the gastric juice volume and output. On the other hand, there was a significant increase however in a dose dependent manner in the values obtained for the free and total acidities as well as pepsin activity with attendant rise in gastric juice volume and acid output. Observations which were consistently supported by the values generated on the gastric ulcer preventive index of *A. marginata* mucin shown on figure 1.0 This may be due to the gastro protective potentials of zinc which has been found present in snail mucin [18] which is consequently translated into decreased inhibition of prostaglandin synthase which results to a minimized secretion of

gastric acid. These results are in accordance with the findings of Cho et al. [19] which show that a 10 day pretreatment with a zinc compound significantly decreased gastric acidities and pepsin activity.

Table 1: Gastric secretions from wistar rats pretreated with *A. marginata* mucin

Grouping	Dose	Free acidity (mEq/L)	Total acidity (mEq/L)	Pepsin activity (µg/ml)	Gastric juice vol.(ml)	Acid output (µEq/L/4hrs)
Group I	2ml/kg distilled H_20	40.60 ± 6.39^{a}	71.00 ± 9.30^{a}	1.18 ±0.41^{ab}	2.58±0.87^{a}	0.11±0.03^{a}
Group II	120mg/kg Indo only	59.00 ± 5.43^{c}	85.20 ± 7.49^{b}	1.39 ± 0.62^{d}	4.40±0.77^{b}	0.26±0.04^{b}
Group III	$Muc_{200mg/kg}$ +Indo	52.00 ± 7.44^{bc}	81.40 ± 8.29^{b}	1.35 ± 0.82^{d}	4.02±0.77^{b}	0.21±0.02^{b}
Group IV	$Muc_{400mg/kg}$ +Indo	45.60 ± 4.82^{a}	73.20 ± 8.40^{a}	1.27 ± 0.50^{c}	2.70±0.64a	0.11±0.02^{a}
Group V	$Muc_{800mglkg}$ +Indo	43.00 ± 5.33^{a}	72.60 ± 5.54^{a}	1.26± 0.48^{bc}	2.66±0.77^{a}	0.11±0.04^{a}
Group VI	$Misto_{20µg/kg}$ +Indo	42.60 ± 5.36 ^{a}	71.80 ± 7.91^{a}	1.15 ± 0.61^{a}	2.62±0.80^{b}	0.10±0.02^{a}

Values are means ± SD of five determinations.
Values with different superscripts in a column are significantly different (P<0.05). Key: muc= mucin, indo= indomethacin, misto= mistoprostol

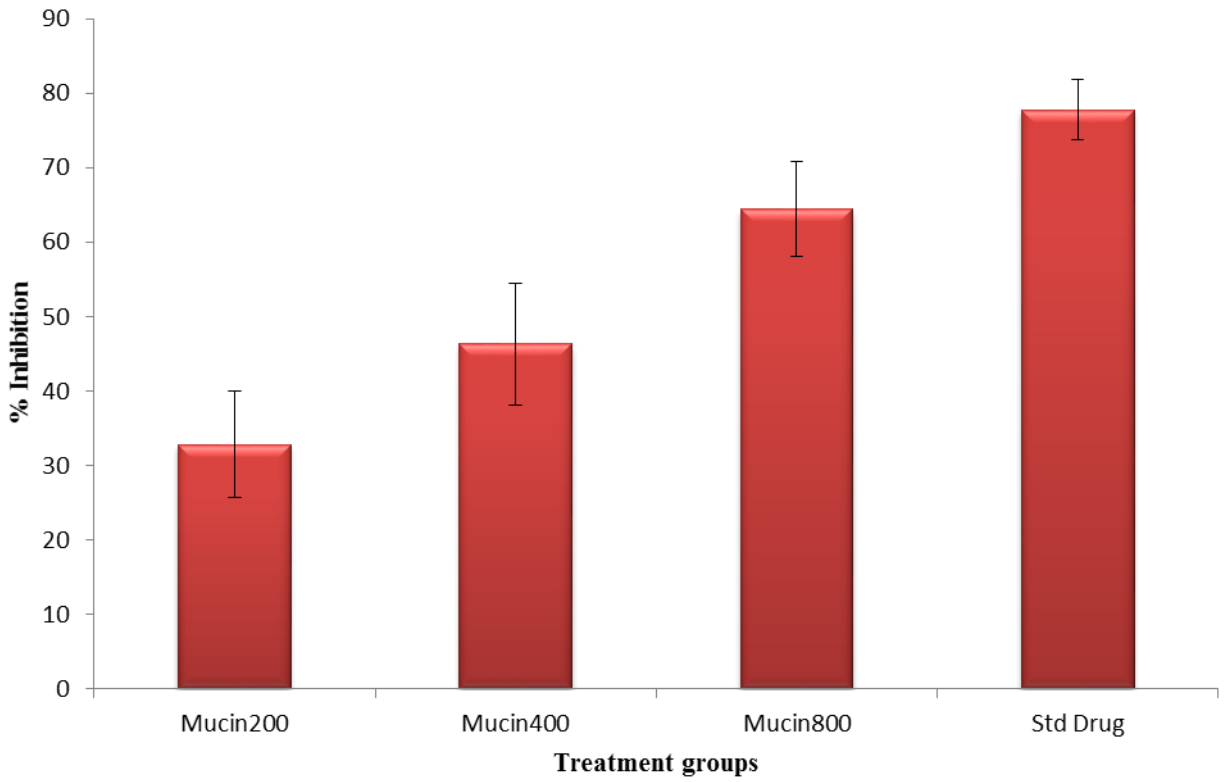

Figure 1 Gastric ulcer preventive index of *A. marginata* mucin

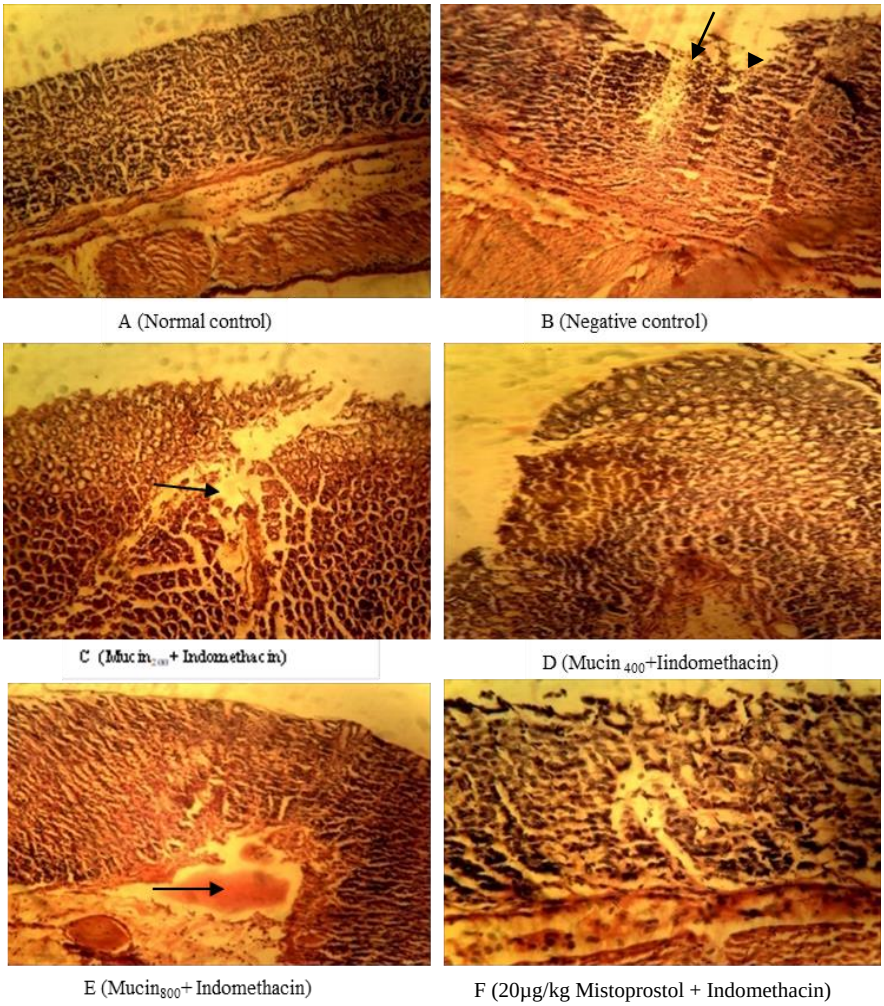

Figure 2 A: is the photomicrograph of stomach tissue of wistar rats administered with 2ml/kg distilled water only showing intact stomach layers. **B** is indicative of stomach tissue of wistar rats administered with 120mg/kg indomethacin only, manifesting a generalized focal erosion of the mucosa. **C** shows the status of stomach tissue of wistar rat pretreated with 200mg/kg mucin indicating a disruption in the full thickness of the mucosa. **D** shows the stomach tissue of normal rats pretreated with 400mg/kg showing a total break in the integrity of the mucosa. **E** shows the stomach tissue of wistar rats pretreated with 800mg/kg of mucin showing reddish patch. **F** is the stomach tissue of rats pretreated with the standard drug mistoprostol (Standard drug) showing a poorly visible lesion a justification for a tangible level of protection**.**

4. Conclusion

The ability of *A. maginata* mucin to reduce the secretion of the aggressive factors in gastric ulcer conditions facilitates healing an indicator of its viability as a candidate for a possible anti-ulcer drug development.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that no conflict of interest exist on this work.

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