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EVALUATION OF THE GASTRO-PROTECTIVE POTENTIAL OF *Archachatina Marginata* MUCIN IN ALBINO RAT

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ABSTRACT

Gastro-protective potential of *A. marginata* mucin in albino Rats was evaluated by standard procedures. Oral administration of indomethacin caused ulceration at the glandular region of the stomach which was characterized by a significantly high ulcer index (8.52 ± 0.82). However, pretreatment with mucin prior to oral administration of indomethacin significantly reduced ulcer index in a dose-dependent manner in which (3.00 ± 0.35) was recorded on 800mg/kg mucin. This observation was consolidated by the results generated on the percentage ulcer inhibitory analysis carried out on mucin which also was found to be dose dependent with 80% inhibition being recorded following the administration of 800mg/kg of mucin. Gastric mucus was significantly reduced in the negative control group (0.21 ± 0.04) compared to the normal control group (0.42 ± 0.10). However, it was significantly increased in groups pretreated with 400 and 800mg/kg mucin (0.29 ± 0.05), (0.31 ± 0.07) respectively. It can be deduced from the findings made in this study that mucin derived from *A. marginata* has gastroprotective potential and hence should be considered for inclusion in the formulation of non-steroidal anti-inflammatory drugs to either reduce or mask its natural potential to cause mucosal injury.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) NSAIDs are one of the most commonly prescribed classes of medication (Abdulla *et al.*, 2013). NSAIDs belong to a wide class of therapeutic agents with analgesic and anti-inflammatory potentials (Grosser *et al.*, 2006). They have also been used in the treatment of conditions such as osteoarthritis and rheumatoid arthritis (McGettigan and Henry, 2011). They include ibuprofen, diclofenac, naproxen and mefenamic acid, indometacin, aspirin, piroxican etc. NSAIDs are responsible for approximately 5-10% of all medications prescribed each year (Onder *et al.*, 2004). The prevalence of NSAID use in patients over 65 years old is as high as 96% in the general practice setting (Pilotto *et al.*, 2003). While the major adverse effects of NSAIDs such as gastrointestinal mucosa injury are well known, NSAIDs have also been associated with hepatic side effects ranging from asymptomatic elevations in serum aminotransferase levels and

hepatitis with jaundice to fulminant liver failure and death (Sarges *et al.*, 2016). This is evident by the fact that in 2008, lumiracoxib a typical NSAID was withdrawn from the market in several countries, mostly due to its potential to cause severe hepatic failure (Techman, 2008). NSAIDs interfere with mucosal defense via direct toxic effects in addition to cyclooxygenase inhibition and subsequent depletion of endogenous prostaglandins (Larkai *et al.*, 1987). This ultimately results to gastric ulcer and its attendant consequences.

Snail is rich in a wide array of bioactive substances which have made it valuable in the treatment of several human ailments such as arteriosclerosis, whooping cough, anaemia, asthma, age related problems, hypertension and rheumatism etc (Abere and Lameed, 2008). Mucins belong to a family of large extracellular, high molecular weight O-glycosylated proteins (Sheiman and Fendrick, 2005; Germano and Elisabetta, 2015). Mucin from the mucilage of *A. marginata* has been reported to possess wound healing potential and thus could be considered for inclusion as an ingredient in the formulation the NSAIDs to either reduce or eliminate its natural potential to cause gastric mucosal injury. (Adikwu and Ikejiuba, 2005).

2. Materials and Methods

Snail and Mucin Extraction

Two hundred and fifty (250) mature African giant land snails (*Acharchatina marginata*) weighing (100-450g) was purchased from Benin Edo State. They were carried in a plastic basket to avoid suffocation and cracking. The fleshy bodies of the snails were dislodged from their shells by inserting a spirally coiled rod into the shells. They were placed in 250ml of water and washed until mucin was thoroughly washed off. The washing was pooled together in a plastic container and subsequently precipitated using chilled acetone. Mucin was air dried, pulverized into fine powder and stored in an air tight container (Adiukwu, 2005).

Experimental Animals

Thirty adult male albino rats (150-200g) were obtained from the faculty of Pharmaceutical Science 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 cycle and ambient temperature. Acclimatization lasted for two weeks.

Acute Toxicity Test (LD₅₀)

Acute toxicity test described by Lorkes (1983) was carried out on snail mucin. Three groups of three rats each were administered 10mg/kg, 100mg/kg and 1000mg/kg of mucin orally. The rats were observed for 24hrs. Being that mortality was not recorded in first phase of the test. Another three groups of one rat each were administered with 1600, 2900 and 5000mg/kg of extract accordingly. The animals were observed for 48 hrs.

Experimental Design

- Group 1:** (Normal control) 2ml/kg b.w distilled water p.o.
- Group 2:** (Negative control) administered 120mg/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

Induction of Gastric Ulcer

Animals were pretreated for 10 day after which they were starved for 48 hours in a metal cage to avoid coprophagy. Animals were allowed access to water *ad-libitum*. 120mg/kg body weight single

dose of indomethacin were administered orally to rats in all groups except the normal control group. Animals were sacrificed by cervical dislocation after seven hours (Urushidani *et al.*, 1979).

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).

Percentage Inhibition

This was calculated according to the method of (Hano *et al.*, 1976) using the formulae below

$$P.I (\%) = \frac{\text{mean ulcer index (indomethacin group)} - \text{mean ulcer index (test group)}}{\text{Mean ulcer index (indomethacin group)}} \times 100$$

Gastric Mucus Evaluation

Gastric wall mucus was determined according to the modified procedure of Corne *et al* (1974). 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 h in Alcian blue and excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, firstly for 15mins and subsequently for 45 mins. Dye complexes with the gastric wall mucus was extracted with 10 ml of 0.5 M MgCl₂ which was shaken intermittently for 1 min at 30 min intervals for 2 h. Four milliliter of blue extract was then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The quality of dye (alcian blue) obtained per gram of wet stomach tissue was then calculated.

Histological Examination

The stomach tissue samples were fixed in 10% buffered formalin overnight and then processed in an automated tissue processor. Stomach tissue were embedded, sectioned by a microtome and stained with haematoxylin and Eosin stain. Each section was examined by light microscope with magnification of $\times 100$

Statistical Analysis

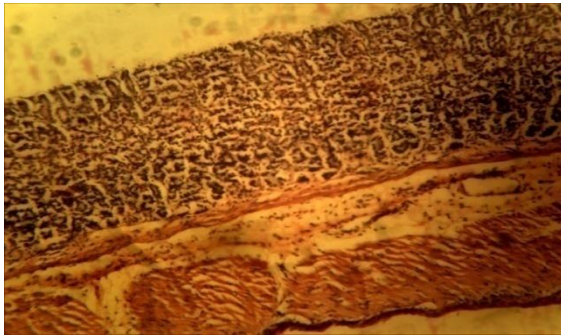
Data were expressed as Means $\pm$ SD. The data were analysed using the analysis of variance (ANOVA). The differences in mean were compared using Duncan Multiple Range Test. $P < 0.05$ was considered significant.

3. Results

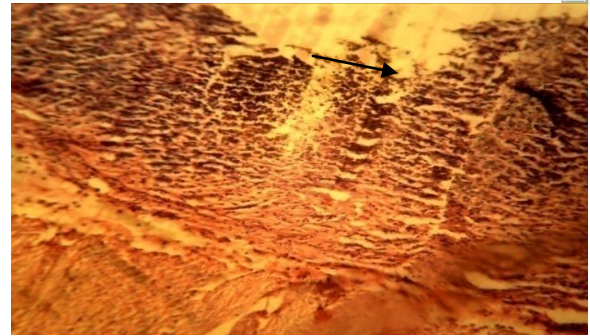
Table 1: Values on the Ulcer Index, Percentage Inhibition and Gastric Mucus of pretreated rats

Treatment groups	Dose	Ulcer Index	% inhibition	Gastric Mucus
Group I (Normal ctrl)	2ml/kg distilled water	0	0	0.42 $\pm$ 0.10 ^d
Group II (Negative ctrl)	120mg/kg indo	8.52 $\pm$ 0.82 ^e	0	0.21 $\pm$ 0.06 ^a
Group III	Mucin200mg/kg+indo	5.70 $\pm$ 0.57 ^d	17	0.24 $\pm$ 0.04 ^{ab}
Group IV	Mucin400mg/kg+indo	4.54 $\pm$ 0.55 ^c	53	0.29 $\pm$ 0.05 ^{ab}
Group V	Mucin800mg/kg+indo	3.00 $\pm$ 0.35 ^b	83	0.31 $\pm$ 0.07 ^{ab}
Group VI	Mistoprostol 20 μ g/kg+indo	1.90 $\pm$ 0.41 ^a	91	0.40 $\pm$ 0.08 ^{cd}

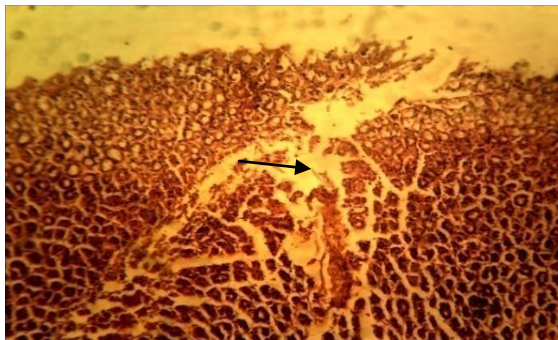
Values are expressed as means $\pm$ SD of five determinations. Values with different superscripts in a column are significantly different ($P < 0.05$).



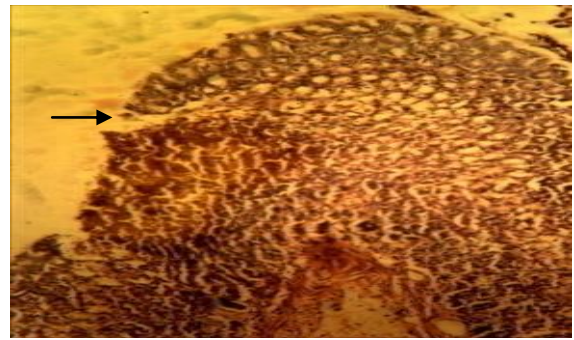
A (Normal control)



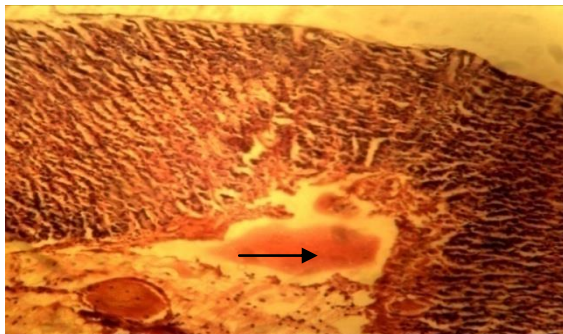
B (Negative control)



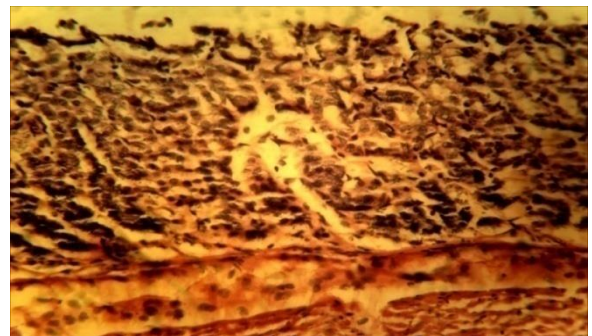
C (Mucin₂₀₀+ Indomethacin)



D (Mucin₄₀₀+Indomethacin)



E (Mucin₈₀₀+ Indomethacin)



F (20µg/kg Mistoprostol + Indomethacin)

Fig. 1 Plates A-F show the photomicrographs of stomach tissue of rats pretreated with varying doses of *A. marginata* mucin. Plate A, is the stomach tissue of rats given distilled water only showing an intact stomach layers, B is the stomach tissue of rat administered with 120mg/kg indomethacin only showing a generalized severe focal erosion of surface epithelium. C is the stomach tissue of rat pretreated with 200mg/kg mucin showing a narrowly constricted ulceration which is restricted to the surface of the tissue. D shows the stomach tissue of rat pretreated with 400mg/kg showing a tiny perforation on the tissue mucosal layer. E represents the stomach tissue of rat pretreated with 800mg/kg of mucin showing a reddish patch. F is the stomach tissue of rats pretreated with mistoprostol (Standard drug) showing a disappearing lesion.

4. Discussion

Snail mucin is composed primarily of sulphated sugar, globular soluble proteins, uronic acids and oligoelements (copper, zinc, calcium and iron). Oral administration of indomethacin caused ulceration at the glandular region of the stomach of rats which were not pretreated with mucin

(negative control). However, pretreatment with mucin significantly reduced ulceration in a dose dependent manner an indication of gastro-protection. The gastro-protective potential of mucin may be due to its stimulatory effects on the immune system (Glade, 1990). This study is consistent with the finding of Adikwu and Enebeke (2007), which showed that the presence of snail mucin aroused the immune system leading to a stronger immune based resistance against aggressive factors.

800mg/kg mucin offered a significant degree of gastro-protection as indicated by the ulcer index, percentage inhibition and gastric mucus (3.00 ± 0.35), (83%) and (0.31 ± 0.07) respectively compared to doses of 200mg/kg mucin (5.70 ± 0.57), (17%) and (0.24 ± 0.04) respectively and 400mg/kg mucin (4.54 ± 0.55), (53%) and (0.29 ± 0.05) respectively. Mistoprostol however, being a standard drug at a much lower dose of 20 μ g/kg offered the most substantial gastro-protection as has been shown by the ulcer index, percentage inhibition and gastric mucus (1.90 ± 0.41), (91%) and (0.40 ± 0.08) respectively. Although the significant difference in the gastro-protective potentials of the standard anti-ulcer drug (mistoprostol) and mucin abound in this study, there is an indication that further increase in the dose of mucin can mask the difference.

5. Conclusion

The non-steroidal anti-inflammatory drugs are essential in the management of acute and chronic pain in addition to inflammation, osteoarthritis and rheumatoid arthritis. Unfortunately, they have been implicated in certain GIT conditions notably gastric ulcer and associated complications. Effort to maximize the benefits associated with the use of NSAIDs for clinical purposes may be traced to the level of drug formulation where inclusion trial may be carried out on *A. marginata* mucin with the view of eliminating or reducing the ulcerogenic effect that comes with its consumption for clinical purposes.

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