

Evaluation of Influence of *Archachatina marginata* Mucin on the Body Weight and Some Gastric Secretions of Wistar Rats Induced with Gastric Ulcer

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Authors' contributions

This work was carried out in collaboration among all authors. Author RAA conceptualized and designed the research. Authors KMR and OOO participated in the laboratory analysis of samples. Author EMU provided the manuscript with which the work was drafted, managed statistical analysis and edited galley proof. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the influence of *A. marginata* mucin on the gastric secretions and body weight of wistar rats induced with gastric ulcer. Thirty (30) adult male wistar rats were divided into six groups of five rats each. Group I (normal control) was administered with 2ml/kg distilled water, while Groups II, III, IV and V were induced with ulceration via oral administration of 120mg/kg indomethacin. After ulceration had been confirmed successfully induced, while Group II was left untreated with mucin, III, IV and V were treated with 200mg/kg, 400mg/kg and 800mg/kg mucin respectively. Meanwhile, Group VI was administered with 20mg/kg omeprazole (standard drug). Body weight of rats was determined after induction (initial) and after treatment (final). The

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values recorded for free acidity (FA), total acidity (TA), pepsin activity (PA) and acid output (AO) for the untreated ulcer rats (Group II) were significantly high ($63.20 \pm 7.66 \text{ mEq/L}$), ($91.40 \pm 6.34 \text{ mEq/L}$), ($1.63 \pm 0.09 \mu\text{Eq/L/4hr}$) and ($0.43 \pm 0.04 \mu\text{g/ml}$) respectively. However, significant reduction in the aforementioned parameters was established with mucin treatment in a dose dependent manner, such that Group V administered with 800 mg/kg manifested the lowest level of FA ($47.40 \pm 7.19 \text{ mEq/L}$), TA (72.00 ± 6.36), PA ($1.39 \pm 0.07 \mu\text{g/ml}$) and AO ($0.16 \pm 0.05 \mu\text{g/ml}$) which was not significantly different from the values recorded for Group VI treated with the standard drug (omeprazole). The values recorded on the body weight of Group II reduced with the induction of gastric ulcer. However, a dose dependent increase was observed in all treated groups. In conclusion, through this study, it has been established that *A. marginata* mucin could be explored for the development of anti-ulcer therapy.

Keywords: Mucin; *Archachatina marginata*; omeprazole and indomethacin.

1. INTRODUCTION

The aggressive and protective factors of the stomach constitute the gastric secretions of the said tissue and consist of pepsin derived from the inactive pepsinogen in the chief cells, hydrochloric acid which is synthesized by the parietal cells of the stomach, the mucus which is formed by neck cells of the gastric mucosa [1]. The integrity of the gastric mucosa is maintained by a delicate balance between these two important factors [2]. It has however been discovered that imposition of certain elements such *Helicobacter pylori* infection as well as the non-steroidal anti-inflammatory drugs can adversely affect this balance [3].

Gastric ulcer as the name implies is an ulcer in the stomach which results when the balance between the aggressive and the protective factors is distorted. It is found in both developed and developing countries of the world [4]. An estimated 80% of the populations of the developing countries suffer from this disease with more women than men being affected [5]. Among other symptoms, loss of appetite that generally characterise ulcer conditions has been implicated which has in turn resulted to reduced food intake and consequently undernutrition

Mucin belongs to a family of large extracellular, high molecular weight O-glycosylated proteins that are formed by tandem repeats of amino acid chain rich in cysteine, serine, and threonine coated with oligosaccharide side chain [6]. Mucin from the mucilage of *Archachatina marginata* has demonstrated wound healing and bactericidal activities being effective against both gram-positive and gram-negative bacteria and thus should be explored in research efforts to develop anti-ulcer therapy [7].

2. MATERIALS AND METHODS

2.1 Snails

Africa giant snails (*Acharchatina marginata*) weighing $100\text{--}450 \text{ g}$ were purchased from Benin City, Edo State Nigeria. The snails were transported in a plastic basket to avoid suffocation.

2.2 Animals

Thirty (30) apparently healthy adult male wistar rats were used for the study. The animals were placed in metal cages in a well ventilated room with a 12/12hr light/dark cycles for period of three weeks to acclimatize.

2.3 Mucin Extraction

The fleshy bodies of the snails were removed from their shells. They were placed in 250 ml of water and washed until the mucin was completely 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 with the aid of a mortar and pestle before being stored in an air tight container [7].

2.4 Median Lethal Dose 50% (LD50)

To perform the acute toxicity test on mucin, three groups of three wistar rats each were used. Various groups were separately administered with 10 mg , 100 mg and 1000 mg/kg of mucin orally. Observation on animals lasted for 24hrs for effects of toxicity. In the absence of mortality in any of the groups, another three groups of one rat each was each administered with 1600 , 2900 and 5000 mg/kg of mucin separately. The animals were observed for 48 hrs for signs of toxicity [8].

2.5 Experimental Design

Thirty (30) apparently healthy 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 all groups except the normal control.

Group 1: Rats were administered 2ml/kg b.w distilled water (Normal control).

Group 2: Rats were administered a single 120mg/kg b.w indomethacin only (Negative control).

Group 3: Rats were administered (Mucin_{200mg/kg} b.w).

Group 4: Rats were administered (Mucin_{200mg/kg} b.w).

Group 5: Rats were administered (Mucin_{200mg/kg} b.w).

Group 6: Rats were administered (Omeprazole_{20mg/kg} b.w).

2.6 Collection of Gastric Juice

Firstly, each rat was anaesthetised by intravenous administration of 50mg/kg of ketamine hydrochloride. After three minutes, midline incision was performed on the rats. The pyloric region was located and ligated using chromic cat gut. The skin was sutured by interlocking pattern with the aid of a chromic cat gut (size 2.0). The rats were taken to a starvation cage and allowed to recover. Animals were sacrificed by cervical dislocation seven hours after indomethacin administration. The stomachs were excised carefully by keeping the esophagus closed. The stomach was opened along the greater curvature and gastric juice was emptied into a suitable container [9,10].

2.7 Estimation of Total and Free Acidity

Exactly 10ml of gastric juice sample was placed in a conical flask before 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 recorded as the free acidity. To the content, 2-3 drops of phenolphthalein indicator 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 [1].

2.8 Acid Output

This was calculated by the method of Ishizuka *et al* [11] thus: Acid output = Acidity × volume of gastric juice.

2.9 Determination of Pepsin Activity

The enzymatic activity of pepsin in undiluted gastric juice was determined according to the method described by Prino *et al* [12].

2.10 Percentage Inhibition

This was calculated according to the method of Hano *et al* [13] using the formulae below

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

2.11 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 prior to staining with haematoxylin and Eosin stain. Each section was examined using a light microscope of magnification ×100.

2.12 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. RESULTS

Table 1 includes values generated from the evaluation of gastric secretions obtained from ulcerated stomach tissues of rats treated with mucin obtained from *A. marginata*. The six groups of rats were distinguished by the treatment administered. Group II (negative control) which was induced with gastric ulcer without treatment, manifested elevated level of free acidity, total acidity, gastric acid output and pepsin activity which was significantly different from the values recorded on the normal control. However, mucin treatment resulted in a dose dependent reduction in the values recorded on the studied parameters.

Table 1. Values on the gastric secretions obtained from the ulcerated stomach tissues of rats treated with *A. marginata* mucin

Treatment groups	Free acidity (mEq/L)	Total acidity (mEq/L)	Acid output (μEq/L/4hr)	Pepsin activity (μg/ml)
Group I (2ml/kg distilled water)	48.40 ± 7.95 ^b	76.80 ± 9.78 ^b	0.16 ± 0.04 ^b	1.36 ± 0.06 ^b
Group II (indomethacin only)	63.40 ± 7.66 ^c	91.40 ± 6.34 ^c	0.43 ± 0.04 ^c	1.63 ± 0.09 ^d
Group III (Mucin ₂₀₀)	60.20 ± 9.80 ^c	86.80 ± 7.66 ^c	0.42 ± 0.03 ^c	1.53 ± 0.07 ^c
Group IV (Mucin ₄₀₀)	45.40 ± 7.79 ^b	72.00 ± 6.36 ^b	0.20 ± 0.04 ^b	1.40 ± 0.06 ^b
Group V (Mucin ₈₀₀)	47.40 ± 7.19 ^b	72.00 ± 6.36 ^b	0.16 ± 0.05 ^b	1.39 ± 0.07 ^b
Group VI (Standard ₂₀)	34.00 ± 5.40 ^a	54.80 ± 5.35 ^a	0.07 ± 0.03 ^a	1.24 ± 0.06 ^a

Values are means ± SD of five determinations.

Values with different superscript in a column are significantly different (P<0.05)

Table 2. Changes in body weights of ulcer rats treated with *A. marginata* mucin

Treatment groups	Mean initial body weight (g)	Mean final body weight (g)	Change in body weight (g)	% Change in body weight
Group I (Normal control)	158.8 ± 5.93	189.40 ± 4.77	30.6 ± 1.16	16.16
Group II (Negative control)	154.80 ± 4.48	123.60 ± 4.33	30.4 ± 0.15	24.7
Group III (Mucin ₂₀₀)	152.20 ± 3.89	156.80 ± 3.42	4.6 ± 0.47	2.93
Group III (Mucin ₄₀₀)	153.20 ± 5.97	155.20 ± 5.35	2.0 ± 0.62	1.28
Group IV (Mucin ₈₀₀)	157.80 ± 1.62	161.40 ± 6.67	3.60 ± 5.07	2.23
Group V (Standard ₂₀)	154.80 ± 5.71	165.50 ± 14.1	10.70 ± 8.42	6.48

Values are means ± SD of five determinations, an arrow pointing downward indicate weight loss in the study group

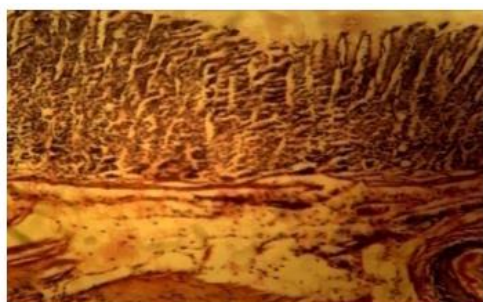
Table 2 shows the changes in body weight of gastric ulcer induced rats treated with *A. marginata* mucin. Ulceration in Group II rats which was left untreated resulted in a significant weight loss which characterised the study group. However, weight gain was recorded on the ulcer induced groups of rat treated with *A. marginata* mucin which could be as a result of enhanced feed intake resulting from improved gastrointestinal health of the treated groups.

4. DISCUSSION

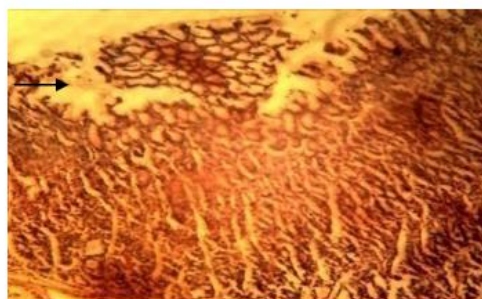
The balance between the aggressive and the protective factors of the stomach accounts for a viable and healthy gastrointestinal tract; this implies that a distortion in this balance will trigger the pathogenesis of gastric ulcer. Loss of weight and undernutrition suffered by gastric ulcer patients as a result of reduced food intake calls for urgent attention among researchers. Table 1. indicates the values on the gastric secretions of stomach tissue of rats treated with *A. marginata* mucin. Oral administration of indomethacin to experimental rats significantly (P<0.05)

increased free and total acidities, pepsin activity and consequently acid output and decreased gastric mucus. However, significant (P<0.05) reduction of the aforementioned parameters was observed with 400mg/kg and 800mg/kg of mucin. This may be attributed to presence of zinc in mucin derived from *A. marginata* which has been scientifically proven to accelerate the restorative pathway in gastric ulcer healing [13] Opoka *et al*, 2010), thus, resulting in the generation of the damaged mucosa and increased secretion of mucus and consequent suppression of gastric acidities, output and volume. This result is consistent with the finding of Kirchhoff *et al* [14] which reveals that zinc salt inhibited acid secretion in isolated rat and human gastric glands.

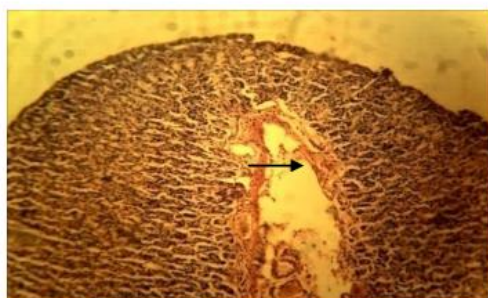
Table 2 shows changes in body weight of ulcer rats treated with *A. marginata* mucin. Reduction in body weight was observed in the group given only indomethacin by 24.7% when compared to the normal control group which increased in body weight by 16.2%. However, weight gain was observed in the treated groups.



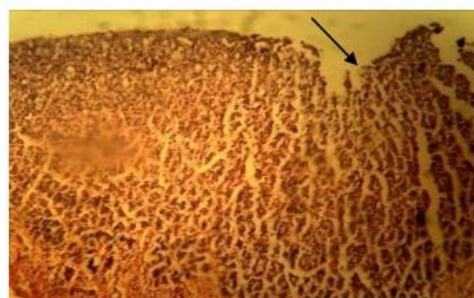
A (normal CTRL)



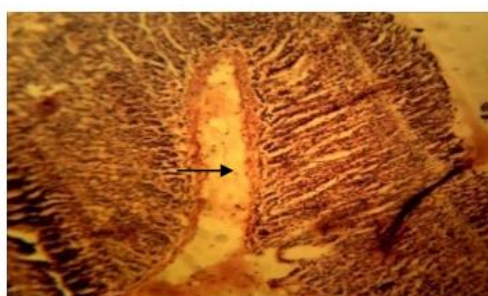
B (negative CTRL)



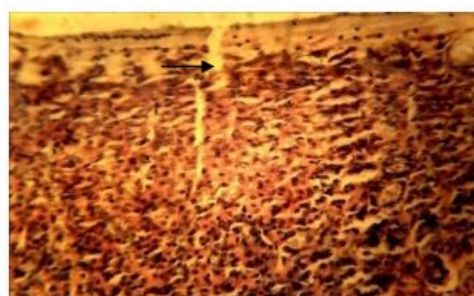
C (Indo+ Mucin_{200mg/kg})



D (Indo+ Mucin_{400mg/kg})



E (Indo + Mucin_{800mg/kg})



F (Indo + omeprazole_{20mg/kg})

Plate A: shows the photomicrograph of mucosa of rats administered with 2ml/kg distilled water. **Plate B:** is the representation of the stomach tissue of the negative control i.e administered with 120mg/kg b.w indomethacin only showing that oral administration of indomethacin caused a serious focal erosion of the mucosa layer of the stomach. **Plate C:** is the photomicrograph of stomach tissues of rats with ulcer treated with 200mg/kg mucin showing a minimal surface of injury. **Plate D:** is the photomicrograph of stomach tissue of ulcerated rats treated with 400 mg/kg mucin displaying a minimal ulcer area. **Plate E:** Treatment with 800mg/kg mucin resulted in a reduced ulcer area while the standard drug (omeprazole) caused an obvious regeneration of the disrupted tissue integrity as shown in plate F.

5. CONCLUSION

The balance between the opposing factors (gastric secretions) of the gastric environment is indispensable to gastrointestinal health and consequently, adequate food intake, absorption

and nutrient metabolism. Distorting this balance however results to gastrointestinal injury and its attendant consequences including weight loss due to loss of appetite and reduced food intake. Results from this study have shown that mucin derived from *A. marginata* has the potential to

restore a derailed gastrointestinal health resulting from gastric ulceration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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