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Original article

Anti-ulcer potentials of phylum mollusca (tropical snail) slime

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Abstract

Objective: The effectiveness of the slimy substance in snail to regenerate and repair damaged areas on its body/shell lead to this investigation. **Methods:** The anti-ulcer´property of snail slime extracted from phylum mollusca (tropical snail) from the giant African snail *Archachatina marginata* (Fam. Arionidae) was investigated using histamine, stress and indomethacin-induced ulcers. The solubility profile of extract was investigated in different solvents and at different temperatures. Chemical analysis was carried out to determine the types of constituents present in the slim, while acute toxicity test was carried out to evaluate its profile of toxicity. The effect of the snail slim on gastrointestinal motility was investigated in mice, while the guinea pig ileum was used to study the effect of the extract on contraction produced by acetylcholine and histamine. The snail slime contained copious quantity of protein, with varying amounts of simple sugars, carbohydrates and fats. The slime was not soluble in most common solvents and increases in temperature, did not appear to increase its solubility. **Results:** The result further indicated that although the snail slime exhibited significant ($P<0.05$) anti-ulcer induced by stress and histamine, it was most potent against ulcer induced by indomethacin. The snail slime potently inhibited gastrointestinal movement in mice in a dose-dependent manner; however, it was not able to inhibit contraction induced by acetylcholine and histamine in guinea pig ileum. **Conclusion:** The snail mucin possesses potent antiulcer properties without any toxic effect. The mechanism responsible for the anti-ulcer property may not be postulated with certainty but cytoprotective and anti-spasmodic activities are most likely to be involved.

Keywords: Anti-ulcer activity; *Phylum mollusca*; Snail slime

INTRODUCTION

Although the number of biological active compounds derived from animal sources is limited, there has been an immense interest, in recent years, in the use of marine creatures as potential sources of drugs and biologically active materials[1]. This resurgence of interest arose because some animal sources has been found promising as a potential source of biologically active compounds; for example, the dried and powdered toad skin contain some cardioactive principles and was used in the treatment of dropsy prior to the widespread adoption of digitals[1].

Sequel to the above, the anti-ulcer property of the slim snail isolated from Phylum mollusca (Tropical snail) is the subject of this study. The land snail (tropical snail) belongs to the class Gastropoda. Different species are abundant in Africa, Europe, India and in some other parts of the world. The land snail is always numerous in the rainy season where they harbor and survive in most humid terrestrial

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habitat. The spirally coil shell, into which the animal can easily retract, form a conspicuous means of identification[2]. The snail is found mainly in forest and around bushes in some areas in the tropic zones. In Nigeria, it is called by different names depending on geographical location. In Eastern Nigeria (Igbo), it is called 'Ejula' while in Northern Nigeria (Hausa) and Western Nigeria Yoruba), it is called 'Dodon kodi' and 'Igbi respectively. Snail contains enormous amount of protein and it is prepared as a special delicacy in some parts of Nigeria.

Snail elaborates a lot of slimy substances even when it moves along its path, the slimy substance, otherwise known as 'snail slim', is however used by the snail to quickly regenerate its own shell and skin when damaged. The regenerative capacity of snail slime, and the fact that peptic ulcer is characterized by gastrointestinal mucosal damage, lead to this investigation for possible anti-ulcer activity.

MATERIALS AND METHODS

Collection and identification of snail

Snails were collected between the months of July and September, from Nsukka markets, in Enugu State, Nigeria and was botanically identified by Mr. Ugwu of Department of Zoology, University of Nigeria, Nsukka.

Animals

All the animals used were obtained from the animal house of the Department of Pharmacology and Toxicology, university of Nigeria, Nsukka and included adult albino mice of either sex (22 - 31g), adult albino rats of both sexes (140 - 210g) and guinea pigs (370 - 450 g). The rats and mice were fed with standard pellets (Guinea feeds PLC, Nigeria) while the guinea pig was fed with a local grass, Penicum maximum L. The animals were maintained under standard 12-h light/dark cycle throughout the duration of the study.

Drugs and chemicals

Acetylcholine hydrochloride, histamine hydrochloride (Sigma, St. Louis MO, USA), indomethacin (Shanghai Med and Health, China), Cimetidine (Synmedic Lab., India), Tween 85 (Janssen, Belgium), glucose (M &B, England), magnesium chloride heptahydrate, sodium chloride (BDH, England), potassium chloride (Hopkin and William, England), sodium carbonate, (Reidel - De Haen Ag, Germany), calcium chloride, acetone.

Preparation of snail slime extract

The snail was washed with clean water to remove dirt and dust particles on the shells. The inner content of the snail was separated from the shells by a mechanical means involving the use of a spirally coiled rod inserted to remove the fleshy body from where the excretory materials were removed. The fleshy parts were then placed in 200 mL of water washed until the mucin was completely washed off. The extract was precipitated using acetone, lyophilized in a lyophilizer, to obtain dry brown flakes of snail slime extract, which was then pulverized into fine powder bottled and stored in a refrigerator.

Tests for chemical constituents

Preliminary chemical analysis was carries out to check for the presence of sugar, carbohydrates, proteins and fixed oil, using standard procedures[3].

Determination of solubility profile of the snail slime extract

The solubility of snail slime in different solvent was determined by dispersing a definite quantity of the snail slime in a definite volume of acetone, alcohol, 0.1N sodium hydroxide, 0.1N ammonium hydroxide, 0.1N sulphuric acid, 0.1N hydrochloric acid and finally dimethylsulphoxide at temperatures of 27, 35 and 40 ℃.

Whole animal experiments

Acute toxicity test

Acute toxic profile of the snail slime was assessed using the method described by Lorke[4].

Test for anti-ulcer activity

The snail slime, suspended in 3% Tween 85, was tested for anti-ulcer activities using three models of experimental gastric ulcers. These included indomethacin[5], histamine[6]-induced ulcers. Two dose levels of the extract were employed in the study in

each of the models. Twenty rats were used for each model. They were divided into four groups of five rats each. Groups one and two were given 5 mL. Of 3% Tween 85 and cimetidine 100 mg/kg respectively while groups three and four were administered 250 and 500 mg/kg of the extract respectively. All administrations were by oral route. Thirty minutes later, ulcers were induced with the respective agents (indomethacin 30 mg/kg p.o histamine 2 mg/kg p.o, and cold restrain for stress model). After 8 h (for indomethacin and histamine), the animals were sacrificed and the stomach removed and opened along the greater curvature. The stomach was raised under a stream of water and pinned flat on a corkbod. The stomachs were observed with a hand lens (×10). Erosins formed on the glandular portions of the stomach were counted and each given a severity rating on a 1 3 scale based on the diameter of the ulcer (viz 1 < 1 mm; 2 > 1mm; 3 > 2 mm)[7]. The overall total divided by a factor of 19 was designated as the ulcer index (UI) for that stomach.

For stress-induced ulcer, 30 min after administration of drugs, each animal was introduced into a metal restrainer and kept for 18 h in a refrigerator maintained at 10-15 ℃. The animals were then sacrificed, their stomachs removed and opened along the grater curvature and the ulcer index calculated as above. Cimetidine was used for comparison. The percentage ulcer protection was calculated as follows[8]:

$$\text{Percentage ulcer protection} = \left[1 - \frac{(\text{Ulcer index for test agent})}{\text{Ulcer index for negative control})}\right] \times 100$$

Gastrointestinal motility test

Twenty albino mice, staved for 24 h but had free access to water, were divided into four groups of five animals per group. The first group received 5% tragacanth mucilage (20 mL/kg) while the second, third and the fourth groups were given 20, 40, and 80 mg/kg of the slime extract respectively. All administrations were by the oral rout. Five minutes after drug administration, 0.5 mL of a 5% charcoal suspension in 5% tragacanth mucilage was administered to each animal orally. The animals were sacrificed, 30 min later and the abdomen opened. The percentage distance of the small intestine (from the pylorus to the caecum) by the charcoal plug in the different groups was determined[9].

Isolated tissue preparation

The experiments were sent up as described previously[10]. Segments of the guinea pig ileum of about 2 cm long were suspended in an aerated 30 mL organ bath filled with Tyrode solution, maintained at 37 ± 1℃. The composition of the tyrode solution was (g/l): NaCl 8.00, KCl 0.20, $CaCl_2$ 0.20, $NaHCO_3$ 1.00, $NaHPO_4$ 0.05, and allowed to equilibrate for 60 min, during which the bathing fluid was changed every 10 minute to prevent the accumulation of toxic metabolite. At the end of 60 min equilibration period, the responses of the tissue to acetylcholine, histamine and extract were established. Subsequently, the effects of the slime extract on the counteraction produced by the agonists on the isolated tissue were established. Thirty seconds contact time was allows for the action of the standard agonists. Each agonist was used on a separate tissue and the experiments were repeated in triplicates. The responses were recorded using isotonic traducer, 7006 (Ugo Basile, Italy) connected to a 2 - channel recorder "Germini" 7070.

Statistical Analysis

All results were expressed as mean ± SE. Student's t-test at $P < 0.05$ was used to assess statistical significance in various groups of animals.

RESULTS

Chemical analysis of the snail slime indicated that it contained abundant quantity of protein, with varying amounts of simple sugars, carbohydrates and fats (Table 1) Determination of the solubility profile of the extract showed that at the temperatures of 27, 35 and 40 ℃, the snail slime was not soluble in acetone, ethanol, sodium hydroxide, suphuric acid, ammonium hydroxide and hydrochloric acid solutions, However, it was slightly soluble in dimethysulphoxide at the temperatures of 35 and 40 ℃; indicating that increase in temperature had little solubility enhancing effect on the snail slime. Acute tox-

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icity test revealed that even at 5 000 mg/kg, the crystal did not induce any obvious sign of toxicity in mice.

Anti-ulcer study demonstrated that the snail slime exhibited significant ($P<0.05$) anti- ulcer in indomethacin, stress- and histamine -induced ulcer (Table 2). Although the anti-ulcer activity of the extract against ulcer induced by histamine was not clearly dose-dependent, the ulcer protective effect displayed by the two dose levels employed were still statistically significant ($P<0.05$). The slime inhibited the gastrointestinal movement in a graded manner and at 40 and 80 mg/kg, it produced significant decrease in intestinal motility in mice (Table3)

The extract neither contracted nor relaxed the isolated guinea pig ileum preparation. However, it dose-dependently potentiated histamine-induced contraction, while slight change in contractile response was noted when it was tested against acetylcholine -induced contraction (Table4).

Table 1 Chemical constitutions of the snail slime extract.

Chemical Constituent	Observation	Inference
Simple sugar	+ +	Present
Carbohydrates	+ +	Present
Proteins	+ + +	Present
Fats	+	Present

+ = Present, + + = moderately Present, + + + = abundantly Present

Table 2 Effect of the snail slime extract on ulcer induced by different ulcerogens (percentage ulcer protection is indicated in parenthesis).

Ulcerogenic Agents	Ulcer index (percentage ulcer protection in parenthesis)			
	Tween85 (5mL/kg)	Cimetidine (100 mg/kg)	Snail Slime(250 mg/kg)	Snail Slime(500 mg/kg)
Indomethacin	8.37 ±1.45	2.67 ±0.33 *	4.67 ±0.67 *	5.33 ±1.44 *
(30 mg/kg p. o)	-	(68.11%)	(36.33%)	(44.21%)
Histamine	6.67 ±1.40	2.50 ±0.67 *	3.50 ±0.69 *	3.93 ±0.97 *
(2 mg/kg p. o)	-	(17.79%)	(47.53%)	(41.08%)
Stress	7.35 ±0.35	2.50 ±0.58 *	5.32 ± 0.58	4.67 ±0.88 *
	-	(65.90%)	(27.29%)	(36, 63%)

Table 3 Effect of the snail slime extract on gastrointestinal motility in mice.

Treatment	Percentage length of intestine (cm, mean ± SEM)	Distance traveled (cm, mean ±SEM)	Distance traveled (cm)
5% Tragacanth Mucilage (5 mL/kg)	67.17 ± 2.44	64.63 ± 0.17	69.42
Snail slime(20 mg/kg)	65.93 ± 1.09	35.30 ±1.70	53.54
Snail slime(40 mg/kg)	60.27 ± 2.97	13.90 ± 1.73 *	22.91
Snail slime(80 mg/kg)	66.77 ± 0.93	7.40 ± 0.49 *	11.08

* Significant $P<0.05$; $n=5$

Table 4 Effect of Snail Slime on Maximal Contraction Produced by Acetylcholine and Histamine in Guinea pig ileum

Treatment	Percentage Maximal Response	
	Acetylcholine	Histamine
Snail lime (1 mg/mL)	100.00 ± 0.00	128.57 ± 1.47
Snail slime (2 mg/mL)	110.00 ± 0.81	142.86 ± 2.46
Snail slime (4 mg/mL)	120.00 ± 0.56	257.14 ± 0.85
Snail Slime (8 mg/mL)	80.00 ± 1.20	328.57 ± 3.69

DISCUSSION

Edible flora and fauna have advantage in toxicity considerations based on their long-term use by humans and one might expect bioactive compounds obtained from them to have low animal and human toxicity. This is sustained in the acute toxicity test, which indicated that even at 5 000 mg/kg; the snail slime did not induce any obvious sign of toxicity in mice. This is an indication of its high safety profile as substances with LD_{50} above 5 000 mg/kg have been classified as being generally safe[4,11].

Indomethacin and related no-steroidal anti-inflammatory drugs induce gastric damage by inhibition of prostaglandin synthesis and chronic administration of these drugs is associated with blood loss from the upper gastrointinestinal tract, ulcerogenesis, and aggravation of pre-existing peptic ulcer[12-14]. The best ulcer inhibitory potency of the snail slime was against ulcer induced by indomethacin. This is an indication of strong cytoprotection and ability to counteract inhibition of prostaglandin synthesis induced by indomethacin.

The abundant quantity of proteins noted in the extract may be partly responsible for the anti-ulcer property impervious protective pellicle on the lining that will help in resisting the attack of propeotytic enzyme[15]. The copious protein content of the snail slimy, from impervious shield on the ulcer creaters, producing anti-ulcer activity. Mucin motif has been found to be used as rectal absorption enhancer[16], the degree of water sorption by the pedal mucus trail of land snail has also been investigated[17]. Amongs other medicinal uses of snail mucin, it has been found to possess antibacterial activity[18].

The result of gastrointestinal motility test indicates that the extract produced significant ($P<0.05$) inhibition of gastrointestinal movement in mice. It has been demonstrated that agent reduce gastric hyper motility are beneficial anti-ulcer agents[19]. Delay in gastric emptying of stomach will prevent speedy evaporation of the stomach content, thus ameliorating ulcer pain and promoting healing of ulcers[20]. Drugs affecting motility, frequency and consistency of diarrhoea also affect secretion[21]. The mechanisms of action of many plants used in the treatment of ulcer in folk medicine have been pharmacologically demonstrated to be partly as a result of inhibition in intestinal motility[22-24]. It is likely that the anti-ulcer activity of the extract may partly be due to its ability to inhibit gastrointestinal movement.

Histamine and acetylcholine are important endogenous spasmogens and agents that inhibit their contractions may represent good antispasmodic agents. Antispasmodic agents such as hyoscine methyl bromide and propantheline are employed in the management of ulcer[25]. However, the result of in vivo pharmacological study ingénue pig ileum did not indicate inhibitory activity against contraction induced by either acetylcholine or histamine. This is contrary to the potent antispasmodic effect noted with the snail slime in the vivo study represented by significant inhibition of gastrointestinal motility. This contradiction may be possible because some drugs have been shown to be effective in vivo but inactive in vitro, an indication that probably such drugs are metabolized in the animal to release the active compound[26].

In conclusion, snail slime from Phylum mollusca significantly protected animals against ulcer induced

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by different experimental ulcer models. The mechanism of action is yet to be clearly understood; it may be related to the cytoprotective and anti spasmodic activities of the snail slime.

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