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EVALUATION OF THE CYTOPROTECTIVE EFFECTS OF THE FORMULATION VARIABLES OF SNAIL MUCIN AND CIMETIDINE IN RATS

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ABSTRACT

The cytoprotective effect of four formulations (Batch A, Batch B, Batch C and Batch D) of snail mucin and cimetidine were evaluated using three animal (albino rats) models (indomethacin, histamine and stress-induced ulcer models). Results show that the cytoprotective effect of the formulation variables increased with increase in the concentration of snail mucin extract. The negative control which was Tween 85 had no significant effect on the ulcer ($p<0.05$). This suggests that a combination of snail mucin and cimetidine in drug delivery may have some therapeutic importance and should be properly harnessed.

Keywords: *peptic ulcer; snail mucin; cimetidine; cytoprotective; animal models*

INTRODUCTION

Peptic ulcer is a breach[1] or a sore[2] in the lining (mucosa) of the digestive tract produced by digestion of the mucosa by acid-pepsin[3]. Peptic ulceration develops in the epithelial lined surfaces exposed to the acid secretion of the gastric glands. The sites most often affected are the stomach itself, the duodenum bulb and the distal part of the oesophagus[4].

It is known that non-steroidal anti-inflammatory agents are capable of causing a variety of acute gastric lesions, which may culminate in gastric erosions or even frank ulceration. Sometimes drug induced mucosal damage is accompanied by extensive haemorrhage into the mucosa[5]. Histamine, by stimulating the cAMP-dependent pathway, leads to increased gastric secretion[6].The role of stress is controversial[7]. Wilson and Waugh, 2000[8] reported that with stress, there is increase in secretion of noradrenaline and adrenaline hormones that cause constriction of the blood vessels supplying the alimentary canal.

The presence or absence of peptic ulcer is determined by the delicate interplay between aggressive factors (secreted gastric acid and pepsin) and defensive factors (mucosal resistance). Peptic ulcer is produced when the aggressive effects of acid-pepsin dominate the protective effects of gastric or duodenal mucosal resistance[5,9].

Mucus is a highly viscous fluid secreted by mucous membranes and glands, consisting of mucin, leukocytes, inorganic salts, water and epithelial cells[10]. It lubricates the walls of the tract and protects them from digestive enzymes[11]. It is highly biocompatible, non toxic and easily biodegradable. In future, it may play a key role in the pharmaceutical industry as a drug delivery agent[12].

H_2-receptor antagonists have been observed to protect experimental animals from gastric ulceration induced by stress, pyloric ligation, aspirin, H_2- receptor agonist, or cholimimetics but with no consistency on gastric emptying rate[13].

Although several chemical challenges still need to be met in this area of research, it is reasonable to expect that with the advent of drug-mucin combinations, the ravages of gastric and duodenal ulcers will be reduced.

EXPERIMENTAL

Materials

The following were procured from their local suppliers and used without further purification; cimetidine, indomethacin, histamine, snail mucin extract and distilled water.

Animals

The giant African snails, *Archachatina marginata*, Fam. Arionidae were procured from Ibagwa-Nkwo market in Nsukka zone of Enugu State. White albino rats (110-180g) of either sex, obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, were used for the study. While in the animal house, they had free access to food and water and were maintained under standard conditions**.** All animal handling and experiments were conducted following the guidelines stipulated by University of Nigeria Research Ethics Committee on Animal Handling and Use.

Methods

Extraction of snail mucin

After procurement, the shells of 50 giant African land snails were knocked open at the apex and a spirally coiled rod inserted to remove the fleshy body. This fleshy part was placed in 250 ml of water and washed

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each time until the mucin was exhaustively washed off. The mucin was pooled together and lyophilized manually. It was subsequently dried in air. The flakes were pulverized and stored in an air-tight container until used[14].

Preparation of exogenous mucin and cimetidine admixtures

Binary admixtures of cimetidine and exogenous mucin were mixed thoroughly in the ratios of 1:1; 1:2; 1:3; 1:4 respectively. Table 1 shows the drug-mucin formulation variables.

Table 1: *combination ratios of drug: mucin*

Batch	Cimetidine (mg)	Snail Mucin (mg)
A	500	-
B	500	500
C	500	1000
D	500	1500
E	500	2000

Ulcer experiments in albino rats

White albino rats (110-180g) of either sex, obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, were used for the study. Three models of inducing experimental gastric ulcers were used to assess the anti-ulcer activity of the formulation batches. The models were: indomethacin, histamine and stress-induced ulcer models. The method followed was as described by Akah and Nwafor [15].

Indomethacin induced ulcer

Rats were fasted for 18 h prior to the beginning of the experiment and there were twenty-four rats for each model. They were divided into six groups of four rats each. The first group (A) received cimetidine only. It served as positive control at a dose of 500mg/kg. The second group (B) received 1:1 combination of cimetidine and exogenous snail mucin. The third group (C) received combination of cimetidine and snail mucin at a ratio of 1:2. The fourth group (D) received combination of cimetidine and exogenous snail mucin at a ratio of 1:3 and the fifth group (E) received cimetidine and exogenous snail mucin at a ratio of 1:4. The sixth group (F) group received 3 % tween 85. Thirty minutes later, ulcer was induced by administering 30mg/kg body weight of indomethacin (dissolved in 3 % tween 85) to the different groups of animals respectively. All administrations were by the oral route.

After 8 h for indomethacin- induced ulcer model, the animals were killed and the stomachs removed and opened along the greater curvature. The stomachs were rinsed under a stream of water and pinned flat on a corkboard. The stomachs were observed with a hand lens (x10).

Histamine induced ulcer

Ulceration was induced in experimental animals using 2mg/kg of histamine administration orally to the animals. The procedure for indomethacin-induced ulcer was followed. Ulceration was observed equally at the junction between the antrum and fundus of the stomach. The ulcer was viewed and counted, each given a severity rating as follows according to Main and Whittle [16] : $\leq$ 1 mm = 1; > 1 $\leq$ 2 mm = 2; > 2mm = 3. The overall total divided by a factor of 10 was designated as the ulcer index (ui) for that stomach.

Based on their intensity, the ulcer can also be given scores as follows: 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer, 3 = perforated ulcers [17].

Ulcer index = arithmetic mean of intensity in a group + number of ulcer positive animals / total number of animals[18]

Cold restrained stress induced ulcer

The method demonstrated by Suzuki [19] was adopted to induce ulcer by stress in the laboratory animals. The cimetidine – mucin ratios were administered 30 min prior to subjecting the animals to stress. The animals were placed in a restrained cage and the cages were placed at refrigerator temperature for 3h. The animals were sacrificed and then the ulcer protection was determined[20, 21].

Percentage ulcer protection = 1- (ulcer index for test agent/ulcer index for negative control) x 100

Gastric motility test in albino rats

Twenty rats of either sex were randomly divided into five animals per group. The animals were starved for 24 h prior to the experiment, but had free access to water. One group received Tween 85 (5 ml/kg), while the remaining four groups received the different doses of the admixtures. All administrations were made by the oral route. Five minutes after drug administration, 0.5ml of a 5 % charcoal suspension in 10 % aqueous solution of tragacanth powder was administered to each animal. The animals were sacrificed 30 min later and the abdomen opened. The percentage distance of the small intestine (from pylorus to caecum) traveled by the charcoal plug in the treated animals were determined[15]

Statistical Analysis

The results were analyzed using students t-test and were regarded as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Result of ulcer experiment

The results are presented in Table 2a and 2b respectively. Indomethacin-induced ulcer was significantly ($p<0.05$) protected by combinations of cimetidine and snail mucin. It showed a dose dependent protection with the least ulcer index from 1:3 and 1:4; cimetidine: snail mucin combination respectively. Histamine -induced ulcer also showed dose-related protection of the rats. This anti-ulcer study on demonstrated that snail-mucin exhibited significant ($p< 0.05$) anti-ulcer in indomethacin and histamine. The

Table 2a: *Effect of the snail mucin – cimetidine combination on ulcer induced by different ulcerogens*

Agent used	ratio	Number of animals used	ulcer mean index for indomethacin	ulcer mean index for histamine
Cimetidine	1:0	4	26.67 ± 1.43	8.67 ± 1.77
Cimetidine:mucin	1:1	4	5.67 ± 0.65	5.67 ± 2.19
Cimetidine:mucin	1:2	4	11.33 ± 0.35	7.33 ± 2.72
Cimetidine:mucin	1:3	4	3.33 ± 2.03	4.00 ± 0.58
Cimetidine:mucin	1:4	4	3.33 ± 2.03	4.00 ± 0.58

Table 2b: *Percentage ulcer protection of cimetidine – snail mucin against stress induced ulcer*

Agent used	ratio	Number of animals used	% ulcer protection
Cimetidine	1:0	4	41
Cimetidine:mucin	1:1	4	43
Cimetidine:mucin	1:2	4	35
Cimetidine:mucin	1:3	4	0
Cimetine:mucin	1:4	4	0

stress-induced ulcer showed higher protection from the 1:1 combination of mucin: cimetidine than from higher dose combinations. There was no protection from the negative control which was tween 85. Although, the antiulcer activity of the snail mucin-cimetidine combination was not dose dependent, the optimal percentage ulcer protective effect of the 1:1 combination was statistically significant. The snail mucin-cimetidine combination also did not possess any toxic effect on the rats based on long term use by humans. 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 [26]. Snail mucin contains abundant protein which may be partly responsible for the anti-ulcer property of the impervious protective pellicle on the lining that will help in resisting the attack of propeotytic enzyme. The copious protein content of the slimy snail form impervious shield on the ulcer creaters, producing anti-ulcer activity [27]. The goals of therapy for ulcers are to relieve pain, to promote complete healing, to prevent reoccurrence and to prevent the development of complications[22]. Although, the increase of cimetidine: mucin combinations decreased ulcer indices in two of the three models, better protection appears to be shown against indomethacin-induced ulcer suggesting better cytoprotective mechanism of action. The combination did not show significant protection in the stress-induced ulcer especially with the higher dose combinations.

The result of the charcoal meal test is shown in Table 3. The administration of increasing concentration of mucin significantly reduced the charcoal meal transit. Since the small intestine is the primary site of drug absorption, the longer the residence time in this region the greater is the potential for efficient drug absorption assuming that the drug is stable in the intestinal fluid and does not react with endogenous materials to form poor absorbable 'complexes'. The residence time in the small intestine as determined by intestinal motility, [23] may be an important factor with respect to drug bioavailability. The longer a drug is in contact with the absorption site(s) the greater the amount of drug absorbed[24]. In ulcer patients, reduction in gut motility helps to ameliorate the ulcer pain and hasten the healing of ulcer wounds[25].

Table 3: *Effect of test agents on gastrointestinal motility in rats*

Treatment	Dose (mg/kg)	% distance travelled
Tween 85	5 ml	71.04 ± 0.12
Cimetidine : mucin 1:1	5	61.54 ± 1.6
Cimetidine: mucin 1:2	7.5	18.14 ± 1.0
Cimetidine : mucin 1:3	10	12.09 ± 1.9
Cimetidine : mucin 1:4	12.5	9.11±0.1

Significant ($p<0.05$) relative to 3% tween 85

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