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Antisecretory Effects of Watermelon (*Citrullus lanatus*) Juice in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FSO and MEB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author AGT managed the literature searches, carried out the analyses of the study, wrote and edited all subsequent drafts of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate the effects of the juice of *Citrullus lanatus* (watermelon) on gastric acid secretion and pH in Indomethacin-induced ulceration in male albino rats.

Study Design: The experiment was divided into two studies. Under each study, four groups of rats were pre-treated with distilled water (control), 25% watermelon, 50% watermelon and 100% watermelon juice respectively for 30 days.

Place and Duration of Study: Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria, between June, 2011 and July, 2012.

Methodology: Sixty-four animals in total were used for the experiment. The animals were divided into two experimental studies: Study I contained thirty-two rats which were used for the study on ulcerogenesis. Study II also contained thirty-two rats which were used for the study on gastric acid secretion. Each of the experimental studies was further divided into four groups in accordance with the study design.

Results: Rats pre-treated with *Citrullus lanatus* juice exhibited significant dose-dependent reduction of gastric lesions formation ($P < 0.05$). Also, ulcerogenesis in the pretreated groups was significantly lower than that observed with the control ($P < 0.05$).

Conclusion: The results suggest that *Citrullus lanatus* (watermelon) juice has a significant gastroprotective effect in Indomethacin-induced gastric ulceration. One of the mechanisms by which this protective effect is carried out is by its inhibition of gastric acid secretion.

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1. INTRODUCTION

Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *Helicobacter pylori*, non steroidal anti-inflammatory drugs etc.) and local mucosal defensive factors (mucus, bicarbonate, blood flow, prostaglandins etc.). Integrity of the gastro-duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors [1]. Studies have shown a correlation between increased gastric acid secretion and predisposition to peptic ulcer [2,3]. Factors that cause a decrease in basal and maximal gastric acid output will therefore have protective capabilities on the gastric mucosa, acting as a defence against ulcerogenesis, a characteristic that is used in the treatment of ulcers [4].

Watermelon (*Citrullus lanatus*), family *Cucurbitaceae* can be both fruit and plant of a vine-like (scrambler and trailer) plant originally from southern Africa, and is one of the most common types of melon. It has been reported that watermelon juice is naturally rich in citrulline [5]. The amino acid citrulline was first extracted from watermelon and analyzed by Wada in 1930 [6,7], and it has been reported that the body records a significant amount of citrulline after consumption of several kg [8]. The citrulline which exists in watermelon is a known stimulator of Nitric oxide. Similarly, the citrulline in watermelon can be metabolized to L-Arginine [9], a known source of endogenous NO which inhibits gastric acid secretion [10], and also enhances plasma concentrations of pancreatic glucagon which is a physiological inhibitor of gastric acid [11]. Several studies have indicated that NO affects the secretion of gastric acid. For instance, *in vitro* studies have shown that NO stimulates secretion of gastric acid in the mouse [12,13] bulldog [14] and in dogs [15]. However, other investigations have shown that NO inhibits gastric acid secretion in rat [16,17], in gastric glands isolated from rabbits [18], and in mucosa from toads [19]. Studies in humans have equally provided conflicting data [20,21]. A recent study has reported that rats treated with methanolic extract of *Citrullus lanatus* (MECL) seeds showed significant decrease in the gastric volume, free acidity and total acidity and significant percentage inhibition of ulcer [22]. Many experimental studies have been performed to evaluate the anti-ulcer and gastroprotective activities of *Citrullus lanatus* [23,24], however this study was undertaken to assess secretion of gastric acid as a possible mechanism for the anti-ulcer activity of watermelon in Indomethacin-induced gastric ulcers in rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Male albino rats of Wistar strain weighing between 200 and 280g obtained from Pre-Clinical Animal House, College of Medicine, University of Ibadan, Oyo state, Nigeria were used for these studies. They were maintained under standard laboratory conditions and were fed with commercially formulated rat pellets (Ladokun feeds, Ibadan) and tap water given *ad libitum*. Excess feeds and water were removed and replaced daily.

2.2 Watermelon Juice Extraction

Watermelon fruits were purchased from Bodija market, Oyo state, Nigeria. Each watermelon was washed to remove dirt and cut into smaller pieces. The thick outermost back and the

seeds were removed. The remaining red-colored endocarp was blended using an electric blender. A clean sifter was used to separate the juice from the solid particles. Fresh preparations of the watermelon juice were prepared daily.

2.3 Experimental Design

A total of sixty-four (64) animals were used for the study. The animals were divided into two groups, each of thirty-two (32) animals, each group being for a different study. The first study involved animals that underwent experimental Indomethacin-induced peptic ulceration. These were used to assess the degree of ulceration in control and pretreatment test groups. Animals in the second study were assessed for both basal and maximal (histamine-induced) gastric acid secretion. Each of the studies was further divided into a control (distilled water) as well as experimental (25, 50 and 100% watermelon pretreatment) groups.

2.3.1 Determination of ulcerogenesis

This was carried out as earlier described by Elegbe [25]. Twenty-four hours before the experiment, food was withdrawn but the animals were allowed free access to water. Ulcer was induced by single-dose oral administration of Indomethacin (40mg/kg; Merke, Sharp and Dohme). This was dissolved in distilled water to form a suspension and administered to all animals under this study. Four hours after administration, the animals were sacrificed and their stomachs removed and examined for ulcers macroscopically using a hand lens. Gastric lesions and scoring of ulcers were assessed as previously described by Alphin and Ward [26] and modified by Elegbe [25]. This is shown in Table 1 below.

Table 1. Ulcer scoring [26]

Ulcer score	Criteria
0	Normal Stomach/No ulcer
0.5	Punctuate or pinpoint ulcers
1.0	Two or more small haemorrhagic ulcers
2.0	Ulcers greater than 3mm in diameter.

2.3.2 Determination of gastric acid secretion

The study was carried out using the continuous stomach perfusion technique described by Ghosh and Schild [27] and modified by Amure and Ginsburg [28]. This was used together with the titration method described by Olowokorun [29]. However, for basal acid secretory response, early morning gastric contents were collected and thereafter, gastric acid secretory response to histamine was collected.

The animals were anaesthetized with 25% w/v urethane (ethylcarbamate) at a dose of 0.6ml/100g body weight. A tracheal cannula was then inserted via an incision in the neck to ensure normal breathing throughout the course of the experiment. An abdominal incision through the linea alba was made to expose the stomach, and a semitranssection made at the junction of the pylorus with the duodenum. A pyloric cannula was inserted and tied to collect gastric contents. An orogastric cannula was inserted for perfusion of pre-warmed (temperature 37°C) 0.9% normal saline (pH 7) at a rate of 1ml/minute using the Langerdoff's apparatus. The animals were kept warm by a 100 watts electric lamp to prevent

hypothermia. Cotton wool soaked in normal saline (9g NaCl in 1 litre of distilled water) was placed on the opened abdominal cavity to prevent dehydration.

Gastric acid was collected through the pyloric cannula at 10 minutes intervals. In order to determine acidity, 10ml of the stomach perfusate was titrated against 0.0025N Sodium Hydroxide (NaOH) solution with phenolphthalein as indicator. Titrable acidity was expressed in micro (μ) equivalence/litre/10mins. The histamine-induced gastric acid was collected 30 minutes post-surgery at which time a steady (basal) acid secretion had been obtained.

2.3.4 Measurement Of pH

Samples of gastric contents (1ml) were analyzed for hydrogen ion concentration using a pre-calibrated Beckman pH meter. The electrodes were rinsed with distilled water several times and later calibrated using standard buffer solutions of pH 4 and pH 7. This calibration is accurate at ± 0.1 pH

2.4 Statistical Analysis

The results of the experiment were analysed using Analysis of Variance (ANOVA) with Newman Keul's post-hoc analysis test

3. RESULTS AND DISCUSSION

Table 2 below shows the effects of watermelon juice on ulcerogenesis in rats in which gastric ulceration was induced using Indomethacin.

Table 2. Effects of watermelon juice on ulcerogenesis

Grouping	Number of animals per group	Mean ulcer score (Mean \pm SEM)	P-value P=0.05
Control	8	8.69 \pm 0.10	
25% WPT	8	2.31 \pm 0.06	S
50 WPT	8	1.75 \pm 0.10	S
100 WPT	8	0.25 \pm 0.10	S

WPT = Watermelon juice pretreatment; s= significantly different from control.

From Fig. 1, the animals pretreated with 100% watermelon juice had a mean pH of 3.41, which was lowest in the pretreated groups while the other two showed an increase in pH comparable to those pretreated with Sodium Nitroprusside, which had a higher mean pH (3.73) than the control.

The anti-ulcerative activity of watermelon was investigated using Indomethacin. The numbers of ulcers formed (determined by the Mean Ulcer Score) by this anti-inflammatory drug was significantly lower in the animals pretreated with watermelon. All the animals on watermelon supplementation showed a significant reduction in Mean Ulcer Score when compared with rats on distilled water only (Table 2).

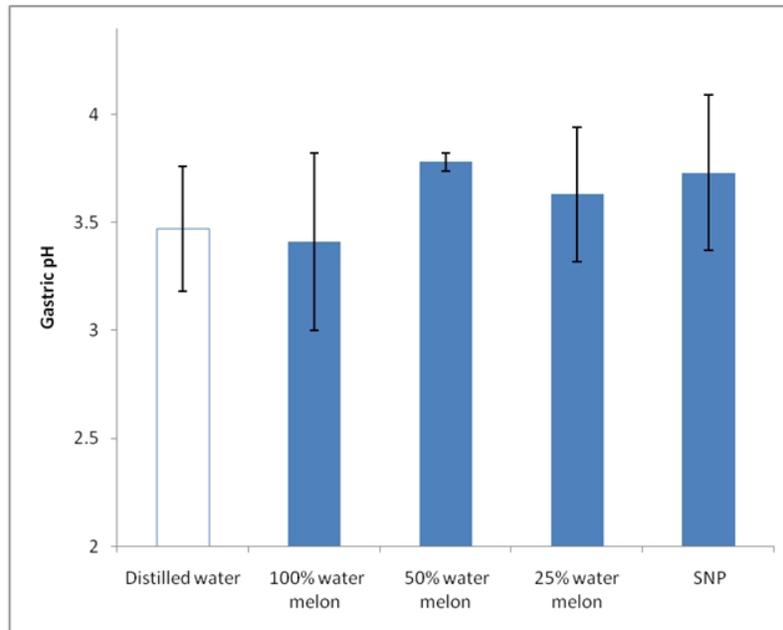


Fig. 1. Effect of watermelon juice on mean gastric acid pH in rats. Values shown are Mean \pm SEM for 8 animals in each group

Table 3. Effect of watermelon juice on gastric acid secretion in rats. Values shown are Mean \pm SEM for 8 animals in each group

Groups	Gastric acid secretion μ Eq/100 body weight
A (Distilled water)	88.75 \pm 29.86
B (100% watermelon)	10.00 \pm 0.01*
C (50% watermelon)	10.50 \pm 2.21*
D (25% watermelon)	31.25 \pm 11.06*
E (10mg/kg Sodium Nitroprusside, SNP)	83.25 \pm 27.52

*Significantly different from the control (Distilled water only) at $P < 0.05$

As can be observed in Table 3 above, gastric acid secretion in all the treatment groups was significantly lower than that in animals given distilled water only (control animals).

Pathophysiology of ulcer is due to an imbalance between aggressive factors (gastric acid, pepsin, *Helicobacter pylori*, non steroidal anti-inflammatory drugs (NSAIDs) etc, and local mucosal defensive factors (mucus, bicarbonate, blood flow and prostaglandins). The integrity of gastroduodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors [1]. When levels of acid and proteolytic enzymes overwhelm the mucosal defence mechanism, ulcer occurs [30]. Also, gastric acid output has considerable effect on the rate of ulcer healing [31].

In the experimental animals, watermelon showed a protective effect on the stomach mucosa against experimentally-induced ulceration. There was a significant reduction ($P < 0.05$) in ulcer formation in test animals pretreated with watermelon juice in concentrations of 25%,

50% and 100% when each was compared individually with the control. This reduction was however not dose-dependent.

The findings of this study also suggest that watermelon causes a significant reduction in gastric acid secretion. When compared individually with the control group, all treatment groups (100%, 50% and 25% watermelon pre-treatment) exhibited a decrease in gastric acid output. This implies that watermelon increasingly inhibits gastric acid secretion as the dose increases. The H⁺ content (pH) of the secreted gastric juice however did not show a significant reduction in all test animals. Sodium nitroprusside, a well-known NO-releasing compound caused a slight reduction in gastric acid secretion, but when compared with the control however this was not significant ($P > 0.05$).

It was earlier reported that Nitric oxide inhibits gastric acid secretion in rats [12,13]. Watermelon is an edible source of L-Citrulline, a compound vital in the vital production of Nitric oxide and its consumption increases the level of citrulline significantly [4]. It is therefore safe to assume that one of the mechanisms by which watermelon causes a decrease in gastric acid secretion is by increasing citrulline levels thereby stimulating an increase in nitric oxide production. This will lead to a decrease in gastric acid secretion as confirmed by Brown et al. [16] and Kato et al. [17], which in turn accounts, at least in part, for the observed and documented gastroprotective effect of watermelon in Indomethacin-induced peptic ulceration. When related to the insignificant decrease observed in animals to which Sodium nitroprusside was administered, it is obvious that the lowering of gastric acid secretion seen in test animals pretreated with watermelon is mainly due to other mechanisms apart from the action of nitric oxide. It is pertinent to state here that other mechanisms of gastroprotection such as increase in mucus secretion, stimulation of prostaglandins, increase in mucosal blood flow etc, could also be involved in the antiulcerative actions of watermelon, however, these are beyond the scope of the present study but should and will be taken into consideration in future investigations.

4. CONCLUSION

In conclusion, the results of this study have shown that watermelon exhibits a gastroprotective, antiulcerative effect on the stomach. The observed significant reduction in mean ulcer count in this work may likely be explained by the antisecretory effect of watermelon, which significantly reduces the formation of ulcers. An inhibition of gastric acid secretion can be adduced to be one of the factors responsible for this protective effect. The inhibition is therefore hypothesized to probably be due to the high levels of Citrulline contained in watermelon, Citrulline being a stimulant for nitric oxide, which has been reported to have antisecretory effects. The study is by no means exhaustive, as other possible mechanisms of nitric oxide's protective capabilities were not explored (e.g. improvement of mucosal blood flow). Also, watermelon contains a lot of active substances, of which Citrulline is only one. Future research work will be focused on the establishment of the roles of these other substances, as well as other pathways of Citrulline and Nitric oxide, in conferring protection against ulcer formation.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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