European Journal of Medicinal Plants
4(4): 394-403, 2014

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Gastroprotective Effects of Masfon–*Aloe vera* Drink: Its Effects on Gastric Acid and Mucus

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Authors' contributions

This work was carried out in collaboration between all authors. Author OVO designed the study and wrote the first draft of the manuscript. Authors IDE and NVU performed the statistical analysis, managed the literature searches and wrote the protocol. Authors AAB and OEE revised the study critically for intellectual content. All authors read and approved the final manuscript.

Original Research Article

Received 9th November 2013
Accepted 27th December 2013
Published 9th January 2014

ABSTRACT

Masfon – *Aloe vera* drink was employed in this study to ascertain its effect on gastric acid secretion, mucus output and cytoprotection. The study utilized forty five (45) wistar albino rats which were divided into 3 batches of 15 rats each. Batch 1 served for gastric acid secretion, while batches 2 and 3 were for mucus output and cytoprotection studies respectively. Each batch was further divided into three groups of 5 rats each (control, low dose and high dose). The study duration was 21 days. The control received 0.3 ml of normal saline (0.9% NaCl solution) while the low dose (LD) and high dose (HD) experimental groups received Masfon - *Aloe vera* drink (1 ml and 3 ml/kg body weight orally, once daily respectively). The study was done at the Department of Physiology, University of Calabar, Nigeria. Results showed the mean basal gastric acid output (µmol/L/hr) for both low dose (9.92±1.51) and high dose (13.36±1.25) groups were significantly ($P<0.01$ and $P<0.001$) greater than control (5.20±0.05). Following histamine administration, the mean gastric acid output in the experimental groups were, control (21.64±3.58), low dose (24.64±2.76), and high dose (19.60±1.93). Simultaneous administration of histamine + ranitidine showed a decrease in mean acid output which was significant ($P<0.01$) in the low dose (11.56±1.96) compared to the control (4.64±0.64)

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and high dose (5.88±0.89) groups. Results for gastric ulceration showed that the mean ulcer score for low dose (9.60±0.73) and high dose (9.60±0.75) groups were significantly (P<0.001) reduced when compared to the control group (14.30±0.75). The mean mucus output was 0.07±0.01 in the control group, 0.06±0.01 in the low dose group and 0.05±0.01 in the high dose group. Masfon–*Aloe vera* drink administered at these concentrations is anti–ulcerogenic via a mechanism that does not involve a reduction or increase in gastric acid and mucus respectively.

Keywords: Cytoprotection; histamine; Masfon-Aloe vera drink; Wistar albino rats.

1. INTRODUCTION

The healthy gastric mucosa maintains structural integrity and function despite continuous exposure to noxious (aggressive) factors including 0.1 mol/L HCl and pepsin that are capable of digesting gastric tissue [1]. The integrity of the mucosa is maintained under normal conditions by defense mechanisms that include: pre – epithelial factors (mucus – bicarbonate–phospholipid "barrier") an epithelial "barrier" (surface epithelial cells connected by tight junctions and generating bicarbonate, mucus, phospholipids, prostaglandins etc), continuous cell renewal accomplished by proliferation of progenitor cells and continuous blood flow through mucosal micro vessels [1,2].

Gastric ulceration arises when there is an imbalance between protective and aggressive factors [3]. Gastric ulcer, also called stomach ulcer, simply refers to perforations in the normal gastric mucosa. The incidence varies with the age, gender and geographical location. This clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss [4,5].

Aloe vera is a cactus like, drought-resisting, succulent plant that belongs to the family Liliacaea (sub-family of the Asphodelaceae). It is native to North Africa and cultivated in warm climatic areas [6,7]. It is commonly called burn plant, lily of the desert, elephant's gal etc. The leaves of *Aloe barbadensis miller* produces two exudates namely: gel and latex, both of which contain the plants bioactive compounds [8]. Documented research in *Aloe vera* is widespread and cuts across diverse body systems such as the cardiovascular [9], integumentary [10], Immune [11], endocrine [12,13] and respiratory [14].

The novel extract of Aloe vera termed Masfon–*Aloe vera* drink was used for this study. It is a high multi-mineral extract of the aloe vera plant (Ekabua, K, University of Calabar, 2009, Personal communication). The present study was carried out to investigate the effect of this plants extract on gastric acid and gastric mucus and also to determine if it is cytoprotective. *Aloe vera* (Aloe barbadensis) has been used extensively by herbal practitioners worldwide as a soothing and healing remedy for peptic ulcers and most other ailments of the gastrointestinal tract [15]. A few studies carried out in the past has found *Aloe vera* to be cytoprotective [16]. In previous studies, crude Aloe vera extract was found to significantly increase gastric acid while also offering cytoprotection [15,17]. This study was thus embarked upon with the intent of determining if this novel Aloe vera extract is cytoprotective, and to also investigate if : (i) unlike crude *Aloe vera* gel it reduces gastric acid and (ii) it has any effect on mucus output.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty five Westar albino rats weighing between 150 and 250 g were purchased from the Animal house of the Department of Physiology, College of Medical Sciences and University of Caliber, Nigeria. The animals were allowed to acclimatize in the research laboratory for two weeks. They were housed under standard environmental conditions. Ethical conditions concerning animal use and handling was strictly followed (Ref. No. UCAL/2013/91).

2.2 Plant Extract

The plant extract that was employed for the study is termed Masfon–*Aloe vera* drink. This extract which was recently formulated is enclosed in a two liter plastic bottle and is marketed by Abishua ventures, Calabar (Ekabua, K, University of Calabar, 2009. Personal communication).

2.3 Experimental Design

The experimental animals were divided into 3 batches of 15 rats each. Batch 1 served for gastric acid secretion, while batches 2 and 3 were for mucus output and cytoprotection studies respectively. Each batch was further divided into three groups of 5 rats each (control, low dose and high dose). All study groups were fed with normal rat chow and water for 21 days. In addition, the control received 0.3 ml of normal saline (0.9 % NaCl solution) while the low dose (LD) and high dose (HD) experimental groups received Masfon-*Aloe vera* drink (1 ml and 3 ml/kg body weight orally, once daily respectively. The dosage of Masfon–Aloe vera drink used was derived from a dose response curve of an earlier study conducted on *Aloe vera* gel, where an ED_{50} of 0.1 ml/100g was documented [17].

2.4 Measurement of Gastric Acid Secretion

The measurement of gastric acid secretion was done using the continuous perfusion method by Gosh and Schild as modified by Osim et al. [18]. The animals were fasted for about 18 hours prior to the commencement of the experimental procedure. Each was then anaesthesized with 25 percent ethyl carbonate (urethane) at a dose of 0.6 ml/100g body weight. The trachea of the animal was then exposed and cannulated to allow for better respiration. The abdomen was dissected along the linea alba to expose the stomach, and a pyloric cannula was inserted into the stomach at its pyloric end and was held fast with thread. An orogastric cannula was finally passed through the mouth to reach the stomach.

The stomach was then perfused with 0.9 percent NaCl at the rate of 1 ml/minute through the orogastric cannula. The perfusate was allowed to flow freely to wash off food remains in the stomach after which six basal aliquot samples were then collected at an interval of 10 minutes. The aliquot samples were then titrated against a base (0.01N NaOH) using phenolphthalein as indicator. The end point was taken as the first light pink colouration that did not fade on shaking and this was recorded. After the basal collections, all the experimental groups were then challenged with injections of histamine (100 mg/kg body weight, subcutaneously) first, and then with histamine and ranitidine (intramuscularly at a dose of 11.4 mg/kg) simultaneously. Following each of the above injections, six aliquot

samples of gastric effluent were collected at 10 minute intervals and titrated as done in the basal stage. Acid output was then determined and calculated as described by Ibu [19].

2.5 Cytoprotection Studies

Gastric ulceration was induced in rats as described by Tekeuchi et al. [20] .The animals for the cytoprotection studies were fasted for 18 hours prior to the period for commencement of the study. The animals were then anaesthesized with light diethyl anaesthesia. An incision was made first along the trachea for inserting a tracheal cannula, then another incision was made along the linea alba to expose the junction of the stomach with the pylorus. A pyloric incision was then made through which 1.5 ml of acid alcohol (equal volume of conc. HCL and 70 percent ethanol) was introduced intragastrically to induce gastric ulceration. A ligature was tied at the pylorus to keep the acid alcohol confined within the stomach walls and the linea alba was then closed up using interrupted suture. The animals were then allowed to stay for two hours after which the animals were opened up and the pyloric ligature was removed. The acid alcohol was flushed out with normal saline in all the three groups. The stomachs were then dissected out and laid bare by cutting along the greater curvature. Each stomach was then spread out on a flat board by using pins to hold fast the edges. Then with the aid of a magnifying glass and a vernier calliper, the ulcer score was determined following the method where ulcers were classified into four grades thus.

Grade 0.0–no lesions.
Grade 1.0–haemorrhagic erosions (less than 5).
Grade 2.0–haemorrhagic erosions (greater than 5 or small linear ulcers).
Grade 3.0–many small linear ulcers (greater than 2 mm or a single linear).
Grade 4.0–multiple linear ulcers of marked size >3 mm.

The ulcer score was calculated by multiplying each grade with its frequency of occurrence. The sum of all the values formed the ulcer score for each animal [21].

2.6 Determination of Mucus Output

The adherent gastric mucus was determined by the method as described by Tan et al. [22]. The animals were fasted for 18 hours prior to commencing the experimental procedure, after which they were sacrificed and their stomachs were removed. The stomachs were then opened along the greater curvature and spread out on a flat board by using pins to hold the edges fast. By using a spatula, the gastric mucus was scraped off the surface of the mucosa and introduced into a pre-weighed sterilized sample bottle containing 3 ml of distilled water. The sample bottle containing distilled water and the collected mucus was now weighed on a sensitive electronic balance. Mucus output was calculated as the difference in weights of sample bottle containing water and sample bottle containing water and mucus.

2.7 Statistical Analysis

Results were expressed as mean±SEM. The data were analyzed using One way analysis of variance (ANOVA), followed by post hoc multiple comparisons test. $P<0.01$, 0.001, 0.05 was considered as statistically significant. Computer software SPSS version 17.0 and Excel Analyzer was used for the analysis.

3. RESULTS

3.1 Results for Gastric Acid Secretion

Significant differences were observed in the mean basal gastric acid output of the three groups. The basal gastric acid output for both the low dose (9.92±1.51 µmol/L/hr) and high group (13.36±1.25 µmol/L/hr) were significantly ($p<0.01$ and $p<0.001$) higher than that of control (5.20±0.05µmol/L/hr). The basal output of the high dose extract treated group was also significantly ($p<0.05$) higher when compared with the low dose group (Fig. 1).

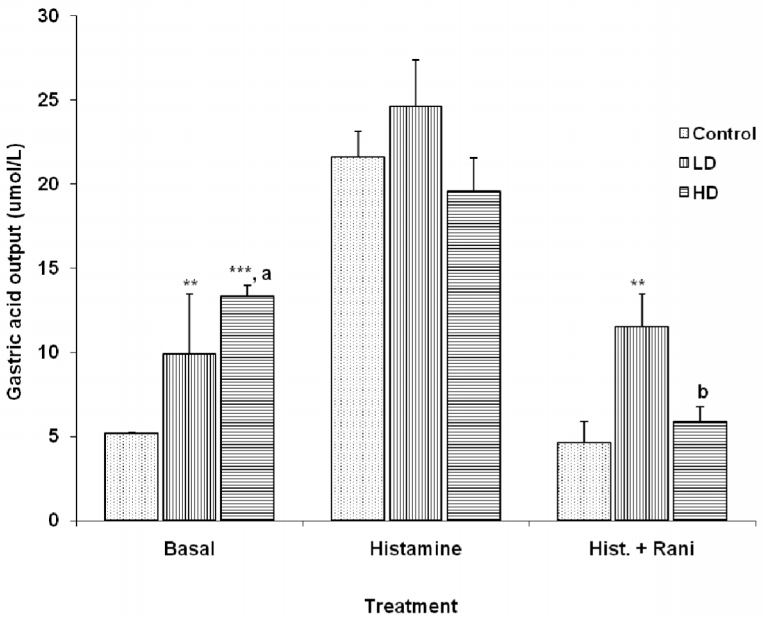

Fig. 1. Comparison of gastric acid secretion in control, LD and HD groups following administration of histamine and histamine + ranitidine in rats. Values are mean±SEM, n=5

***p<0.01, ***p<0.001 vs control; a = p<0.05, b = p<0.01 vs LD*

Following administration of histamine on all three groups, the increase in mean gastric acid output (µmol/L/hr) which was not significantly different among the experimental groups was 21.64±3.58, 24.64±2.76 and 19.60±1.93 in the control, LD and HD groups respectively (Fig. 1). This increase when compared with those obtained at the basal level showed significant ($p<0.001$ for control and LD, $p<0.01$ for HD) differences in all three groups (Fig. 2).

When histamine + ranitidine were administered simultaneously, the decrease in mean gastric acid output (µmol/L/hr) observed in all three groups, 4.64±0.64 for control, 11.56± 1.96 for LD and 5.88±0.89 for HD was significant ($p<0.001$) when compared to acid output following histamine administration (Fig. 2).

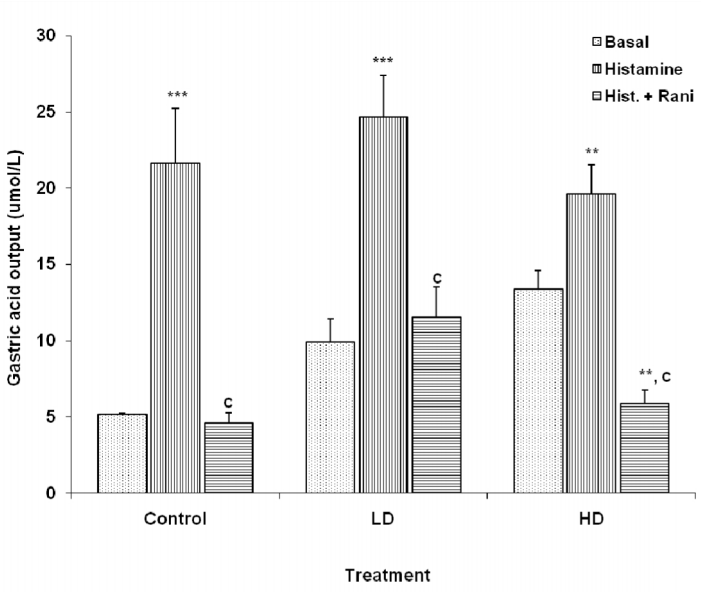

Fig. 2. Comparison of the effect of histamine and histamine+ranitidine on gastric acid secretion in control, LD and HD groups. Values are mean±SEM, n=5

***p<0.01, ***p<0.001 vs control; c = p<0.001 vs Histamine*

3.2 Results for Gastric Ulceration Studies

The mean ulcer score was lower in LD and HD compared to control (9.60±0.73 and 9.60± 0.75 vs 14.30±0.75, respectively; p<0.001). (Fig. 3)

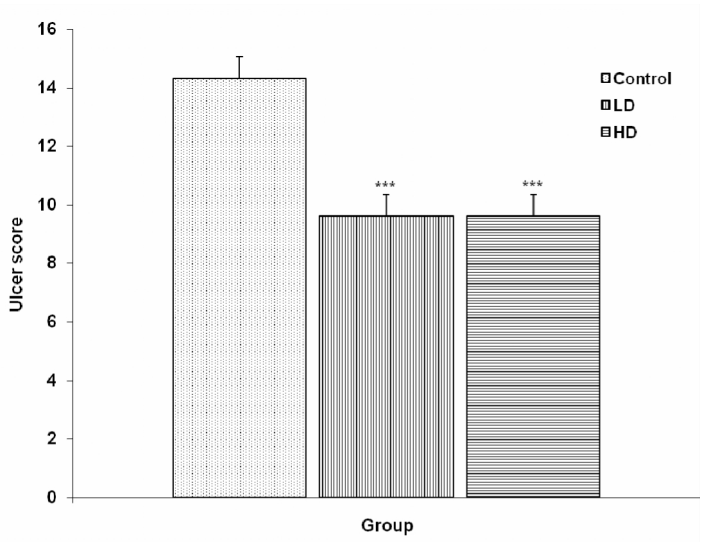

Fig. 3. Comparison of ulcer score in the different experimental groups. Values are mean±SEM, n=5

****p<0.001 vs control*

3.3 Gastric Mucus Output Result

The results of the mucus output in the different experimental groups were expressed as Mean±SEM. The mean mucus output which was not significantly different amongst the three groups was 0.07±0.01 in the control, 0.06±0.01 in the low dose and 0.05±0.01 in the high dose (Fig. 4).

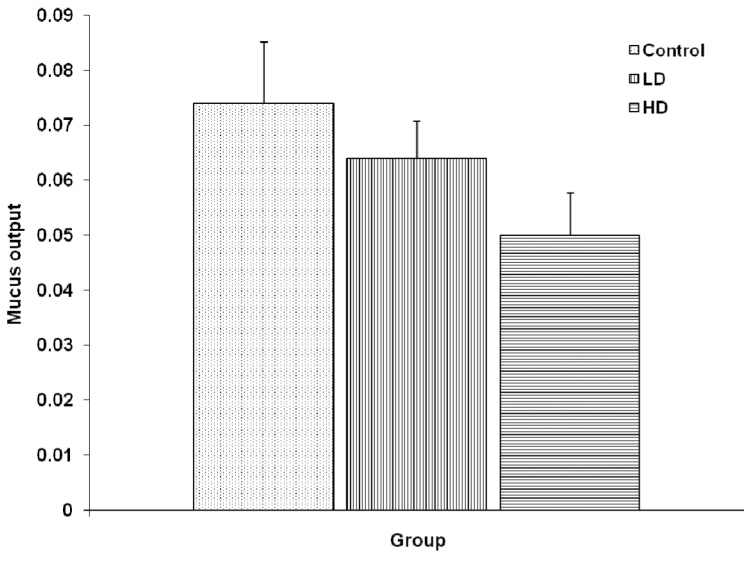

Fig. 4. Comparison of mucus output in the different experimental groups. Values are mean±SEM, n=5

4. DISCUSSION

The incidence of ulcer is a worldwide concern due to its high mortality, morbidity and economic loss [4]. This study showed that administration of Masfon-*Aloe vera* drink resulted in a significant increase in gastric acid output in the extract treated groups compared to control. This result agrees with findings from a previous study that showed a significant increase in gastric acid output in rats treated with *Aloe vera* extracts [17]. The mechanism through which Masfon-*Aloe vera* drink produced this increase in acid output is unknown, but it is possible that phytochemicals in this plants extract might have acted as secretagogues.

The results for mucus output and gastric ulceration showed decreases in both mucus output and gastric ulceration of the extract treated groups when compared to the control; a decrease that was significant only in the case of the gastric ulceration studies. Gastric ulceration arises when there is an imbalance between protective and aggressive factors. A previous report agrees with this present study that an experimental substance can reduce gastric mucus and still offer cytoprotection [23]. There are two layers of mucus, the loosely adherent and the firmly adherent mucus layers [24]. While the loosely adherent mucus layer can be easily excised, the firmly adherent layer is anchored firmly to the epithelium.

Literature posits that the layer of mucus closest to the epithelium (firmly adherent mucus) is responsible for maintaining juxtamucosal pH through neutralization of inwardly permeating protons by secreted bicarbonate ions (Kaunitz, JD, University of California, 1998, Personal communication). It is possible that though Masfon-Aloe vera drink did not increase the mucus of the stomach that was actually excised for this study, it might have actually increased the layer of adherent mucus that has been implicated in cytoprotection. Invitro studies have reported that nitric oxide supplied by luminal nitric oxide donor increases the thickness of the firmly adherent layer [24]. Acemannan constituent of *Aloe vera* extracts have been reported to increase cell's production of nitric oxide [25]. So it is most possible to infer that Masfon-*Aloe vera* drink may have actually increased the firmly adherent mucus layer as a result of the increased nitric oxide release, a situation which explains the cytoprotection offered by the extract. Furthermore, scavenging of free radicals has been documented as a method of cytoprotection [26,27]. *Aloe vera* extracts have been reported to contain agents that scavenge free radicals. They include, vitamins A, C and E [28]. Hence, it is probable that the presence of these anti-oxidants in Masfon-*Aloe vera* drink may have further added to the cytoprotection offered to the rat gastric mucosa.

These results suggest that the plant extract though cytoprotective must be closely regulated in individuals with ulcer. It is not known from this study design the mechanism through which this plants extract offered protection to the gastric mucosa. Also, rats induced invivo with ulcer were not used in this study; this could have shown effectively the efficacy of the plants extract on ulcer.

5. CONCLUSION

Masfon-*Aloe vera* drink administered at these concentrations is anti-ulcerogenic via a mechanism that does not involve a reduction or increase in gastric acid and mucus respectively.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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