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Effects of *Piper guineense (*Schumach) Leaf and *Xylopia aethiopica* Seed Extracts on Gastric Acid Secretion in Ibuprofen-Treated Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOA designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript and literature searches. Authors CCE and CJN managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Gastric ulcer can develop from good foods especially spices and seasonings. *Xylopia aethiopica* and *Piper guineense* are comon spices used to prepare favourite delicacies nowadays. Due to their pepperish flavour, they could trigger gastric acid secretion by mucosal irritation. Therefore, the present work tested the effects of methanol extracts of *Xylopia aethiopica* seed and *Piper guinneense* on gastric acid secretion in ibuprofen treated wistar rats.
Methods: Rats were given oral administration of 180 mg/kg/day of ibuprofen and/or 200 mg/kg of methanol extract of *Piper guineense* leaf and/or 300 mg/kg of methanol extract of *Xylopia aethiopica* seed extract respectively for 14 days. Determination of gastric acid was evaluated with free and total acidity levels, and gastric volume using Topfer's method of gastric analysis.
Results: Gastric acid levels and volume were significantly reduced in *Xylopia aethiopica* plus ibuprofen treated groups C and F ($P < 0.05$) compared to ibuprofen only treated group B rats. *Piper guineense* plus ibuprofen potentiated gastric acid secretion in group D rats compared to group B at $P < 0.05$. However, *Xylopia aethiopica* weakly reduced gastric acid secretion in ibuprofen plus *Piper guineense* treated group E rats although not statistically significant ($P > 0.05$) compared to control. Group G rats administered only *Piper guineense* showed significant increase ($P < 0.05$) in gastric acid secretion compared to control.
Conclusion: Results showed that *Xylopia aethiopica* seed extract reduced gastric acid secretion whereas *Piper guineense* increased gastric acid secretio in ibuprofen treated rats.

Keywords: Xylopia aethiopica; Piper guineense; ibuprofen; gastric acid; gastric volume.

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been employed in several experimental animal studies for induction of gastric ulcer [1-3]. NSAIDs do so by attenuating hydrophobic surface barrier properties of the stomach [4,5]. Ibuprofen is a non-selective inhibitor of cyclooxygenase - I and - II [6] due to its inhibition of cyclooxygenase - I that catalyzes the synthesis of prostaglandins - I2 and E2 (PGI-2 and PGE-2) which keep the gastrointestinal tract integrity by reducing gastric acid secretion, increasing thickness of mucus layer, stimulating bicarbonate secretion, and enhancing mucosal blood flow [7,8]. Experiments in laboratory animals have shown that depletion of COX_1-dependent PGE_2 causes a decrease in gastric mucosal blood flow [9], thus likelihood of gastric ulcer.

Piper guineense from piperaceacea is a herbaceous climber plant 4-10 m in length is commonly found in Nigeria and African tropical zones. It is locally called "*Uziza*" by the Ibos, "*Ata-Iyere*" by the Yorubas and "*Monsoro*" among Hausas. The fruits and leaves are commonly sold in Nigeria as condiments for food flavouring [10], whereas *Xylopia aethiopica* from Annonacea family is a tropical evergreen tree 20 m in length bearing aromatic seeds. The dried fruit is commonly called "*Uda"* by Ibos, "*Eru-nji*" by Yorubas and "*Kimbi*" amongst Hausas. Dry fruits are used in Nigeria, Ghana and Cameroon as spices [11].

Piper guineense leaf or seed and *Xylopia aethiopica* seed are common spices used together in "pepper soup" preparation in South Nigeria and West Africa. However, the use of *Piper guineense* leaf or seed may have its adverse effect on gastrointestinal system due to the pepperish-like strong pungent aroma and flavour that could cause mucosal wall irritation. Reports have shown that *Piper guineense* stimulates gastric secretion [12] as well as duodenal ulcer [13] in rats. Since *Xylopia aethiopica* extracts have been reported to reduce gastric acid secretion and increased gastric mucosal secretion [14,15].

Therefore, the present work tested the effects of *Xylopia aethiopica* seed and *Piper guineense* leaf extracts on gastric acid secretion in ibuprofen-treated rats.

2. MATERIALS AND METHODS

2.1 Experimental Design

Forty-two wistar rats (100 - 200 g) were used in the study. The rats were purchased from Animal House, University of Nigeria, Nsukka campus and transported to the Animal House, Madonna University Elele, Rivers State. They were acclimatized for 2 weeks and had access to

normal rat chow and tap water *ad libitum*. The rats were randomly divided into 7 groups (n = 5). The were kept in standard cages (Henan, China) (2 per cage). Seven rats were used for preliminary induction of gastric acid secretion using *Piper guineense*.

Group A rats were used as Control. Group B rats received oral administration of 180 mg/kg/day of ibuprofen as published in Pfizer safety data sheet [16]. The dose administration of *Xylopia aethiopica* and *Piper guineense* was done according to dose described by Agbai and Nwanegwo [17].

Group C received oral administration of 180 mg/kg/day of ibuprofen plus 300 mg/kg/day of *Xylopia aethiopica* seed extract. Group D received oral administration of 180 mg/kg/day of ibuprofen plus 200 mg/kg/day of *Piper guineense* leaf extract. Group E received oral administration of 180 mg/kg/day of ibuprofen, 300 mg/kg/day of *Xylopia aethiopica* plus 200 mg/kg/day of *Piper guineense* extracts. Group F received 300 mg/kg/day of *Xylopia aethiopica* seed extract only. Group G received 200 mg/kg/day of *Piper guineense* leaf extract only. All oral administration was performed using gavage for accurate administration. Care was taken to avoid damage to the alimentary canal. The duration of administration was 2 weeks.

2.2 Animal Ethics

The rats received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health [18].

2.3 Drug and Plant Extraction

Three packets (84 coated tablets in a pack) of ibuprofen (Bristol, UK) were used. Twenty coated tablets of ibuprofen (400 mg per tablet) were grounded daily into a powder form, sieved and extracted in ethanol to remove excipients. The filtrate was then kept in a refrigerator at 4^{o}C for an hour and administered orally.

Fresh leaves and seed of *Piper guineense* and *Xylopia aethiopica* were purchase from Afor-Ogbe market in Mbaise, Imo State on 08/06/2015. The plants were identified in the herbarium of the Department of Pharmacognosy, Madonna University Nigeria with voucher numbers (MUE/PGSY/004) and (MUE/PGSY/011) respectively. Fresh leaves of *Piper guineense* were sorted and cleaned. They were sun-dried and grounded into a coarse powdered form for extraction. The black pods of *Xylopia aethiopica* were crushed to remove the seeds. The seeds were also grounded into a coarse powdered form for extraction. 200 g (100 g each: ratio of 1:1) of each extract (*Piper guineense* leaves and *Xylopia aethiopica* seeds) was collected and suspended in 100 ml of methanol separately (taking into cognisance of ethanol induced gastric lesion [19]) and stirred continuously to make a sohxlet extraction. The mixture was filtered using a Whatman paper (No 1). The filtrate was dried with Rotatory evaporator (Buchi) in a semi solid mass and was stored in air-tight container and kept in a refrigerator (4^{o}C). The extraction lasted for 2 days.

Phytochemical analysis of *Piper guineense* and *Xylopia aethiopica* were determined according to methods by Trease and Evans [20]. Phytochemical screening showed that *Piper guineense* extract has bioactive compounds as follows; hydrogen cyanides (8.76%), saponins (1.91%), flavonoids (1.80%), tannins (1.19%) and alkaloids (0.81%), whereas *Xylopia aethiopica* extract bioactive compounds are high percentage of hydrogen cyanide (7.82%), tannins (5.01%) and saponins (3.02%). Others are alkaloids (1.20%) and flavonoids (0.78%).

2.4 Gastric Acid and Volume Collection

At the end of experiment, the rats were sacrificed under chloroform anaesthesia. The rats were dissected and stomach harvested in order to determine free and total acidity and gastric volume according to Topfer's method of gastric analysis as described by Hubbard [21].

2.5 Measurement of Free Acidity (Topfer'method)

In order to determine the free acidity, the contents of the harvested stomach were drained into a graduated tube through a small nick along the greater curvature of the stomach. The tube contents were centrifuged at 3000 rpm for 10 minutes. The centrifuged contents were decanted and measured. 1 ml of the supernatant liquid was drawn up with pipette and put in a beaker. It was further diluted with distilled water to 10 ml. The solution was titrated against 0.01 N sodium hydroxide using Topfer's reagent (Dimethyl-amino-azo-benzene) as an indicator to

the end-point when the solution turns to orange colour. The volume of sodium hydroxide needed was taken as correspondence to free acidity level.

2.6 Measurement of Total Acidity and Gastric Volume (Topfer's method)

10 ml of gastric juice specimen was transferred in a porcelain evaporating dish. About 1-2 drops of Topfer's reagent was added. A colour change was observed (a bright red colour appears if free hydrochloric acid is present). 1-2 drops of phenophthalein was added into the gastric juice with Topfer's reagent titrated with 0.01 N of sodium hydroxide from a labelled burrette. Mixing was done after each addition until the last trace of red colour disappeared and replaced with a canary yellow colour. The numbers of millilitres of sodium hydroxide was read from the burrette. This represents the amount of free hydrochloric acid. The titration was continued until until the red colour of phenophthalein appeared deep pink, and titrated to the point of which the further addition of sodium hydroxide could not deepen the colour. The volume of sodium hydroxide was taken as correspondence to total acidity. The total volume of gastric content was measured in measuring cylinder and corresponded to the gastric volume.

2.7 Statistical Analysis

Data was analysed using a One-Way ANOVA followed by a Post hoc test (Tukey's test) using SPSS 18 version. Results were presented as Mean ± SEM and P value < 0.05 was accepted as statistically significant.

3. RESULTS

Results showed statistically significant reduction ($P < 0.05$) in free acidity, total acidity and gastric volume levels in group A (18.62 ± 0.43; 53.27 ±1.67;1.19 ± 0.05) and group F (19.94 ± 0.87; 45.69 ± 0.65; 0.69 ± 0.13) compared to group B (24.83 ± 0.35; 63.39 ± 0.70; 2.97 ± 0.29), group C (21.15 ± 0.45; 58.96 ± 0.41; 1.91 ± 0.06), group D (31.07 ± 0.63; 77.98 ± 1.03; 4.17 ± 0.15), group E (26.22 ± 0.41; 59.82 ± 0.55; 2.71 ± 0.33) and group G (28.03 ±0.88; 69.91 ± 1.39; 2.80 ± 0.35).

There was no statistically significant difference ($P > 0.05$) in free acidity, total acidity and acid volume between group A and group F.

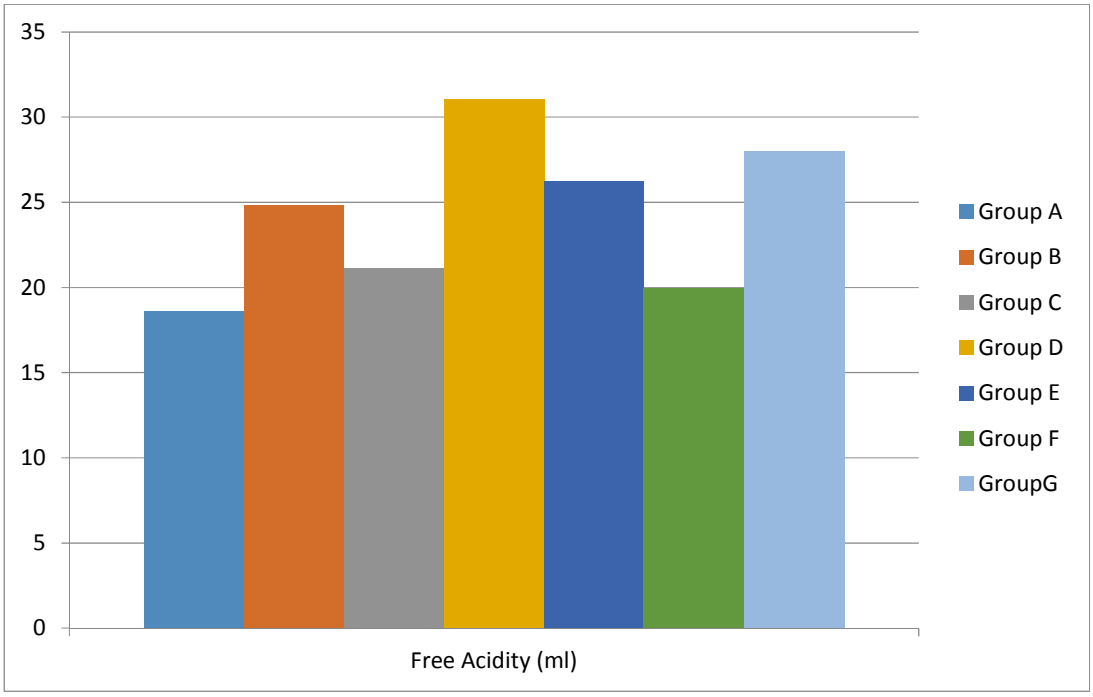

Fig. 1a. The effect of *Piper guineense* leaf extract, *Xylopia aethiopica* seed extract and ibuprofen on free acidity levels

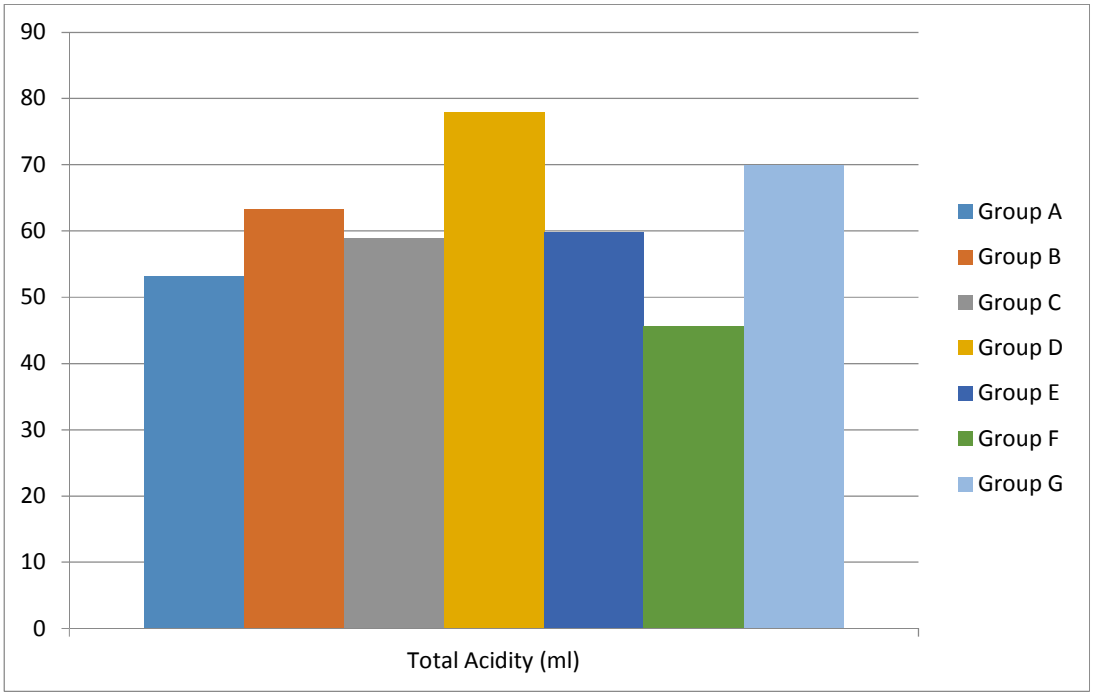

Fig. 1b. The effect of *Piper guineense* leaf extract, *Xylopia aethiopica* seed extract and ibuprofen on total acidity levels

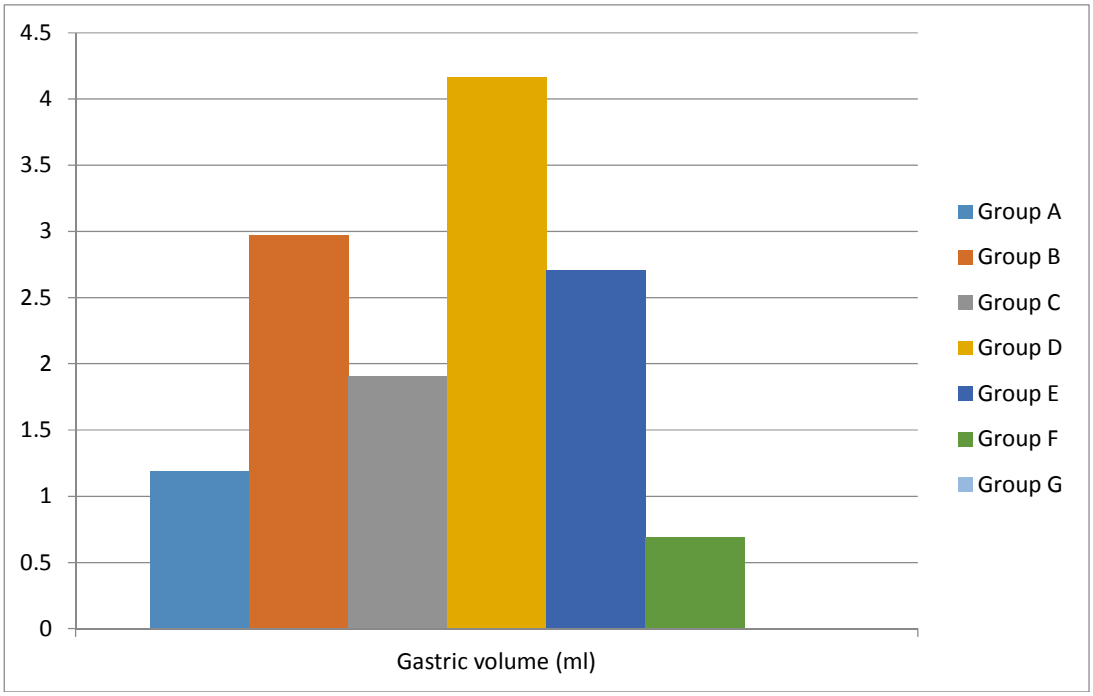

Fig. 1c. The effect of *Piper guineense* leaf extract, *Xylopia aethiopica* seed extract and ibuprofen on gastric volume

Table 1. Shows the effect of *Piper guineense* leaf extract, *Xylopia aethiopica* seed extract and ibuprofen on gastric acid and volume secretion

	Free acidity	Total acidity	Gastric volume
Group A (Control)	18.62 ± 0.43	53.27 ± 1.67	1.19 ± 0.05
Group B	24.83 ± 0.35*	63.39 ± 0.70*	2.97 ± 0.29*
Group C	21.15 ± 0.45*	58.96 ± 0.41*	1.91 ± 0.06*
Group D	31.07 ± 0.63*	77.98 ± 1.03*	4.17 ± 0.15*
Group E	26.22 ±0.41*	59.82 ± 0.55*	2.71 ± 0.33*
Group F	19.94 ± 0.87	45.69 ± 0.65	0.69 ± 0.13
Group G	28.03 ± 0.88*	69.91 ± 1.39*	2.80 ± 0.35*

$P < 0.05$ denotes statistically significant. All values are expressed as mean ± SEM; n = 5 in each group

4. DISCUSSION

As expected, oral administration of ibuprofen and *Piper guineense* leaf extract respectively and/or combined caused marked increase in the free acidity and total acidity levels and gastric volume. Studies have shown that ibuprofen caused an increase in free acidity and gastric volume [22] and total acidity output [23]. Ibuprofen inhibits cyclo-oxygenase-I enzyme that catalyzes the synthesis of PGE-2 and PGI-2. These prostaglandins maintain gastrointestinal tract integrity via reduction of gastric acid secretion, stimulating bicarbonate release, increasing mucosal blood fkiiilow and mucosal wall thickness [6,7].

Raji et al. [12] reported that 200 mg/kg of *Piper guineense* extract induced gastric ulceration via histamine receptors (H2-receptors). However, the increase in the free acidity, total acidity and gastric iifollowing administration of *Piper guineense* extract could be due to stimulation of H2-receptors. In other words, strong pungent pepperish spices are thought to induce ulcer. Myers et al. [24] showed that intragastric administration of red and black pepper meals markedly increased in parietal secretion. Study also showed that aframomum *melegueta* elevated gastric acid secretion mediated via muscarinic and histaminic receptors [25]. Therefore, it is not surprising that rats treated with only *Piper guineense* extract increased gastric acid secretion because of its pepperish flavor. In addition, combination of ibuprofen and *Piper guineense* extract potentiated gastric acid secretion by causing significant increase in free and total acidity levels and gastric volume corroborating with Raji et al. [12].

In contrast, oral administration of only *Xylopia aethiopica* extract resulted in a significant reduction in free acidity and acidity and gastric volume. Several studies have shown that *Xylopia aethiopica* seed extract reduced gastric acid secretion and increased gastric mucus production [14,15]. On the one hand, *Xylopia aethiopica* seed as having strong pungent musky flavour cannot be overemphasized for acting as irritant to the mucosal wall. This irritation normally triggers profuse secretion of muscus to safeguard the gastric mucosa. Studies have shown that mild irritants are effective in providing gastric mucosal protection by increasing gastric production of PGE-2. [26], and increased mucin secretion and decreased cell shedding [27].

In a similar vein, the presence of diterpene, a compound in *Xylopia aethiopica* leaf has been implicated in the mechanism by which *Xylopia* mediated gastroprotective effect [28]. Diterpene was reported to increase gastric volume and it's pH value, reduced titrable acidity without effect on gastric mucus [29]. Conversely, the gastric volume was significantly reduced by the *Xylopia aethiopica* seed extract in the present study. Therefore, the mechanism of action underlying *Xylopia aethiopica* extract could involve PGE-2 synthesis that inhibited gastric acid secretion although mechanism via diterpene cannot be undermined, but since gastric volume was significantly reduced pointed to the PGE-2 activity. PGE -I and PGE-2 have been reported to inhibit both gastrin and pituitary adenylate cyclase-activating peptide histamine secretion [30].

Furthermore, it is clear that *Piper guineense* leaf extract induced increased gastric acid secretion whereas *Xylopia aethiopica* showed weak-reducing effect on gastric acid secretion in *Piper guineense* and ibuprofen treated group E rats compared to group D administered ibuprofen and *Piper guineense* only. It was evident in the table that *Xylopia aethiopica* extract did not only reduce gastric acid levels in the ibuprofen treated but also on both ibuprofen and *Piper guineense* treated.

5. CONCLUSION

It can therefore be concluded that *Xylopia aethiopica guineense* seed extract inhibited gastric acid secretion whereas *Piper guineense* leaf extract triggered secretion, although weak reduction of gastric acid levels was observed in *Xylopia aethiopica* treated rats in the presence of ibuprofen and *Piper guineense.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments followed standard protocol and was approved by the Research committee, Department of Human Physiology and Professor (Sir) A. C. Ugwu, external examiner to the Department of Physiology, Madonna University Nigeria. All experiments 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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