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GASTRIC ULCEROGENIC ACTIVITIES OF *PIPER GUINEENSE* EXTRACT IN RATS

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Summary: Administration of *Piper guineense* fruit extract caused a dose – dependent mortality rate in rats. Doses of 200 $mgkg^{-1}$ intravenously, (i.v); 300 $mgkg^{-1}$ intraperitoneally, (i.p) and 3200 $mgkg^{-1}$ orally(p.o) produced 100% mortality rate – giving LD_{50} of 85.1 $mgkg^{-1}$ i.v ; 224 mgkg i.p and 1122 $mgkg^{-1}$ p.o respectively. Piper extract significantly stimulated gastric ulceration, total gastric acidity and *in situ* gastric acid secretion in rats. Piper-induced gastric ulceration appears to be similar to that induced by indomethacin (40 $mgkg^{-1}$ b.w). Atropine produced no effect on the stimulatory action of piper on gastric acid secretion. However, gastric acid secretion in the presence of histamine, and cimetidine appears to be augmented by piper extract (25 $mgkg^{-1}$ b.w). The results suggest that piper could stimulate gastric acid secretion (and probably gastric ulceration) via the H_2 – receptor potentiation.

Key Words: Piper guineense, ulceration, rats

Introduction

Piper guineense also called black pepper (Aschum and Thonn) is widely used in Nigeria as food spice and appetizer. However, the dry fruit of *Piper* has been reported to be toxic to many insect species (Harvil et al, 1943; Synerholm et al; 1945; Matubara and Tanimura 1966). The insecticidal properties of black pepper to rice, cowpea and boll weevils have also been reported (Su, 1977; Scott and Muckibben , 1978). These studies also revealed that the *Piper* fruit could meet home storage requirements for grains (e.g. beans)

In spite of the widespread consumption of *Piper* fruits as a food spice, there is no information on its biological activities in mammals. The represent work was carried out to investigate the effects of *Piper* extract on gastric activities as part of our efforts in the search for antiulcer agents from plant sources.

Materials and Methods

Animals: Male Wistar strain albino rats (180 – 200 g) obtained from the central animal house, College of Medicine, University of Ibadan were used for the study. The animals kept in wire-mesh cages were acclimated to laboratory conditions (12D: 12L cycles; 24 ± 1°C) and had free access to food and water. These rats were randomly assigned to three experimental phases as follows:

Toxicity Study:

(i) *Oral toxicity study:*

30 male rats (180 – 200g) divided into six equal groups were treated with 4% tween 80 (vehicle for the extract and served as the control), 200, 400, 800, 1600 and 3200 $mgkg^{-1}$ b.w (p.o) respectively each as a single dose. The 24 h mortality rate was determined.

(ii) *Intraperitoneal toxicity study:*

30 male rats (180-200g) divided into six equal groups were treated with 4% between 80, 100, 150 200, 250 and 300 $mgkg^{-1}$ b.w (i.p) respectively each as a single dose. The 24 hour mortality rate was determined daily.

(iii) *Intravenous toxicity study:*

35 male rats (180 – 200g) divided into seven equal groups were treated with 4% tween 80, 25, 50, 75, 100, 150, and 200 $mgkg^{-1}$ b.w (i.v) respectively each as a single dose. Prior to the extract administration, each animal was anaesthetized with 0.6ml/100g b.w i.p urethane and the femoral vein was cannulated for extract

administration. Thereafter the cannular was carefully removed and the incision sutured. The mortality rate within 24 h period was determined. All animals in (i),(ii) and (iii) above were observed for general behaviour over a period of one week.

2. Experimental Gastric Ulceration

Male albino rats (180 – 200g) were randomly divided into six goups of five animals each. One group served as the control and received 4% tween 80 (0.5ml); a second group received propranolol (40 $mgkg^{-1}$ b.w, Sigma,) and served as the reference drug; piper extract was administered at doses of 100, 200 and 300 $mgkg^{-1}$ b.w to groups 3,4 and 5 respectively and 50 mg kg^{-1} b.w cimetidine (p.o) to group 6. One hour after administration of drug or extract, acute gastric mucosa lesions were induced in rats using indomethacin 40 $mgkg^{-1}$ i.p; Mark, Sharp and Dohme). Indomethacin was dissolved in 2% sodium carbonate in water. Four hours later the animals were killed as previous described (Raji and Bolarinwa, 1997). The stomach of each rat was removed and total gastric acidity and ulcer scores were determined as earlier described (Raji et al, 2001)

Total gastric acidity and ulcer score:

The stomach was opened along the greater curvature and gastric contents were drained into a centrifuge tube and centrifuged for 10 minutes. The supernatant was then titrated with NaOH (0.01M) to an end-point using phenolphthalein as an indicator. The total gastric acidity was expressed as μEq/100g body weight of each rat.

Assessment of the degree of ulceration was carried out by examining the inner surface of the stomach with a dissecting binocular microscope. The gastric lesions formed were scored and the mean ulcer index and percentage inhibition of ulceration were calculated as earlier described (Raji et al; 2000).

3. Gastric Acid Secretion

In this section, 25 $mgkg^{-1}$ b.w *Piper guineensis* extract was used, because it produced the maximum effect and did not caused death of any rat when administered intravenously. Thus, the effect of *Piper guineensis* extract (25 mg kg^{-1} b.w) on basal, histamine, atropine, and cimetidine actions of gastric acid secretion in adult male albino rats was studied as earlier described (Raji et al; 2000, 2001).

Plant Material and Extract Preparation

The dried fruit of *Piper guineense* was bought from a local market, Bodija, Ibadan, Nigeria. The fruits were thoroughly washed in cold water, and sun-dried for two days. The dried fruits were ground using an electric blender. The ground *Piper* fruits (200g) were then exhaustively extracted with 95% ethanol by means of a soxhlet apparatus. The extract was distilled under pressure at 55 ^{0}C to give 15g (7.5% yield) of dark brown semi-solid extract, which was stored in a dark bottle and kept in a refrigerator at 4^{0}C for the study. Fresh solution of the extract was prepared in 4% tween 80 when required.

Statistical analysis:

This was done suing student's *t*-test and analysis of variance (ANOVA) where applicable. The significance of difference was accepted at $P<0.05$. Data are presented as mean ± SEM.

Results

Toxicity: Administration of *Piper guineense* extract orally, intraperitoneally and intravenously produced dose – dependent increases in mortality rate in rats. The LD_{50} was 1122 for oral, 224 for intraperiotoneal and 85.1 $mgkg^{-1}$ b.w for intravenous administrations of the extract respectively. Administration of *P. guineense* extract at 3200 $mgkg^{-1}$ b.w orally, 300 $mgkg^{-1}$ b.w intraperitoneally and 200 $mgkg^{-1}$ b.w intravenously caused 100% mortality in rats.

Experimental Gastric Ulceration

The results shown in Table 1 indicate that *Piper guineense* fruit extract caused a dose – dependent gastric ulceration, which was accompanied by a similar dose dependent increase in total gastric acidity. To compare gastric ulceration induced by indomethacin with that of *Piper* extract alone,another groups of rats were treated orally with the *Piper* extract without prior indomethacin administration. The results are also shown in TableI and they indicate that *Piper* extract alone caused a dose – dependent gastric ulceration in rats.

Piper Guineense *Extract And Gastric Acid Secretion* In Situ

Tables 2 and 3 show the effects of *Piper* extract on gastric acid secretion. An increase in gastric acid secretion from a mean basal value of 0.69±0.06 to a maximum of 0.82±0.07 and 1.08±0.03 mEq/L were obtained for 1 $mgkg^{-1}$b.w *Piper* extract within 30 minutes of

administration. This increase (18.8%) is much less than that obtained with histamine alone (52.2%). However, a dose of 25 mgkg^{-1} *Piper* extract produced a greater gastric acid stimulation (71.0%) than histamine (1mgkg^{-1} b.w). Gastric acid secretion in the presence of histamine, atropine or cimetidine, appears to be augmented by 25 mgkg^{-1} b.w *Piper* extract (Table 3).

Table1: Experimental gastric lesions in rats following oral administration of Piper guineense extract

Treatment group (n=5)	Ulcer index*	% Inhibition of ulceration**	Total gastric acidity (mEq/100g b.w)
Control, 4% Tween 80	18.3±1.52	-	12.53±0.74
Propranolol (40 mgkg^{-1} b.w)	7.8±1.38	57.4	7.40±0.25
P. guineense (100 mgkg^{-1}b.w)	17.6±1.41$^{\Psi}$ (16.9±1.18)	3.8	12.34±0.61$^{\Psi}$ (11.9 ± 0.59)
P. guineense (200 mgkg^{-1}b.w)	18.5±1.46$^{\Psi}$ 18.1±1.40)	-1.1	13.20±0.68$^{\Psi}$ (12.8±0.56)
P. guineense (300 mgkg^{-1}b.w)	19.3±1.39$^{\Psi}$ (18.4±1.28)	-5.5	13.20±0.52$^{\Psi}$ (12.9±0.51)
Cimetidine (50 mgkg^{-1}b.w)	4.7±1.45	74.3	6.35±0.41

* Ulcer index = $\frac{\text{mean degree of ulceration x \% of group ulcerated}}{100}$

** Inhibition of ulceration = $\frac{\text{ulcer index in control – ulcer index in test X100}}{\text{Ulcer index in control.}}$

$^{\Psi}$Significantly different from the cimetidine group (P<0.05).
Values in brackets were obtained from rats treated with *Piper* extract without prior indomethacin treatment.

Table 2:Effect of Piper extract on gastric acid secretion in situ

Treatment group	Maximal gastric Acid out-put Meq/30 min	Net Acid output	% Change in Acid output
Basal	0.69±0.06	00	00
4%Tween 80	0.67±0.05	-0.02	-2.9
P. guineense (1mgkg^{-1} b.w)	0.82±0.07*	0.13	18.8
P. guineense (25mgkg^{-1} b.w)	1.18±0.06*	0.49	71.0
Histamine (1mgkg^{-1} b.w)	1.08±0.03*	0.39	52.2

*Significantly higher than the control and basal values (P< 0.05).

Table 3: Effect of Piper extract on histamine, atropine, and cimetidine – induced gastric acid secretion in rats.

Treatment group	Maximum gastric acid output Meq/30 min	Net acid output (NAO)	% Change in Acid secretion
Basal	0.69±0.02	00	00
Histamine alone (1mgkg^{-1} b.w)	0.09±0.06*	+0.40	+58.0
Histamine + *Piper* (25 mgkg^{-1} b.w)	1.39±0.04*	+0.70	+101.4
Atropine alone (0.5 mg/100g b.w)	0.67±0.02	-0.02	-2.9
Atropine + *Piper*	0.82±0.03*	+0.13	+18.8
Cimetidine alone (0.12m gkg^{-1}b.w)	0.42±0.04**	-0.27	-39.1
Cimedine + *Piper*	0.53±0.03**	-0.16	-23.2

Footnote to Table 3 : Net acid output. This was determined by subtracting basal acid output from acid output by in each experimental group. Plus (+) indicates extent of stimulation and minus (-) indicates extent of inhibition of gastric acid secretion. Significantly (*) increased (P<0.01) and (**) decreased (P<001) when compared with basal value. Note that gastric acid output by 4% Tween 80 was not significantly different from the basal acid secretion in all the experimental groups.

Discussion

Results of acute toxicity studies indicate that oral, intraperitoneal and intravenous administrations of *Piper* extract at high doses tested were lethal to rats. This observation suggests that excessive administration of *Piper guineense* as a spice in food may have both immediate and long term, adverse effect.

The ethanolic extract of *Piper* guineense was found to increase gastric acid secretion. The extract also caused a significant increase in gastric ulceration, which was accompanied by an increase in total gastric acidity. The mechanism by which *Piper* extract produced these effects is not well known at present. However, since atropine a muscarinic receptor blocker (Hersay and Sachs, 1995) did not affect the action of *Piper* on gastric acid secretion, it is most unlikely that the extract acts through the muscarinic receptors. That the action of *Piper* extract on gastric acid secretion and therefore gastric ulceration is probably mediated via H_2 receptor was demonstrated by the significant potentiation of histamine and cimetidine – induced gastric acid secretion in this study. The stimulatory action of histamine on gastric acid secretion is mediated by the H_2 receptor (Hersey and Sachs, 1995). Moreover cimetidine, a potent H_2 receptor blocker has been demonstrated to inhibit gastric ulceration in rats (Raji et al 2000, 2001). Since H_2 receptors are located on the parietal cell, it is probable that *P. guineense* extract acts on the parietal cell directly, in order to stimulate gastric acid secretion and consequently inducing gastric ulceration.

Members of the plant family *piperaceae* contain chemicals like amides, flavonoids and lignans. The active constituents in the acetone extract of *Piper guineense* has been found to contain *pipercide*, *guineensine*, and the alkaloid *piperine* (Harvill et al, 1943). Gas chromatography of petroleum ether extract of *Piper guineense* yielded four active fractions, one of which was a pure component identified as *pellitorine* (Gbewonyo and Candy, 1992). These compounds have been found to be toxic to insects (Gbewonyo et al 1993). The mechanism of insecticidal activities of *Piper* has not been elucidated. It is not known which of the constituents of *Piper* produced gastric ulceration and increases in gastric acid secretion in rats. Phytochemical screening (Sim, 1968) of the fruit extract of *Piper guineense* in the present study revealed presence of alkaloids, flavonoids and amides. Future studies will attempt to identify, the active principle with these gastric activities.

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