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Ethanolic Leaf Extract of *Psidium Guajava* L. [Myrtaceae] Protects the Stomach against Ischemia-Reperfusion Induced Gastric Mucosal Injury

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Abstract

Objective: Decoction of the root, bark or leaves of *Psidium guajava* universally known guava has a long history of dietary and medicinal uses in Africa. This study was designed to investigate the gastric secretory and protective properties of leaf extract of guava on ischemia-reperfusion (I-R) induced gastric mucosal injury in rats.

Methods: Male Wistar rats (n = 40) were divided in eight groups. Group 1 served as control group, group 2 animals were subjected to I-R without treatment, groups 3, 4, 5 and 7 were pretreated with 25, 50, 100 mg/kg of the extract orally 10 mg/kg indomethacin intraperitoneally [i.p.] 30 min before I-R respectively. Group 6 animals were pretreated with indomethacin [10mg/kg, i.p.] without I-R while group 8 animals were administered with 100mg/kg guava extract 30 minutes before administration of indomethacin [10mg/kg, i. p.] and subjected to I-R. Extent of mucosal damage was assessed by calculating the ulcer index while adherent mucus was determined by the Alcian Blue method.

Results: The results obtained indicated that oral pretreatment of rats with extract of guava leaves significantly reduced and reversed I-R induced mucosal injury ($P < 0.001$), reduced gastric acid and adherent mucus on the gastric mucosa ($P < 0.05$) respectively. Indomethacin aggravated the mucosal injury induced by I-R which was reversed by 100 mg/kg extract of guava.

Conclusion: In conclusion, the stimulation of mucus secretion by guava extract may be responsible for its gastro-protective properties against I-R induced mucosal injury**.**

Keywords: guava extract; gastric mucosa; ischemia-reperfusion; indomethacin; *Psidium guajava*

1. Introduction

The gastric mucosa forms a barrier between the body and luminal environment which contains aggressive agents such as hydrochloric acid, pepsin and toxins produced by microorganisms like *Helicobacter pylori*. Damage to the gastric mucosa barrier due to ischemia and reperfusion [I-R] injury is a common and serious condition.[1] The gastric mucosal injury induced by I-R has been regarded as a useful experimental model for the study of stress ulcer formation.[2]

I-R usually results from conditions such as circulatory shock, trauma, sepsis, circulatory insufficiency or thrombosis.[3] Considerable evidence has accumulated to show that, generation of reactive oxygen species, such as superoxide, hydrogen peroxide and hydroxyl radicals, by invading neutrophils mediate the injury associated with I-R.[4,5] Oxygen derived free radicals generated during reperfusion initiate a series of events that causes mucosal damage and disruption of the barrier. As a result, a number of pharmacological interventions are under development to prevent the cascade of events that eventually leads to the gastric mucosa barrier being compromised. These include application of anti-oxidants and use of drugs to block the effect of inflammatory mediators and acid.[6]

Pharmacological agents which posses anti-oxidant and chelating properties like flavonoids have been isolated from fruits and vegetables of variety of plants. Flavonoids are a group of about 400 naturally occurring polyphenolic compounds, broad based class of low molecular weight, secondary metabolites found in plants. Documented evidence has shown that, they exert their anti-oxidant effects by acting as free radical scavengers; hydrogen donating compounds and single oxygen quenchers.[7] Flavonoids have been shown to posses anti-ulcer activity by preventing gastric mucosal lesions in several ulcer models.[8,9] As a result, natural products are being proposed as therapeutic alternative to conventional anti-ulcer treatment whose effectiveness is often limited.

The roots, bark, leaves and fruits of *Psidium guajava* L [Mytraceae], which is universally known by its common English name guava, have a long history of use for dietary and medicinal purposes throughout the tropics. The extracts from these parts of the plant have been used by many tribes to treat diarrhea, gastroenteritis, dysentery, ulcers gastroenteritis and regulate menstrual periods.[10,11] The roots, bark, leaves and fruits of *Psidium guajava* in addition to tannins, polyphenols, alkaloids, glycosides, saponins contains flavonoids, particularly quercetin.[12-14] Much of the guava's therapeutic activity has been attributed to these flavonoids. The long history of guava's use as natural medicine by traditional healers has led to modern-day researchers to study the effect of guava extracts on the treatment of different ailments but its effect on gastric mucosal injury and thus its application for the treatment of peptic ulcer diseases has not been documented.

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The present study was designed to evaluate the effect of aqueous leaf extract of guava on gastric mucosal injury induced by I-R and to clarify the mechanisms underlying the gastro-protective action of the extract by determining various parameters of gastric function. In addition, we compared the effects of I-R in control rats with those observed in rats pretreated with non-selective cyclo-oxygenase [COX] inhibitor indomethacin.

2. Materials and Methods

2.1. Plant Material

Leaves of *Psidium guajava* were collected from the Botanical Garden of Ahmadu Bello University, Zaria, Nigeria. The specimen was identified and authenticated by Mr. Musa Mohammed of the Department of Biological Sciences herbarium unit of the same institution. A voucher specimen (No. 7075) is deposited at the herbarium unit of for future reference.

2.2. Preparation of Ethanolic Extract

Fresh leaves [500g] were air dried for five days before they were grounded to a fine powder. The powder was macerated in 250 ml of ethanol [80% v/v] for 48 hours. The extract evaporated and filtered with Whatman paper No. 3 was kept in sealed containers and stored at 4°C until used. The yield was 10.4% [w/w]. The extract was dissolved in physiological saline on the morning of the experiment. The plant extract was administered orally to the rats with the help of a specially designed oral needle connected to a polythene tube. The doses selected for the study the study were 25, 50 and 100 mg/kg. Volume of administration was 1 ml per 100 g of body weight.

2.3. Animals

Male Wistar rats [150-200g] were purchased from the animal house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. They were housed in groups of five in large cages with mesh bottoms to prevent coprophagy and acclimatized to standard laboratory conditions (25 ± 2^{o}C, 12 hr light/dark cycle). The animals were fed on standard laboratory chow and allowed free access to tap water *ad libitum*. They were randomly distributed into different experimental groups of five rats per group. The procedures described in this study were approved by an Institutional Committee on Animal Research in accordance with National Guidelines for Animal Research before commencement of the study. All animals were deprived of food but not water 24 hours before the experiments.

2.4. Chemicals

All chemicals and drugs were purchased from Sigma chemicals CO [St. Louise, MO, USA].

2.5. Gastric Lesions Induced by Ischemia-Reperfusion

Gastric mucosal injury was induced by the ischemia-reperfusion [I-R] technique of Wada et al.[15] The rats were anesthetized with pentobarbital [50mg/kg i. p.] and tracheotomized. A midline laparotomy was performed, the stomach exteriorized and the pylorus ligated. Ischemia was induced by clamping the celiac artery using an atraumatic microvascular clamp for 30 minutes. Reperfusion was established by removal of the clamp. After a 60 minutes reperfusion period, the animals were sacrificed by cervical dislocation. The stomach was removed and opened along the greater

curvature. The gastric juice was collected in 5ml eppendorff tubes.

2.6. Evaluation of Gastric Mucosal Lesions

After removal of the stomach and the content emptied, it was rinsed with saline and examined for hemorrhagic lesions with a x2 hand lens. The severity of gastric mucosal damage was graded according to the length of the lesion as follows; Grade 0=no visible lesion; grade 1=hemorrhagic lesions of <1mm; grade 2= hemorrhagic lesions of 2-4mm and grade 3= hemorrhagic lesions of >4mm. The ulcer index for each animal was calculated as the total number of lesions multiplied by their grade.[16] In sham operated animals [control], the abdomen was opened and the celiac artery manipulated without clamping.

2.7. Gastric Secretion Parameters

Two parameters of gastric function [adherent mucus and total acid secretion] were determined at the end of each experiment. Total acid level in the gastric juice was determined by titration to pH 7.0 with NaOH [5mmol/L] and the results expressed as mEq/L.

Adherent gastric mucus content was estimated by the method described by Corne et al.[17] Briefly, after evaluation of gastric lesions, the stomach was rinsed in cold saline. The glandular part of the stomach was excised, weighed and immersed for 2 hours in 20 ml of 0.1% w/v Alcian blue in 0.16 M sucrose solution buffered with 0.05 M sodium acetate [pH 5.8]. The excess dye was removed by rinsing twice for 30 min in 0.25 M of sucrose. The mucus-dye complex was extracted by immersing the gastric tissue in 0.5 M $MgCl_2$ solution, which was intermittently shaken for1 min at 30 min intervals for 2 hours. The blue extract was shaken with diethylether and the resultant emulsion was then centrifuged at 5000 x g for 10 min. The amount of mucin in the gastric sample was quantified at 600 nm with a spectrophotometer. The results are expressed as absorbance per gram of wet tissue [g tissue].

2.8. Assessment of Plant Extract on Mucosal Injury

The animals were randomly divided into eight groups and each group consisted of five rats.

Group 1: It represented the sham control group. Normal saline was administered orally and the animals were not subjected to I-R.

Group 2: Animals in this group were subjected to I-R and administered with normal saline orally.

Groups 3, 4 and 5: Guava extracts [25, 50 and 100 mg/kg] were administered orally 30 minutes before being subjected to I-R.

Group 6: The animals were pretreated with indomethacin [10mg/kg, i. p.] without I-R.

Group 7: The animals were administered with indomethacin [10mg/kg, i. p.] and subjected to I-R.

Group 8: The animals were administered with 100mg/kg guava extract 30 minutes before administration of indomethacin [10mg/kg, i. p.] and subjected to I-R.

2.9. Statistical Analysis

All data are expressed as mean ± S.E.M. Comparisons between groups were made using Student's t-test for paired data. A P value of <0.05 was considered significant.

3. Results

3.1. Effect of Guava on Ischemia-Reperfusion Mucosal Damage

The result of this study showed that ischemia for 30 minutes followed by reperfusion for 60 minutes resulted in gastric mucosal injury [ulcer index of 3.6 ± 0.2 mm; Fig. **1]**. The gastric mucosa of the sham operated (control) animals had no lesions. Pretreatment with the extract of guava reduced the severity and extent of gastric mucosa damage by I-R [P < 0.001; Fig. 1]. Intraperitoneal injection of indomethacin 60 minutes before I-R worsened the mucosa damage induced by I-R [P < 0 .001; Fig. 1**]**.

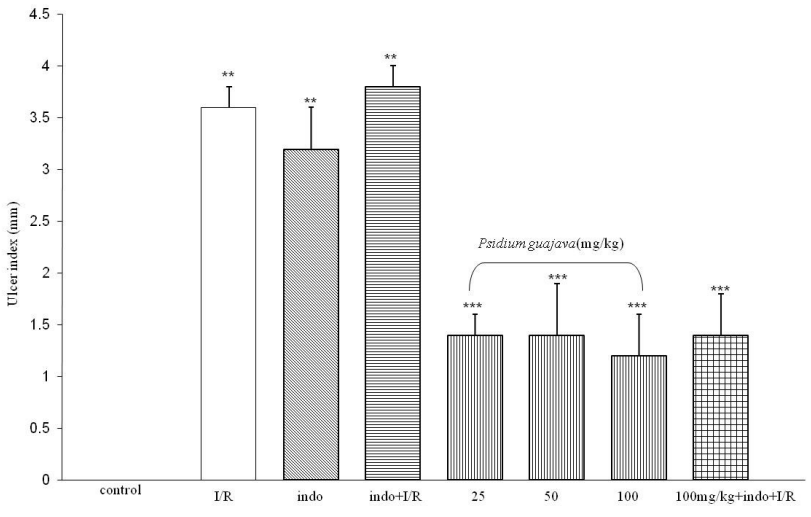

Figure 1: Effect of leaf extract P. guajava [25, 50 and 100 mg/kg] on ischemia-reperfusion [I-R] induced gastric mucosa injury in rats. The animals were pretreated with the extract of indomethacin [10 mg/kg] before I-R. They sacrificed at the end of the reperfusion period and the stomach was examined for hemorrhagic lesions. Each bar represents the mean ± S.E.M of five rats. ** P < 0.001 vs Control; *** P < 0.001 vs I-R without drug administration.

Treatment with 100mg/kg guava administered 30 minutes before dosing with indomethacin and 60 minutes before ischemia, reversed the damage when compared to when indomethacin was given alone before I-R [Fig. 1**]**.

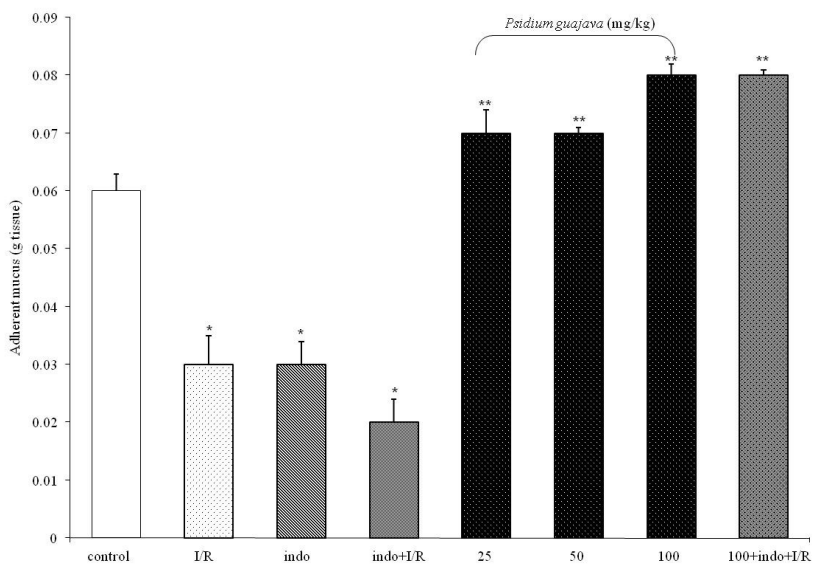

Figure 2: Effect of P. guajava [25, 50 and 100 mg/kg], ischemia-reperfusion [I-R] and indomethacin [indo] on adherent mucus content of the rat stomach. The animals were pretreated with the extract and indomethacin 30 and 60 minutes respectively before 30 minutes of Ischemia and 60 minutes reperfusion period. The animals were sacrificed at the end of the reperfusion period. The stomach was removed and processed for adherent mucus content. Each bar represents mean ± S.E.M of 5 rats. * P < 0.05 vs Control; ** P < 0.005 vs I-R without administration of drugs.

3.2. Effect of Guava on Gastric Secretions

Rats that were subjected to I-R and indomethacin suppressed mucosal release of mucus [P < 0.05; Fig. 2**]**. Pretreatment of extract of guava significantly increased the amount of mucus adherent to the gastric mucosa as compared with the control levels. Likewise 100mg/kg of guava extract reversed the effect of indomethacin administration on mucus output [Fig. 2]. As shown in table 1, I-R decreased the amount of gastric acid in the lumen [P < 0.05]. In guava pretreated animals, gastric acid content of the gastric juice were restored back to control levels.

Table 1: Mean gastric acid output [mEq/L] in the gastric juice of sham [control], ischemia-reperfusion, indomethacin and Psidium guajava [25, 50 and 100 mg/kg] treated animals. Rats were anesthetized with pentobarbital [50 mg/kg i.p.] and subjected to I-R. P. guajava and indomethacin were administered 30 minutes before I-R. Data shown represent mean ± S.E.M of each group of rats. * P < 0.05 vs Control.

Groups	1	2	3	4	5	6	7	8
Acid Out-put (mEq/L)	0.38 ± 0.02	0.17 ± 0.04*	0.12 ± 0.02*	0.25 ± 0.04	0.24 ± 0.04	0.31 ± 0.02	0.11 ± 0.02*	0.31 ± 0.03

4. Discussion

It is known that reactive oxygen metabolites produced by invading neutrophils mediate the macrovascular and parenchymal injury associated with I-R.[4,18] and that gastric mucosal perfusion is an essential component in the ability of the mucosa to protect itself against injury.[19] The presence of a firm mucus layer adherent to the gastric mucosa creates a stable, unstirred layer to support surface neutralization of acid and act as protective physical barrier against luminal pepsin from reaching the underlying epithelium.[22,23] Several mechanisms have been postulated to explain how mucus protects the gastric mucosa against injury. One of such mechanisms is that mucus possesses anti-oxidant properties.[24]

Mucosal lesions induce by I-R has been prevented by pharmacological compounds that posses anti-oxidant or anti-neutrophil properties.[1,20] The most common flavonoid found in guava leaves is quercetin and much of guava therapeutic activity has been attributed to these flavonoids and quercetin in particular. Quercetin has been shown to possess anti-oxidant activity[21] and protect the gastric mucosal against injury induced by I-R.[9]

The gastro- protective property of the extract against I-R induced mucosal injury reported in this study may be related its stimulation of mucus secretion by the mucus cells in the gastric mucosa. Evidence from this study has shown that extracts from the leaves of guava prevent indomethacin induced mucosal injury. Indomethacin is a prostaglandin inhibitor and studies have shown that prostaglandin pretreatment completely prevent the aggravation of I-R injury by indomethacin.[25] Apart from stimulation of mucus secretion,[26] prostaglandins have been found to inhibit the generation of reactive oxygen metabolites in activated neutrophils.[27]

Regarding the role of gastric acid in I-R induced mucosal damage, our results are consistent with earlier studies which reported reduction of gastric acid concentration within 30 min of ischemia.[6] This exclude gastric acid as a major contributor to gastric mucosal damage induced by I-R but still the plant extract was able to restore the gastric acid to control levels. The significant of this observation remains to be identified but it is possible that ischemia may have reduced the metabolic demand of acid secretion and guava extract was able to restore this process.

In conclusion, our results demonstrate that in rats, I-R damage the gastric mucosa by causing a decrease in gastric mucus secretion and that, extract from guava leaves substantially protects the gastric mucosa against injury induced by I-R. This gastro- protective property of the plant may be mediated

through the release of mucus. The anti-ulcer activity of *Psidium guajava* demonstrated in the present study provides support for the traditional use of this plant in the treatment of gastric ulcers and intestinal associated with I-R.

5. References

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