

## Ulceroprotective Effect of Methanol Extract of *Psidium guajava* Leaves on Ethanol Induced Gastric Ulcer in Adult Wistar Rats

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**Abstract:** The present study investigates the anti-ulcer activity of methanol extract of *P. guajava* on ethanol induced gastric ulceration in adult Wistar rats. Twenty nine adult non pregnant female Wistar rats weighing between 160-225 gm were used for the study. Five rats were used for the acute oral toxicity test in accordance with the OECD guidelines 425. The remaining 24 rats were randomly divided in 4 groups of 6 rats each. Groups I and II which served as negative and positive controls orally received 2 mL/Kg body weight of distilled water and 50 mg/kg ranitidine respectively for a period of 10 days while groups III and IV were administered 500 and 1000 mg/kg of methanol extract of *P. guajava* leaves respectively also for 10 days. The next day, ulcer was induced by oral administration of 1 mL of 90% ethanol in all the groups after overnight fasting. The animals were sacrificed 1 h after induction of gastric ulceration and the stomachs were removed, processed and examined using a 6X hand held magnifying lens and mean ulcerative indices were scored accordingly. The result for the acute oral toxicity test showed no mortality nor was there any physical sign of toxicity. Significant dose dependent decrease in ulcer indices was observed among the *P. guajava* leaves extract treated groups as compared to the control groups. The study concludes that methanol extract of *P. guajava* is non toxic in acute doses and confers ulceroprotective activity on ethanol induced gastric ulcer in adult Wistar rats.

**Key words:** Methanol extract, *P. guajava* leaves, ulceroprotective, wistar rats

### INTRODUCTION

Guava (*Psidium guajava*) belongs to the family *myrtaceae*. It has spread widely throughout the tropics because it thrives in the variety of soil, propagated easily and bears fruit relatively quickly. The fruit contain numerous seed that mature into fruit within 4 years. It is an important food crop and medicinal plant in tropical and subtropical countries where it is widely use as food and in folk medicine. The fruit as well as its juice is freely consumed for its great taste and nutritional benefits. In many parts of Africa, the leaves, bark and roots of guava are used traditionally for the management, control and or treatment of an array of human disorder. Much of the traditional uses have been validated by scientific research (Kamath *et al.*, 2008). The medicinal use of guava today is supported by numerous biomedical studies concerning the properties of the leave extract. Guava leaves are used as a quick remedy for the several gastrointestinal conditions; the leaf infusion is taken in Ghana, Senegal and Nigeria for stomach complaints such as constipation and dysentery (Jaiarj *et al.*, 1999). Guava leaf is used traditionally in Africa folk medicine for several ailments including diabetes mellitus, diarrhoea, cough, painful menstruation and hypertension. It is also used to treat tooth decay, gum infection, sore throat to disinfect

wounds and as an antiseptic (Geidam *et al.*, 2007). A number of chemicals have been isolated from the plant including quercetin, guaijaverin, flavonoids and galactose-specific lecithins. *Psidium guajava* or guava is very rich in antioxidants and vitamins and also high in lutein, zeaxanthine and lycopene (Tee *et al.*, 1997; Hobert and Tietze, 1998) and many of these have shown promising activity in many human trials (Abdelrahim *et al.*, 2002). The extensive studies on the plant has shown active pharmacological activity of its major components and the results indicate potent anti-diarrheal, antihypertensive, hepatoprotective, antioxidant, antimicrobial, hypoglycaemic and antimutagenic activities (Nwinyi *et al.*, 2008).

The stomach is a site of massive production and concentration of Reactive Oxygen Species (ROS), far higher than other tissues or biological fluids (Graziani *et al.*, 2005). The generation of these ROS plays a major role in the development of multiple pathologies, such as gastritis, peptic ulcerations or gastric adenocarcinoma (Oliveira *et al.*, 2003). Peptic ulcers are sharp circumscribed lesions with loss of mucus membrane of mucosa of the stomach duodenum or any other part of the gastrointestinal tract expose to pepsin or acidic contents of the gastric juice (Anderson *et al.*, 1994). It is brought about by the activity or imbalance between the mucosal

aggressive factors and mucosal defensive factors inherent in the upper gastrointestinal tract (Guyton and Hall, 2006). The gastric epithelium is often attacked by physical, chemical or microbiological agents acting from the gastric lumen. The numerous injurious luminal agents and irritants are both exogenous and endogenous in origin. Some of the many factors are involved in gastric and duodenal ulcer pathology includes stress, smoking, nutritional deficiencies, alcohol ingestion and nonsteroidal anti-inflammatory drugs (Nash *et al.*, 1994; Basil and Howard, 1995). This study is aimed at determining the ulceroprotective effect of orally administered extract of *Psidium guajava* leave extract on ethanol induced gastric ulcer in Wistar rats.

## MATERIALS AND METHODS

Guava leaves were collected from an orchard in Zaria between January and February 2011. The leaves were authenticated in Biological Science Department of Faculty of Sciences of Ahmadu Bello University Zaria. The batch number obtained was 3253. The study was carried out in the Department of Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The leaves were shade dried and were grounded using an electrical grinder. The powdered leaves were weighed and the mass obtained was 292.5 g. Maceration method was used for extraction. The powdered leaves was soaked for 24 h in 1 L of methanol and placed on a mechanical shaker. After 24 h, the mixture was then filtered (drain) to collect the methanolic solution. The solution was poured into an evaporating dish and was placed on a water bath to hasten the rate of evaporation at about 45-50°C.

**Animals:** Twenty nine healthy Adult female rats of Wistar strain weighing between 160-225 g were obtained from Faculty of Pharmaceutical Sciences Ahmadu Bello University animal house and were kept in a temperature-controlled environment on a 12:12 h light/dark cycle with free access to food and water. The food was withdrawn 24 h before the experiment, but the animals were allowed free access of water. The procedures were in accordance with the guidelines for the care and use of Ahmadu Bello University Zaria. All chemicals and solvents were of analytical grade. The ulcerogenic drugs and necrotizing agents were freshly prepared before administration.

**Acute toxicity studies:** Acute toxicity study was carried out using five female albino rats following the OECD guidelines 425 (OECD, 2001). They were observed for signs of toxicity and mortality up to a dose of 2,000 mg/kg/ body weight and thereafter for 14 days.

The rats were divided into four groups of 6 rats each. The administration of extracts was carried out

intragastrically i.e., using a gavage. Negative Control received 2 mL/kg of distil water ONLY, Group 2-Positive control received 50 mg/kg ranitidine, Group 3 received 500 mg/kg of extract, Group 4-received 1000 mg/kg of extract. Administration of extract and control drugs was for 10 days after which 90% ethanol was administered to the overnight fasted rats of all groups the next day; at a dose of 1 mL per animal, irrespective of the weight of the animal through oral route (Main and Whittle, 1975). One hour after ethanol administration, all rats were sacrificed by an overdose of chloroform and the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water. They were then spread on a paraffin plate and the inner surface was examined with a 6x hand held magnifier. The scores for each single lesion were then totalled (Valcavi *et al.*, 1982). Mean ulcerative index was calculated as follows:

- Presence of oedema, hyperaemia and single submucosal punctiform haemorrhages.
- Presence of submucosal haemorrhagic lesions with small erosions.
- Presence of deep ulcer with erosions and invasive lesions (Pandit *et al.*, 2000):

Ulcer index = (number of lesion. I) + (number of lesion. II)  $\times$  2 + (number of lesion. III)  $\times$  3

The percentage inhibition was determined as follows:

= (Control mean lesion index-Test mean lesion index) /X 100Control mean lesion index

**Statistical analysis:** The data were represented as Mean $\pm$ SEM. The data on antiulcer activity of leaves of *Psidium guajava* were analyzed by one way Analysis of Variance (ANOVA), 'P' value less than 0.05 was considered as statistically significant. EZAnalyze 3.0 and Microsoft Excel 2007 were used for analysis and production of charts.

## RESULTS AND DISCUSSION

The administration of crude methanolic extract of *Psidium guajava* in doses: 500 and 1000 mg/kg body weight causes a decrease in ulcer index of ethanolic induce ulceration in the stomach of Wistar rats.

Ethanol induced gastric ulcer was employed to study the ulceroprotective effect of guava leaves extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric

Table 1: Showing mean ulcer index and % protection of methanolic extract of *psidium guajava* leaves and ranitidine on alcohol induced ulcer in wister rats

Treatment and doses	No of animals	Mean ulcer index $\pm$ SEM	% protection
G1 (distil water 2 mL/kg)	6	17.7 $\pm$ 1.86	NIL
G2 (ranitidine 50 mg/kg)	6	4.8 $\pm$ 0.98	73
G3 (500 mg/kg)	6	12.0 $\pm$ 1.41	32.2
G4 (1000 mg/kg)	6	6.3 $\pm$ 1.21	64.4

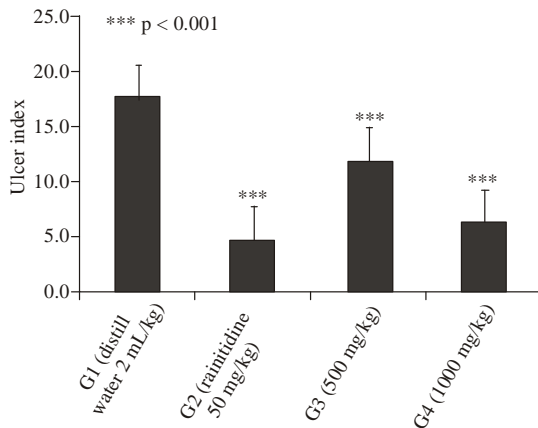


Fig. 1: Showing Mean ulcer index and SEM

mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Soll, 1990; Surendra, 1999; Raju *et al.*, 2009). Diet is said to exert multiple protective biological effects on the mucosa of the gastrointestinal tract. Fruits and vegetables especially seem to play a preventive role against the development of gastric erosions, ulcerations and cancer. The present study showed that guava leaves extract had a significant protective effect (64.4%) at 1000 mg/kg of extract on the stomach wall when compared to the control (Table 1). This was however less than the test drug ranitidine which was 73% (Table 1). There was also a significant decrease in ulcer index between the control and test groups. Group 1 had 17.7 $\pm$ 1.86; groups 2, 3 and 4 had 4.8 $\pm$ 0.98, 12.0 $\pm$ 1.41 and 6.3 $\pm$ 1.21, respectively (Fig. 1). This may lend credence to the use of guava leaves in the treatment of various gastrointestinal ailments. *Psidium guajava* or guava is very rich in antioxidants and vitamins and also high in lutein, zeaxanthine and lycopene (Tee *et al.*, 1997; Hobert and Tietze, 1998). It has been also demonstrated that substances with antioxidant properties, such as flavonoids and polyphenolic compounds, may protect against gastric-damaging effects caused by ethanol (Matsumoto *et al.*, 2004; Dekanski *et al.*, 2009) and guava is very rich in some of these. Reactive Oxygen Species (ROS) is a

known risk factor of such diseases as gastritis, peptic ulcerations or gastric adenocarcinoma. Antioxidants, such as coenzyme Q10 and quercetin, a member of flavonoids present in guava, red wine and tea, are thought to play a significant role in protecting cells from oxidative stress induced by reactive oxygen species. The biological activity of quercetin appears to be due to its potent antioxidant activity. Quercetin scavenges oxygen-free radicals and inhibits the enzyme xanthine oxidase (Miller, 1996), lipid peroxidation, the inflammation-producing enzymes (cyclooxygenase, lipoxygenase) as well as the subsequent inhibition of inflammatory mediators including prostaglandins (Kim and Kim, 1998). Inhibition of histamine release also contributes to quercetin's anti-inflammatory activity (Bronner and Landry, 1985). In an in vitro study, quercetin was shown to inhibit growth of *Helicobacter pylori* in a dose-dependent manner (Beil *et al.*, 1995) and these bacteria is a known contributor to peptic ulcer disease. A combination of all or some of these factors especially the strong antioxidant activity of the guava constituents may be responsible for the Ulceroprotective effect of the extract on the gastric mucosa.

## CONCLUSION

Natural products of plant origin are still a major part of global traditional medicine especially in gastrointestinal disorders. Some studies have shown that drugs commonly used for peptic ulcers such as H2-blockers (ranitidine, famotidine etc.), M1-blockers (pirenzepine, telenzepine etc), proton pump inhibitors (omeprazole, pantoprazole etc.) have danger of drug interaction, adverse effect and increased incidence of relapses during ulcer therapy (Goel and Bhattacharya, 1991); hence the need for better alternatives. This study shows that methanolic extract of *Psidium guajava* leaves showed significant decrease in the mean ulcer index in the pre-treated group as well as good degree of protection and so has the potential to protect the stomach against ulcerogens.

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