

Anacardium Occidentale and Psidium Guajava effectively reduce Ethanol induced Gastric ulcer in male Wistar rats

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Abstract

Ulcer is one of the world's major gastrointestinal disorders affecting a good percentage of the populace in industrialised and developing countries. Plant extracts are some of the most attractive sources of new drugs and have shown promising results for the treatment of gastric ulcer as they possess a variety of natural therapeutic products. This study aimed to look at the effect of combining *Anacardium Occidentale* and *Psidium Guajava* on the management of gastric ulcer. The extracts of the leaves of *Anacardium occidentale* and *Psidium guajava* were tested in ethanol-induced ulcer models in rats. Oral administration of these plant extracts and reference drug was done for two weeks for 30 male Wistar rats divided into five (5) groups. The treatment with the combination of the extract stock solutions at a dose of 150mg/kg each exhibited a significant ($p < 0.05$) decrease in gastric volume, 2.1×10^{-1} mEq/L and ulceration, 2.0 ± 0.26 than the individual extract groups when compared to control 4.0×10^{-1} mEq/L and 5.2 ± 0.60 respectively while cimetidine at the dose tested had the highest percentage reduction. The efficacy of the extracts presented here confirms that the combination of *Anacardium occidentale* and *Psidium guajava* possess better synergistic ulcer healing and gastro protective effects than the individual components. This affirms their efficacy in the management of ulcers.

Keywords: Gastric ulcer, *Anacardium occidentale*, *Psidium guajava*, Cimetidine, Ethanol, Gastric acid Secretion, Male Wistar rats.

Introduction

In this modern era, 75–80% of the world population still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body,

and less side effects (Kumar, 2011). Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity (Patel and Jain, 2010). Plant extracts are some of the most attractive sources of new drugs and have shown promising results for the treatment of gastric ulcer (Schmeda-Hirschmann and Yesilada, 2005). Also, drugs currently used in the treatment of peptic ulcers are antacids, anticholinergics, proton pump inhibitors and H₂-receptors antagonists.

Anacardium occidentale (*A. occidentale*), known as cashew (English), kaju (Yoruba) is a tropical shrub which belongs to the family Anacardiaceae. Its leaves are green with pink-streaked yellow flowers on the spikes. The species is indigenous to Brazil and also grows in tropical areas of Central America and South America and in the West Indies in tropical forests and greenlands as well as Africa. It has however been used locally to treat malaria, toothaches and inflammation of the gums.

Psidium guajava (*P. guajava*), known as guava (Hausa), guafa (Yoruba), gwaibwa (Igbo) is an evergreen shrub with white flowers which belongs to the family of Myrtaceae. It is native to the Caribbean, Central America, South America as well as Africa. It is also grown in Nigeria. However, leaf extracts of *P. guajava* are used in folk medicine to treat gastrointestinal disorders, cough and diarrhea. Phytochemical investigation showed the presence of various flavonoids, mainly quercetin glycosides and saponins in ethanol extract. Flavonoids are free radical scavengers, play important role in gastric ulcer, increase mucosal prostaglandin content and decrease histamine secretion from mast cells by the inhibition of histidine decarboxylase (Borrelli and Izzo, 2000). Quercetin was also found to prevent gastric mucosal lesions (Martin *et al.*, 1993). Various saponins have also been found to possess antiulcer activity (Houng *et al.*, 1998; Matsuda *et al.*, 1998). However, flavonoids and saponin are mainly responsible for antiulcer activity of *Anacardium occidentale*.

Pharmacologically, *P. Guajava* leaves have hepatoprotection, antioxidant, anti-inflammatory, anti-spasmodic, anti-cancer, antimicrobial, anti-hyperglycemic and analgesic effects (Ryu *et al.*, 2012; Metwally *et al.*,

2010; Roy *et al.*, 2006; Ojewole, 2006; Nair and Chanda, 2007; Hui-Yin and Gow-Chin, 2007; Peng *et al.*, 2011; Chen *et al.*, 2010). Ulcer is an inflammatory mucosal erosion of the epithelial lining equal to or greater than 0.5 cm. It is an open sore of the skin or mucus membrane characterised by sloughing of inflamed dead tissue (Chan FKL and Graham, 2004). Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcers such as mouth ulcer, oesophageal ulcer, peptic ulcer, and genital ulcer. Of these, peptic ulcer is the most common. Peptic ulcers are erosions of the lining of the stomach or duodenum (Debjit *et al.*, 2010). In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain, and cramps along with vomiting of blood. Peptic ulcer is one of the world's major gastrointestinal disorders affecting 10% of the world population (Zapata-Colindres, 2006). About 19 out of 20 peptic ulcers are duodenal. An estimated 15000 deaths occur each year as a consequence of peptic ulcer (Lau *et al.*, 2011). Annual incidence estimates of peptic ulcer haemorrhage and perforation were 19.4–57 and 3.8–14 per 100,000 individuals, respectively. The average 7-day recurrence of haemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2% (Lau *et al.*, 2011).

As many as 70% of such ulcers are associated with *Helicobacter pylori* (a helical-shaped bacterium that lives in the acidic environment of the stomach). The incidence of ulcer depends on the battle between the mucosa defensive factors like mucus secretion, prostaglandins, glycoproteins, anti-oxidant enzymes (catalases, superoxide mutase, glutathione) and mucosal irritating or offensive factors like acid, pepsin secretion and lipid peroxidation (Prabha *et al.*, 2009). Other factors such as stress, smoking, nutritional deficiencies, ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) and hereditary predisposition have been demonstrated to increase the incidence of peptic ulcer (Barros *et al.*, 2008).

This study therefore aimed to look at the effect of commonly consumed fruits of *A. occidentale* and *P. guajava* on ulcer protective indices in Wistar rats. It is expected to contribute to the knowledge base on gastric ulcer and its management with the combination of *A. occidentale* and *P. guajava* composition, and to stimulate continuous research towards

Statistical analysis

Methods

Experimental animals

Male Wistar rats weighing about 180-200g were used for this study. They were obtained from 180-185g Wistar rats brought to the University of Ibadan College of Health Sciences Animal House where they were exposed to a constant temperature of 22°C, dark light cycle in a cage of optimum ventilation and illumination with adequate food (guinea piglets) water and protection. Also they were kept clean always in accordance to the guide for the care and use of laboratory animal by National Research Council of USA, 1966.

Experimental plants

The plants, *Commersonia bartramia* and *Phyllanthus* species, were collected from plants growing along highway continuously and were identified by Mrs. Isaac Omidi, Botanist of the Department of Plant Biology, University of Ibadan, Ibadan.

Preparation of Extracts

The roots used in this study were air-dried and grinded separately to powder form. The powder form of *C. bartramia* weighing 10g was mixed with 400ml of distilled water and was left for 24 hours after which it was stirred and evaporated to dryness in an oil bath at 50-60 degrees which gave a yield of 20g.

The powder form of *P. guineensis* weighing 10g was mixed with 400ml of distilled water and was left for 24 hours after which it was stirred and evaporated to dryness which gave a yield of 10g of plant material. These extracts were diluted to 100% alcohol solution for pharmacological studies. Preliminary phytochemical screening of the extracts of *P. guineensis* revealed the presence of flavonoids, tannins, saponins, carbohydrates, alkaloids, glycosides and cardiac-glycosides. Acute toxicity studies of the alcoholic extract of the *P. guineensis* did not exhibit any signs of toxicity up to 2g/kg body weight. A high dose or mortality of the animals was observed, hence, 100mg/kg dose of the extract was selected for evaluation of anti-ulcer activity.

Experimental procedure

In each group of mice, 100 individuals were used. 50 male white mice were used and divided into five (5) groups of 20 (20) mice each. Group 1 (control group) received 500 kg of normal saline. Group 2 and group 3 received 100 mg/kg body weight of *A. extractum* and *P. granatum* respectively. Group 4 received a combination of *A. extractum* and *P. granatum* extract such as shown in table 1.0. While Group 5 (reference group) received 100 mg/kg of cimetidine throughout the period of administration. The reference drug which was bought at Ayo Pharmacy, Ibadan was administered according to weight of animals.

The experimental procedure commenced by acclimatizing the animals for two weeks at the animal house, College of Health Sciences, University of Ibadan. This was followed by two weeks oral administration of the plant extracts and reference drug. All the groups were fasted on the 14th day for twenty-four hours but had free access to water. Afterwards, 1.0 ml of 70% ethanol (9:1 v/v H₂O:ethanol) was given orally to all groups to induce ulcers. Four hours later, the animals were sacrificed after being anaesthetized with chloroform. The lower end of the esophagus and the pylorus were clamped and the stomach was cut open along the greater curvature and the gastric content was collected and it was then washed clear using normal saline and spread on a plain white material to be viewed with the magnifying lens.

Grading of Ulcer

The severity of gastric mucosal lesions expressed as ulcer index was determined as described by Jones et al. in 1965 and was graded as follows:
Grade 0 = No lesion (No visible damage)

Grade 0.5 = *Microscopic*

Grade 1.0 = Slight Lesion (the presence of one ulcer and generalized erythema)

Grade 2.0 = Severe Lesion (at least two ulcers approximately 2mm in length)

Grade 3.0 = Very Severe Lesion (ulcers 1-4mm in length on 80% of the folds)

Grade 4.0 = Almost full of Lesions (lesions which followed approximately 80% of the folds)

Grade 5.0 = Multiple ulcers following almost entire length of gastric fold.

Measurement of Gastric acidity

Samples of gastric contents were centrifuged for 10 minutes at a speed of 200m/s. A quantifiable ml of supernatant from each rat was analysed for hydrogen ion concentration by titrating it against 0.01M of NaOH to a pH of 1.0. This was detected by using phenolphthalein (2 drops) as indicator, which when added to the supernatant gave a colourless liquid but when further titrated against 0.01M of NaOH at pH of 1.0, the colour of the supernatant with the indicator gave a pink colour. The acid content was expressed in Meq/L using the formula below;

$$C_a V_a = C_b V_b$$

Where; C_a = Concentration of acid
 C_b = Concentration of base
 V_a = Volume of acid
 V_b = Volume of base

Histopathological Study

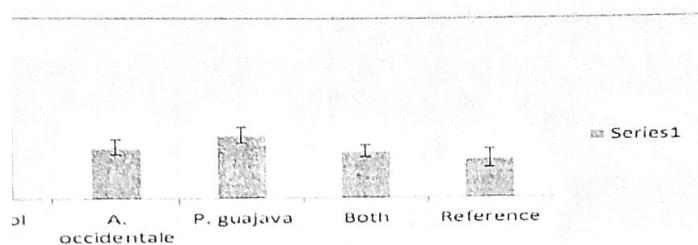
The gastric tissues were fixed in formalin followed by sectioning to examine the stomach and tissues histopathologically. This was to study the ulcerogenic or anti-ulcerogenic effects of *A. occidentale* (150mg/kg), *P. guajava* (150mg/kg) and the combination of *A. occidentale* and *P. guajava* extract stock solution in 1:1 ratio. The tissues were processed using a tissue processor and the processed tissues were embedded in paraffin block and little thick sections were cut using a rotatory microtome. These sections were stained using hematoxylin and eosin stain. The slides were then examined under the microscope for patho-morphological changes such as inflammation, congestion, edema, hemorrhage and erosion or positive results such as regeneration and protection of mucosal layer and their photomicrographs were taken.

Results

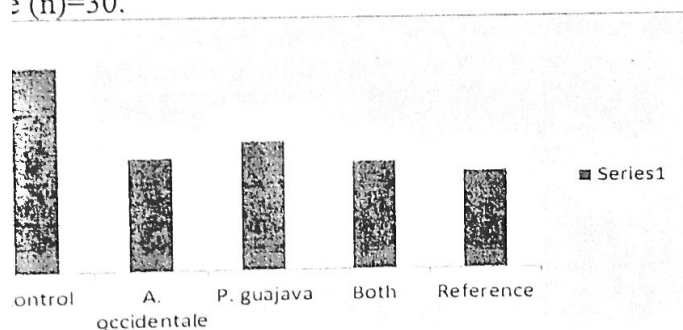
After treatment by extracts, the ulcer count index and gastric acid secretion were observed to be significantly decreased (* $p < 0.05$), and this reduction was compared to control group (group 1). This is presented in Table 1, Figure 1 and Figure 2.

ts of aqueous extracts of *Anacardium occidentale* and
a on ethanol induced ulceration and gastric acid secretion
rats.

	Mean score of ulcer	Mean of gastric acid secretion ($\times 10^{-3}$) mEq/L
al saline	5.2 $\pm$ 0.60	4.0
identale (150mg/kg)	2.2 $\pm$ 0.31*	2.2*
java (150mg/kg)	2.7 $\pm$ 0.33*	2.5*
identale (150mg/kg)+ P.guajava g/kg)	2.0 $\pm$ 0.26*	2.1*
idine (100mg/kg)	1.7 $\pm$ 0.42*	1.9*



ect of aqueous extracts of *A. occidentale* and *P. guajava* on
d gastric ulcer showing the values expressed in mean $\pm$ SEM
e (n)=30.



ect of aqueous extracts of *A. occidentale* and *P. guajava* on
ecretion showing the values expressed in mean $\pm$ SEM for
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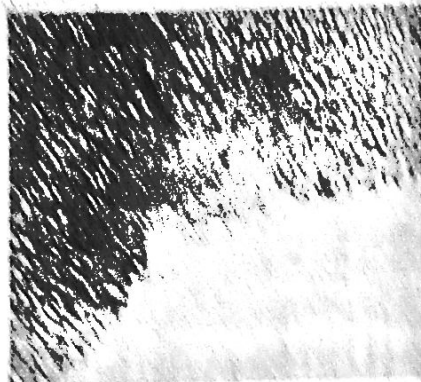
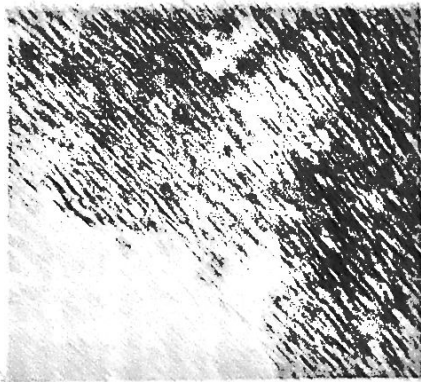


Figure 2 (a) and (b) are a view of the gastric mucosa (gastric 1) where gastric mucosa was removed with biopsy. Figure 2 shows inflammation of the gastric mucosa which is a typical finding in the gastric mucosa.

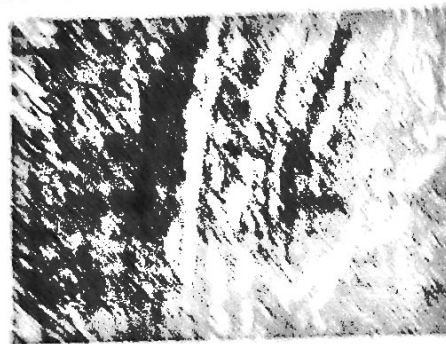
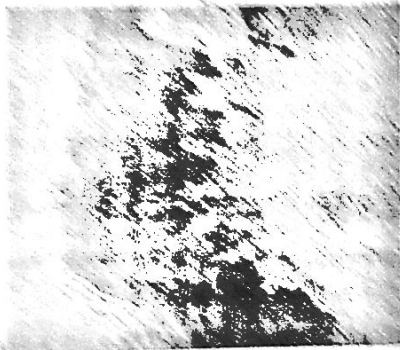
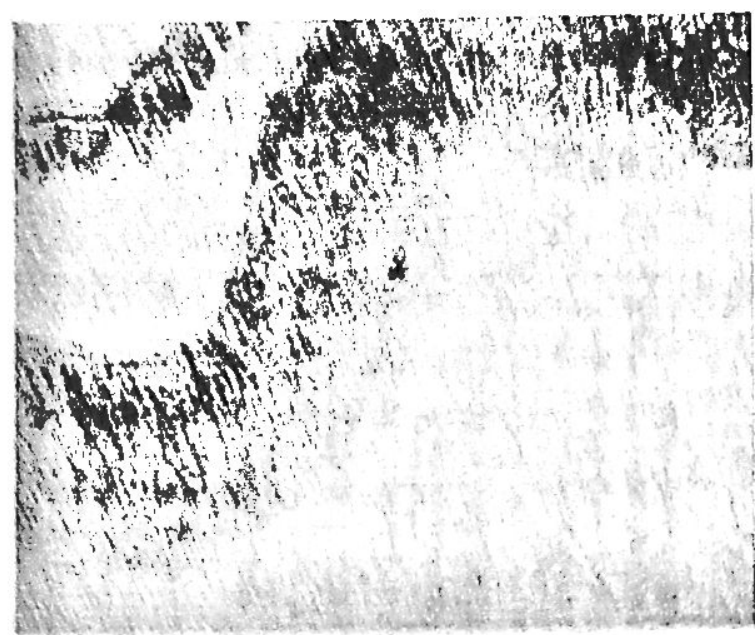


Figure 3 (a) and (b) are a view of the gastric mucosa (gastric 2) where gastric mucosa was removed. Figure 3 shows inflammation of the gastric mucosa which is a typical finding in the gastric mucosa.





(a)



(b)

Figure 8: (a) Slide of group 4 where gastric tissue was treated with the combination of *A. occidentale* and *P. guajava* extracts. It shows the protection of the gastric mucosa. (b) Slide of group 4 where gastric tissue was treated with the combination of *A. occidentale* and *P. guajava* extracts. It depicts some areas of ulceration but majority of the areas are reflecting the protection of the gastric mucosa.



(a)



(b)

Discussion

Since the advent of medicine, chemical substances derived from plants have been used to treat human diseases. Roughly, 50% of new chemical substances introduced during the past two decades are from natural products. Also, recent technological advances have renewed interest in natural products in drug discovery. At the same time considering the fact that chronic degenerative diseases have reached epidemic proportions in many countries and increased the socio-economic burden on the public health system, it is necessary to find non-allopathic alternatives that minimise risk factors of these diseases and help in their treatment. This prompted the use of *A. occidentale* and *P. guajava* which has been demonstrated to possess a wide variety of bioactive compounds in their extracts with beneficial effects on human health. Furthermore, population consumes medicinal plants to treat other kinds of diseases as a result of the high costs of allopathic medications.

In this study, *Anacardium Occidentale* and *Psidium Guajava* effectively reduced ethanol induced gastric ulcer in male Wistar rats. The result showed that the extracts at doses tested had significant effect compared with that of control (normal saline). The combination of *A. occidentale* and *P. guajava* extract stock solutions in ratio 1:1 showed higher percentage reduction in ulcer count than each of *A. occidentale* group and *P. guajava*. The animals treated with *A. occidentale* extract exhibited a significant ($p < 0.05$) decrease of gastric volume, 2.2 ± 0.17 ml/g/L and ulceration, 2.2 ± 0.31 when compared with the control 4.0 ± 0.17 ml/g/L and 5.2 ± 0.60 respectively. This is similar to a study in São Paulo, Brazil, where antitumorogenic effect of a 70% ethanolic extract of *Anacardium occidentale* leaves was investigated against HCl/ethanol induced ulcer and found that the extract inhibits gastric lesions significantly in dose dependent manner (Kanan and Baccin, 2007).

The results further revealed the fact that the animals treated with extracts of *P. guajava* exhibited a significant ($p < 0.05$) decrease in gastric volume, 2.5 ± 0.17 ml/g/L and ulceration, 2.7 ± 0.33 when compared with the control. The results were compared to a standard reference drug commercially available in the market, cimetidine. *Psidium guajava* significantly ($p < 0.05$) reduced the ulcer index and offered significant protection against ethanol induced ulcer.

Moreover, the result also showed that *P. guajava* extract at the dose of 150mg/kg had the least percentage of reduction whereas *A. occidentale* extract at the dose of 150mg/kg had higher percentage reduction in ulcer count.

The ulcerogen used in this study is acceptable in animal experimentation as observed in previous studies (Szabo *et al.*, 1993) where it shows that gastro intestinal tract ulceration could be induced by administration of 1ml/kg of ethanol. Cimetidine on the other hand, has long been known to be an anti-ulcer drug (Silen, 1981). It is effective in the treatment of ulcer and this was established by this study as the mucosa of the rats were protected.

Ethanol-induced gastric ulcer can arise as a result of direct damage to mucosal cells, development of free radicals and hyperoxidation of lipid (Terano *et al.*, 1989) but scavenging these free radicals can play a role in healing of these ulcers (Halliwell and Gutteridge, 2007). In this study, the gastric acid secretion was found to be significantly reduced compared to the control further confirming that the extracts at the doses tested have anti-ulcerogenic activity since an increase in gastric acid have been implicated in the pathogenesis of ulcers (Davenport, 1967).

This study, combining the use of *A. occidentale* and *P. guajava*, has brought information that may provide validation for its medicinal uses but it should be researched more extensively in clinical trials so it could be used for prevention and as an adjuvant in the treatment of numerous disorders. This can be ensured by directing efforts towards isolation and characterisation of the active principles and clarification of the relationship between structure and activity, so much so that detailed analysis of the active constituents of *A. occidentale* and *P. guajava* could be directed towards clinical relevance and global competitiveness. As the current economic downturn in Nigeria bites deeper, there is an increased tendency for the rural populace to tilt towards the patronage of non-allopathic medications such as *A. occidentale* and *P. guajava* for the treatment of diseases. Therefore, the importance of *A. occidentale* and *P. guajava* as drugs of therapeutic effect cannot be underestimated.

Conclusion

In conclusion, the findings of the present study confirmed that the leaf extracts of *A. occidentale* and *P. guajava* possess gastro protective effects against the experimentally induced gastric ulcer models. Therefore, the combination of the leaf extracts of *A. occidentale* and *P. guajava* produced a better synergistic gastroprotective effect than their individual extracts. This makes their combination a good alternative in the management of gastric ulcer.

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