



## GASTROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *Raphia mambillensis* SEEDS ON INDOMETHACIN- INDUCED ULCERATED *Wistar* RATS

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### ABSTRACT

*Raphia mambillensis* (Raffia palm) seeds are traditionally used in South Western Nigeria to treat different ailments including gastrointestinal-related disorders. This research was conducted to assess the gastroprotective efficacy of Ethanolic Extract of *Raphia mambillensis* seeds (EERMS). Animals were pre-treated orally with different doses of EERMS and reference drug (ranitidine) for 5 days and then induced for gastric ulceration using indomethacin (10mg/kg body weight) for 3 days. Various parameters of gastric secretion were examined after the trial. EERMS (50mg and 100mg/kg b.w.) significantly ( $P < 0.05$ ) ameliorated the indomethacin-induced gastric mucosal damage as shown by the reduced ulcer index, gastric acidity, and volume of treated groups when compared with the untreated group. This is highly comparable with the group treated with the reference drug. The presence of a high concentration of Phenolics, Tannins, and antioxidant minerals may be attributed to the antiulcerogenic activity of EERMS. The results suggest that the EERMS confers protection on the gastric tissue against indomethacin-induced ulcerated rats.

**Keywords:** *Raphia mambillensis*, ranitidine, mucosal damage, ulcer index, indomethacin

### INTRODUCTION

The gastrointestinal tract must conduct several coordinated processes to digest food, absorb nutrients, and ultimately deliver a constant supply of water, electrolytes, and nutrients to the entire body (Chang and Leung, 2014). Peptic Ulcer Disease (PUD) is a common gastrointestinal condition associated with risk factors such as alcohol, lifestyle, *Helicobacter pylori*, smoking, and nonsteroidal anti-inflammatory drugs (Lee *et al.*, 2017). Indomethacin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are effective anti-inflammatory, analgesic, and antipyretic medications. Their use has however been limited due to their side effects like development of peptic ulcer disease (Pountos *et al.*, 2011). The pathophysiology of gastric injury caused by

NSAIDs is based on various processes, including suppression of prostaglandin synthesis, the creation of free radicals, and the depletion of gastric NO<sub>2</sub> content (Matsui *et al.*, 2011).

The treatment of peptic ulcers depends on either fighting injurious factors such nonsteroidal anti-inflammatory drugs (NSAIDs) or increasing mucosal defenses. (Jose *et al.*, 2019). Drugs such as histamine (H<sub>2</sub>) receptor antagonists, proton pump inhibitors, antacids, and anticholinergics are the popular medications used to treat PUD. However, the majority of these medications have a variety of unfavorable side effects and drug interactions (Namdev and Jain, 2019). Researchers' increased interest in finding alternative medications for several metabolic

ailments, particularly from plant sources, has led to a surge in research on medicinal plants (Gouws and Hamman, 2018).

Plants are one of the most appealing sources of new drugs, and some have shown therapeutic potential for the treatment of peptic ulcers with minimal side effects (Ndip *et al.*, 2007). In Nigeria, such a plant used locally for the management of Peptic ulcer disease is *R. mambillensis*.

*R. mambillensis* is a trunk less palm with huge fronds. The Palm is up to 7 meters in height. It is found in Cameroon, the Central African Republic, Nigeria, and Sudan (Cheek *et al.*, 2000 and is also used traditionally to treat female infertility (Soladoye *et al.*, 2014).

Its sap is used to promote lactation in nursing mothers, to treat different viral and bacterial infections (Mbuagbaw and Noorduyn, 2012). The seeds are burnt into ash and used with pap for ulcer treatment in South-Western Nigeria. This study was conducted to evaluate the gastroprotective activity of ethanolic seed extract of *R. mambillensis* on indomethacin-induced ulcer in experimental rats.

## MATERIALS AND METHODS

### Plant materials and chemicals

*R. mambillensis* seeds were obtained from the local drug market in Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin; with the voucher number UILH/002/1214. Ranitidine and indomethacin used for the research were products of Zantac Pharmaceutical and Ortin laboratories Nigeria Ltd, respectively. Other chemicals used were of analytical grade.

### Animals

Thirty Wistar rats (*Rattus norvegicus*) of both sexes weighing between 100-150g were obtained from the animal unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. They were kept at 12h light and 12h dark condition in the animal house of the Department of Biological Sciences, Al-Hikmah University, Ilorin, Kwara State, Nigeria for 7 days before the commencement of the experiment. They were given feed pellet (Top Feed Mills Company, Ibadan Nigeria) and water *ad libitum*. The research was conducted according to the

principles of Laboratory Animal Care (NIH Publication, No. 85-23).

### Preparation of *R. mambillensis* Extract

*R. mambillensis* seeds were crushed with mortar and pestle into pieces and then ground into a coarse powder using an electric blender (Century electric blender; Model CB-8231-R). One hundred grams (100g) of the coarse powder was soaked in 1000ml of 95% ethanol for 24hrs. A muslin cloth and what man no. 1 filter paper were used to successively filter the extract and then concentrated using a rotary evaporator.

### Phytochemical analysis

#### Determination of total phenols

The total amount of total phenol present in EERMS was determined using the Folin Ciocalteu reagent method (Olajire and Azeez, 2011). In brief, EERMS (0.25 mL) was diluted with 5 mL of distilled water and 1.25 mL of Folin-Ciocalteu phenol reagent. And was allowed to stand for 5 minutes, 1 ml of Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture and was made up 12.5 mL using distilled water and allowed to stand for 90 minutes. Absorbance was measured at 780 nm, and phenolic content was expressed in milligrams of gallic acid equivalent per gram of dried plant material.

#### Determination of total flavonoids

The total flavonoids concentration of the EERMS was estimated using the Aluminium chloride colorimetric method (Olajire and Azeez, 2011). In a 10 mL volumetric flask, 1.5mL of test sample (EERMS) was combined with 5 mL distilled water and 0.3 mL 5 % NaNO<sub>2</sub>. Methanolic 2% AlCl<sub>3</sub> solution (1.5 mL) and 2 ml of 1 M NaOH was added after 5 minutes, and the volume brought to 10 mL with distilled water. The mixture was shaken for 5 minutes at 200 rpm on an orbital shaker, and the absorbance at 367nm was measured after 10 minutes of incubation. The flavonoid content was reported as milligrams of quercetin per gram of extract.

#### Determination of total tannins

The level of total tannins present in EERMS was determined using the method of Edeoga *et al.* (2005) with slight modification. EERMS (5 ml) was pipetted into a test tube and mixed with 2 ml of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was

measured at 500nm. The total tannin content was expressed as mg quercetin/g.

#### Determination of mineral content

Selected minerals (Na, K, Ca, Mg, Mn and Zn) in EERMS were determined using Atomic Absorption Spectrometer (Model 2280 Perkin Elmer, Spain).

#### Experimental Protocol

Thirty (30) rats of both sexes were completely randomized into five groups (n=6). They were orally pretreated as follows using 1% Dimethylsulphoxide (DMSO) as a vehicle:

Group 1: Normal control received 1ml of 1% DMSO

Group 2: Ulcer control received 1ml of 1% DMSO

Group 3: Drug control received 20mg/kg body weight of Ranitidine

Group 4: Received 50mg/kg body weight of EERMS

Group 5: Received 100mg/kg body weight of EERMS.

The experiment was carried out over seven days. On the fifth to the seventh day of the trial, animals in groups 2-5 were given 10mg/kg b. w of indomethacin daily to induce ulcer. All rats were anesthetized with diethyl ether four hours after the last indomethacin administration. Each rat's abdomen was opened to remove the stomach.

#### Volume and pH of gastric juice

The sacrificed rats' stomachs were opened along the greatest curvature. Each rat's gastric contents were emptied into a centrifuge tube and spun for 10 minutes at 1000 rpm. A pH meter and a standard measuring cylinder were used to determine the pH and volume of stomach juice (Da Silva *et al.*, 2015).

#### Determination of the Total Acidity

About 0.2 ml of gastric juice (0.2 ml) was titrated against 0.01 N NaOH using an endpoint of pH 7.0 as determined colorimetrically with phenol red as an indicator. It was calculated as milliequivalents per liter (mEq/L) which is equal to the number of milliliters (mL) of 0.01 N NaOH required to neutralize 1 mL of the gastric juice (Srivastava *et al.*, 2010).

Total acidity =

$\frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{10}$

10

#### Determination of ulcer index (UI)

An incision was made along the greater curvature to expose the mucosa layer of the lumen. The ulcers were measured using a vernier caliper. The sum of all lengths of all the lesions per stomach was calculated as the ulcer index for each group of rats in mm (Rifat-uz-Zaman *et al.*, 2004).

#### Statistical analysis

Graphpad Prism version 5.01 was used for statistical analysis. The Tukey multiple comparison test was used after the one-way analysis of variance. Significant was defined as a P-value < 0.05.

## RESULTS

#### Phytoconstituents of the plant's extract

The concentration of some phytoconstituent present in the ethanolic extract of *R. mambillensis* seeds is shown in Table 1. The extract contains a higher level of phenol and tannin when compared to the small level of flavonoids.

**Table 1:** Total phenols, Flavonoids and Tannins and some selected minerals content of ethanolic extract of *R. mambillensis* seeds

| Phytoconstituent                          | Concentration                   |
|-------------------------------------------|---------------------------------|
| Total phenol                              | 102.35 ± 0.104 mg gallic acid/g |
| Total flavonoid                           | 10.36 ± 0.147 mg quercetin/g    |
| Total tannins                             | 57.28 ± 0.184 mg quercetin/g    |
| Values are mean of three replicates + SEM |                                 |

Table 2 shows the amounts of certain mineral elements found in the ethanolic extract of *R. mambillensis* seed.

**Table 2:** Concentration of selected minerals in the ethanolic extract of *R. mambillensis* seeds

| Mineral Element | Concentration ( $\mu\text{g}/\text{mg}$ ) |
|-----------------|-------------------------------------------|
| Na              | 1662                                      |
| K               | 15963                                     |
| Ca              | 1200                                      |
| Mg              | 990                                       |
| Mn              | 3168                                      |
| Zn              | 85                                        |

#### Effects of the extract on gastric ulcer

When compared to the control group, oral indomethacin for ulcer induction resulted in a considerable increase in stomach volume and acidity, as well as a reduction in gastric pH. Pre-treatment with either ranitidine or EERMS however caused a decrease in the gastric volume and acidity with an increase in gastric pH level as shown in Figures 1 – 3. This difference was however more significant with the lower dose of EERMS (50 mg/kg ) when compared to the reference drug and 100 mg/kg of EERMS. Figure 4 shows the effect of ethanolic extract of *R. mambillensis* on the ulcer index of indomethacin-induced ulcerated rats. The ulcer index of the indomethacin-induced ulcerated rats was higher than the treated group. The reference group and the group treated with 50mg/kg bw of EERMS have the lowest ulcer index.

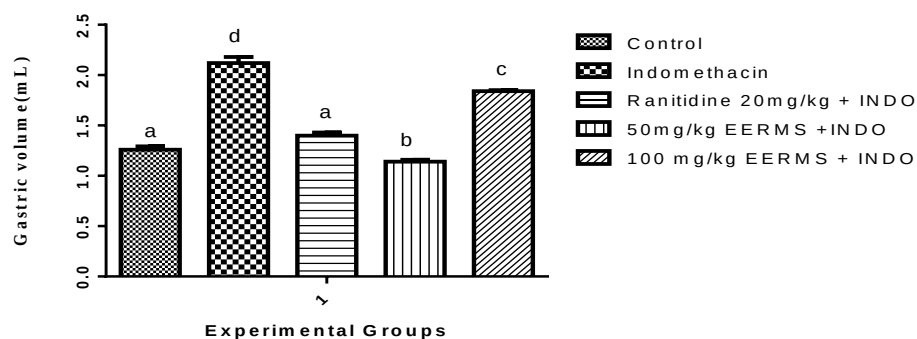


Figure 1: Effect of ethanolic extract of *R. mambillensis* seeds and ranitidine on the gastric volume of indomethacin-ulcerated rats.  
(Values are mean  $\pm$  SEM of six determinations; INDO: Indomethacin)

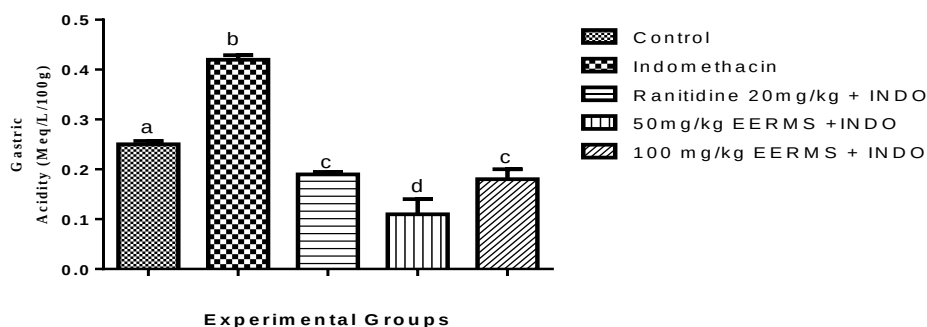


Figure 2: Effect of ethanolic extract of *R. mambillensis* seeds and ranitidine on gastric acidity of indomethacin ulcerated rats.  
(Values are mean  $\pm$  SEM of six determinations; INDO: Indomethacin)

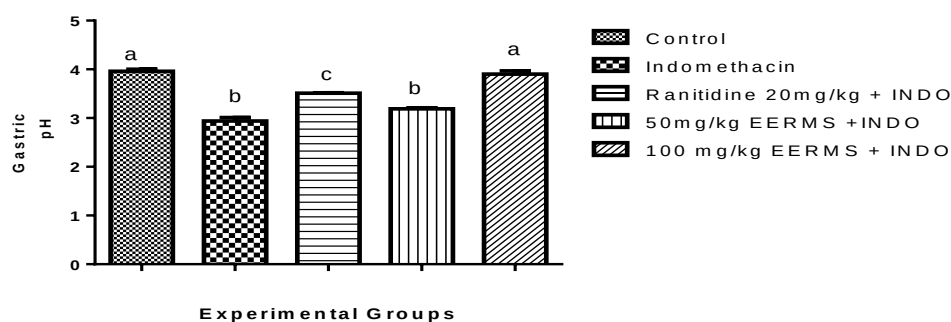


Figure 3: Effect of ethanolic extract of *R. mambillensis* seeds and ranitidine on gastric juice pH of indomethacin ulcerated rats.

(Values are mean ± SEM of six determinations; INDO: Indomethacin)

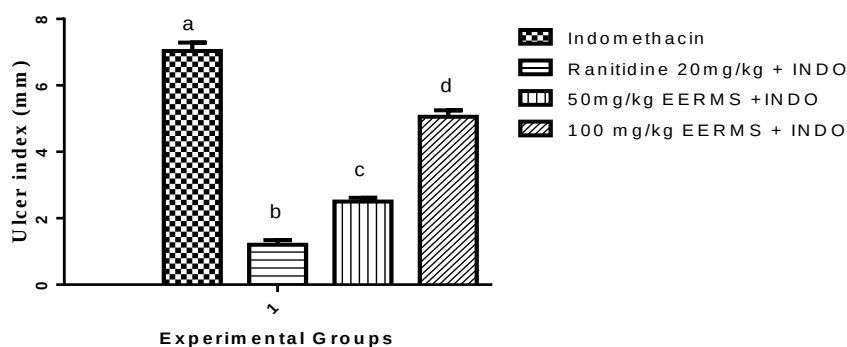


Figure 4: Effect of ethanolic extract of *R. mambillensis* seeds and ranitidine on ulcer index of indomethacin ulcerated rats.

(Values are mean ± SEM of six determinations; INDO: Indomethacin)

## DISCUSSION

Secondary metabolites like flavonoids, tannins are some of the bioactive compounds found in medicinal plants that have a definite physiological function on the human body (Yadav and Agarwala, 2011). Phytochemical screening gives fundamental information regarding a plant extract's therapeutic significance (Shabbir *et al.*, 2013).

Flavonoids are polyphenols that has shown anti-inflammatory characteristics that protect the gastric mucosa from injurious effects of ulcerogens in animals (Airaodion *et al.*, 2019). Flavonoids also help to prevent the formation of ulcers in the stomach lining by increasing the production of mucus which serves as a protective factor against ulcerogens (Nassar *et al.*, 2013). The presence of flavonoids in EERMS can be

attributed to its observed antiulcer effect in the present study.

Phenolic chemicals are common secondary metabolites found in plants. Polyphenolic substances have been shown to have anti-ulcerogenic activity as well as a high level of gastric protection (Sumbul *et al.*, 2011). EERMS has a high level of total phenol that makes it a promising plant as an antiulcer agent.

According to De Jesus *et al.* (2012), tannins' antiulcer action may be related to their ability to bind and precipitate proteins, implying a direct suppression of the H/K<sup>+</sup>-ATPase, which is found in stomach mucosal parietal cells and is implicated in gastric acid production. Traditionally, tannin-rich plants have been used to treat stomach ulcers all over the world (de-Faria *et al.*, 2012). EERMS has a high tannin content that can contribute to the observed antiulcer effect.

The presence of mineral elements in medicinal herbs has also been linked to their therapeutic potentials thus the need to determine their concentrations using techniques such as atomic absorption spectrophotometry (Vieira *et al.*, 2018). The EERMS contain essential elements such as Ca, Na, K, Mg, Mn, and Zn that are known to be involved in many biochemical/enzymatic processes. Calcium has been demonstrated to affect cell proliferation as well as the regeneration of injured stomach mucosa (Liu *et al.*, 2017). Zinc is a cofactor of over 200 metalloenzymes involved in cellular development and protein synthesis, making it essential in tissue regeneration. Zn has also been found to have a crucial role in the healing of gastric ulcers, particularly in the early stages (Nieder *et al.*, 2018). It also aids in the prevention of free radical production and the protection of biological structures from harm (Hotz and Brown 2004).

Long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) causes peptic ulcers by inhibiting prostaglandin synthesis (Kuna *et al.*, 2019). Pretreatment with ethanolic extract of *R. mambillensis* seeds led to a reduction in the volume and acidity of gastric juice indicating an anti-secretory function. Antisecretory drugs act by inhibiting the secretion of acid thus reducing autodigestion of gastric mucosa (Lakshimi *et al.*, 2010). The ulcer index is a score used to assess the physical severity of ulceration (Rahmadi *et al.*, 2021).

The doses of the ethanolic extract of *R. mambillensis* seeds used showed a significant reduction in ulcer index when compared to the ulcerated group thus conferring protection on the gastric mucosa against the deleterious effect of the ulcerogen. The lower dose level of 50 mg/kg bw however showed better protection when compared with 100mg/kg bw. The current study thus found that therapy with EERMS at 50mg/kg promoted considerable gastric mucosal repair, as evidenced by a significant decrease in gastric acidity and gastric ulcer index.

The results of the study showed that the ethanolic extract of *R. mambillensis* seeds as used locally have comparable effect to the reference drug, ranitidine which can be due to the presence of polyphenols, tannins, and microelements in the extract.

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