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Assessment of the Therapeutic and Protective Effects of Fermented Ripe and Unripe *Carica papaya* Fruit Extracts on Histomorphological and Biochemical Alterations in Ibuprofen-Induced Esophageal Ulceration in Wistar Rats

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ABSTRACT: The objective of this paper is to assess the therapeutic and protective effects of fermented ripe and unripe *Carica papaya* fruit extracts on histomorphological and biochemical alterations in ibuprofen-induced esophageal ulceration in wistar rats usin appropriate standard methods. Results revealed decrease in body weight compared to Control. Similarly, there was significant organ weight reduction. Biochemical analysis revealed that *C. papaya* fruit extracts significantly increased ($p<0.05$) the levels of SOD and catalase, and significantly decreased ($p<0.05$) in MDA level. Histological analysis showed normal structural arrangement, with hyperkeratosis and squamous hyperplasia in the control group. Ibuprofen treated group demonstrated severe squamous dysplasia, while pretreatment FRP and FUP groups, showed mild squamous dysplasia while the omeprazole group exhibited moderate squamous dysplasia. Ibuprofen when administered alone or accompanied by other drugs caused some form of gastroesophageal ulcerations which were ameliorated by the administration of fermented *C. papaya* fruit extracts.

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Among the most predominant diseases blighting individuals globally are gastrointestinal disorders (GIDs). Gastrointestinal disorders are common in both the male and female population however, certain types are more prevalent than others (Sperber *et al*., 2020). Ulcers are among the known prevalent gastrointestinal disorders. They are sores or lesions that usually occur along the upper digestive tract. When they form in this area, they are collectively referred to as peptic ulcers. Individually, peptic ulcers are described by their site of occurrence, the most common being gastric ulcers in the stomach and

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duodenal ulcers in the upper portion of the small intestine (Leonard, 2017). Peptic ulcers that occur in the esophagus are referred to as esophageal ulcers (Leonard, 2017). Esophageal ulcer is a distinct disruption in the margin of the esophageal mucosa. This mucosal damage to the esophagus is usually caused by gastroesophageal reflux disease (GERD), or by severe continued esophagitis from other causes (Scida *et al*., 2018; Maesaka *et al*., 2018; Nelms and Pelaez, 2018; Spechler, 2019).

Ulcers can be managed using pharmaceutically manufactured drugs. It is important to seek an alternative to drugs in the management and possible outright treatment using plants. Plants and their parts have been studied and are still being studied, and employed in treatment of ailments in traditional medical practice. Amongst the numerous plants being researched on includes *Carica papaya* popularly known as papaya or pawpaw. The uses of pawpaw plant (seeds, ripe fruits, unripe fruits, latex, root, leaves, flowers and stem bark) and its therapeutic correlation with human wellbeing have been well documented (Emeruwa, 1982; Krishna *et al.,* 2008). Hence, the objective of this paper is to assess the therapeutic and protective effects of fermented ripe and unripe C*arica papaya* fruit extracts on histomorphological and biochemical alterations in ibuprofen-induced esophagealnulceration in wistar rats.

MATERIALS AND METHOD

Research Design: All protocols on animal handling strictly followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) as approved by the Research Ethics Committee, PAMO University of Medical Sciences Port Harcourt, Port Harcourt, Rivers State, Nigeria. Adult Wistar rats with an average weight of 148±12g, groomed at the animal house, Faculty of Basic Medical Sciences (FBMS), PAMO University of Medical Sciences (PUMS), Port Harcourt, Rivers State were used for this study. The animals were kept in standard polypropylene cages, left to acclimatize to their new environment for 14 days, under standard laboratory conditions at the animal house, FBMS, PUMS where they had access to rat chow and water *ad libitum*.

Animal grouping and treatment: 35 adult Wistar rats were used in this study. Animals were randomly separated into 7 groups comprising 5 animals each (n=5) viz: Group 1 (G1) – Control, Group 2 (G2) – Ibuprofen, Group 3 (G3) – Ibuprofen+fermented unripe *C. papaya*, Group 4 (G4) – Ibuprofen+fermented ripe *C. papaya*, Group 5 (G5) – Fermented ripe *C. papaya*+Ibuprofen, Group 6 (G6) – Fermented unripe *C. papaya*+Ibuprofen, Group 7 (G7) – Ibuprofen+omeprazole. Treatment was carried out via oral route of administration viz: G1 – 1ml of distilled water, G2, G3, G4 and G7 were given a single dose of ibuprofen (IBU; 400 mg/kg B.W). After 6 hours of ulcer induction, G3, 4 and 7 were further administered fermented unripe (FUP), fermented ripe (FRP) *C. papaya* extracts (0.75ml extract + 0.25ml distilled water) and omeprazole (OME; 20mg/kg B.W.) respectively. The treatment lasted for 14 days. G5 and G6 were administered FRP and FUP extracts respectively for 12 days at first. 30 minutes after the last extract administration on the 12th day, animals in both groups were further administered ibuprofen (400mg/kg B.W) once daily from day 12 through 14. Prior to the commencement of this experiment, a pilot study was first carried out to determine the toxicity and effective dosage of the fermented extracts. Extracts were administered at the doses 25%, 50%, 75%, and 100%. General observations were made on the fecal matter, eating habits, social activity and agility while histological assessments and biochemical assays were carried out in order to select the most effective dose. Additionally, the animals were weighed using a weighing scale (Atom electronic compact scale), to determine the effect of the treatment on body weight prior the commencement and after the conclusion of the study.

Procurement, Identification and Preparation: Ibuprofen and Omeprazole were purchased from Rhema Pharmacy, Iriebe, Obio-Akpor LGA, Rivers State, Nigeria. Freshly prepared ibuprofen (400mg/kg) and omeprazole (20mg/kg) were dissolved in 1ml of distilled water. Wistar rats were fasted for 24 hours prior to oral administration of ibuprofen (400mg/kg), and allowed for 6 hours. Fresh unripe and ripe *C. papaya* fruits were purchased from Oyigbo Market, Oyigbo LGA, Rivers State. The fruits were identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, with authentication number: UPH/PSB/2021/A13a and UPH/PSB/2021/A13b allocated to the ripe and unripe *Carica papaya* fruits respectively.

C. papaya fermentation was carried out according to the method of Ezike, *et al.* (2009) with slight modification. The fruits were washed thoroughly with running tap water. The unripe and ripe *C. papaya* fruits were separately weighed (ripe; 10kg while unripe; 11kg), peeled, cut into cubes and then grated into a pulp. 200ml of tap water was added to 1kg of the fruits (thus, 2 litres of water and 2.2 litres of water for ripe and unripe C. papaya respectively),

sealed in fermenting jars securely to prevent air interference. The jars were left at room temperature and allowed to ferment for 4 days. The juice extract was decanted to collect the sediments, preserved in a refrigerator and later subjected to phytochemical screening.

Ulcer induction: In line with method described by Goorani, *et al.* (2019) with slight modification, freshly prepared ibuprofen (400mg/kg) was measured and dissolved in distilled water. Wistar rats were fasted for 24 hours prior to oral administration of ibuprofen (400mg/kg) and afterwards, left for 6 hours.

Animal Sacrifice, Tissue Processing and Biochemical Assessment: Rats for histological assessment were euthanized using ketamine anaesthesia and then subjected to transcardial perfusion using 50 ml of 0.1 M PBS (pH 7.4), followed by 500 ml of 10% buffered formalin. With the use of surgical blade, scissors and scalpel, the esophgus was excised and an incision made along its entire length in order to exteorise the mucosa and observe the presence of ulceration. The esophagus was then rinsed in PBS, and post-fixed in 10% buffered formalin solution and subjected to tissue processing and staining (H&E). Rats for biochemical assessment were not subjected to transcardial perfusion. The esophagus was excised, rinsed in PBS, placed in PBS and then preserved in a cooling medium, after which sections of the esophagus were homogenized for biochemical assay.

Statistical analysis: The data obtained were analyzed using GraphPad Prism® software (Version 8.1) and tested for analysis of variance (ANOVA) with Tukey's multiple comparisons test. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The phytochemical screening (Table 1) revealed that both the fermented unripe (FUP) and ripe (FRP*) C. papaya* contain phytochemicals shown to have some therapeutic potentials in managing several ailments. The results of phytoconstituents obtained in the present study for the fermented *C. papaya* fruit were comparable to results reported earlier by Dada *et al*. (2016); Prabhu *et al*. (2017) and Oluchukwu *et al*. (2021) who investigated the phytochemical properties of unripe and ripe *C. papaya* in their unfermented form. With the exception of glycosides, the FUP had more of the phytochemicals than the FRP whereas ripe fruit had more of glycosides. These are indicative of the respective use of the different states of the papaya fruit use in the management of different ailments such as congestive heart failure, hypoglycaemia among others (Eke *et al*., 2014).

Table 1: Phytochemical analysis

Phytochemicals	FRP	FUP
Alkaloids	+	+
Terpenoids	+	+++
Saponins	+	++
Flavonoids	+	+++
Tannins	+	+++
Glycosides	+++	+
Phenols	++	+++

Foot note: FRP (fermented ripe Carica papaya); FUP (fermented unripe Carica papaya)

Ibuprofen, the most often used and prescribed nonsteroidal anti-inflammatory medicine (NSAID), has the potential of causing GIT bleeding, increasing the risk of gastric ulcers, renal failure, epistaxis, apoptosis, heart failure, hyperkalaemia, confusion and bronchospasm (Abraham *et al*., 2005; Kennedy, 2001; Fulcher 2003; Gambero *et al*., 2005; Durkin, 2006; Rossi 2004). Findings from this study revealed that IBU administration brought about a statistically significant loss in body and esophageal weights (Fig. 1) when compared with control and other treatment groups however, experimental rats in protective and therapeutic *C. papaya* treated group gained both body and esophageal weights, when compared to IBU-administered group. Though less commonly, unexplained weight gain has been identified as one of the side effects of NSAID. However, chronic NSAID use has been implicated in several side effects such as loss of appetite, diarrhoea, gastrointestinal ulcerations among several others (Mayo Clinic, 2024). These could have accounted for the loss of body and esophageal weights observed in this study.

When compared to omeprazole treatment, a marked reduction in body weight (Fig. 1) was observed as a of result of the different methods of *C. papaya* treatments except in pre-treated with FUP (G6) that revealed a statistically significant increase when compared with the Control and omeprazole treatment. This agrees with a previous report by Duru *et al.* (2012) who stated that the observed loss in body weight could be as a result of the androgenic activity of *C. papaya* peel in the body of these rats. Furthermore, in comparison to the Control, omeprazole treatment similarly brought about weight loss, which corresponds to a report by Cui *et al.* (2001) who demonstrated that omeprazole treatment reduced body weight and bone mass gain in juvenile male rats. However, protective and therapeutic interventions with FUP and FRP extracts following IBU administration, progressively improved the appetite of Wistar rats which could have been responsible for the slight body and esophageal weight

gains. There was no significant difference in body weight except G6 treated Wistar rats when the positive effects of FUP and FRP extracts were compared to OME group, which showed significant weight gain when compared with OME (G7) treated rats. In like fashion, the esophageal weight gradual improvement across intervention groups showed no significant difference.

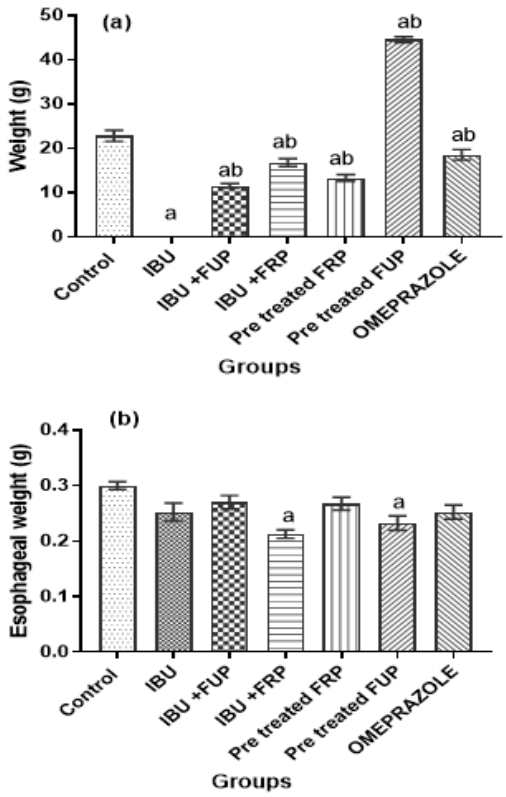

Fig 1: Body weight and esophageal weights of Wistar rats. (a) Body weight; (b) Esophageal weight. Control (G1); IBU (G2); IBU+FUP (G3); IBU+FRP (G4); Pretreated FRP+IBU (G5); Pretreated FUP+IBU (G6); IBU+OME (G7). Note: IBU (Ibuprofen), FUP (fermented unripe *C. papaya*), FRP (fermented ripe *C. papaya*) and OME (Omeprazole). **a**p<0.05 and **b**p<0.05 denote statistically significant when compared **Fig. 1:** Animal body weight and organ weigt. (a) Body weight (b) Esophageal weight. Control (G1); IBU (G2); IBU+FUP (G3); IBU+FRP (G4); Pretreated FRP+IBU (G5); Pretreated FUP+IBU (G6); IBU+OME (G7). Note: IBU (Ibuprofen), FUP (fermented unripe *C. papaya*), FRP (fermented ripe *C. papaya*) and OME (Omeprazole). **a**p<0.05 and **b**p<0.05 denote statistically significant when compared with Control and IBU groups respectively.

Fig. 2 shows esophageal lipid peroxidation and antioxidant expression. An imbalance between the rates of free radical production and scavenging capabilities of cells or tissues causes oxidative stress, which has an attendant effect on cellular function and degeneration and leads to a variety of diseases (Oxidative Stress, 2010). As part of the assessment for the healing mechanisms of the extracts of FUP and FRP, anti-oxidant and oxidative stress parameters were also studied. The generation of reactive oxygen species (ROS) and oxidative damage are crucial steps in the pathogenesis of ulcer (Naito *et al*., 2014).

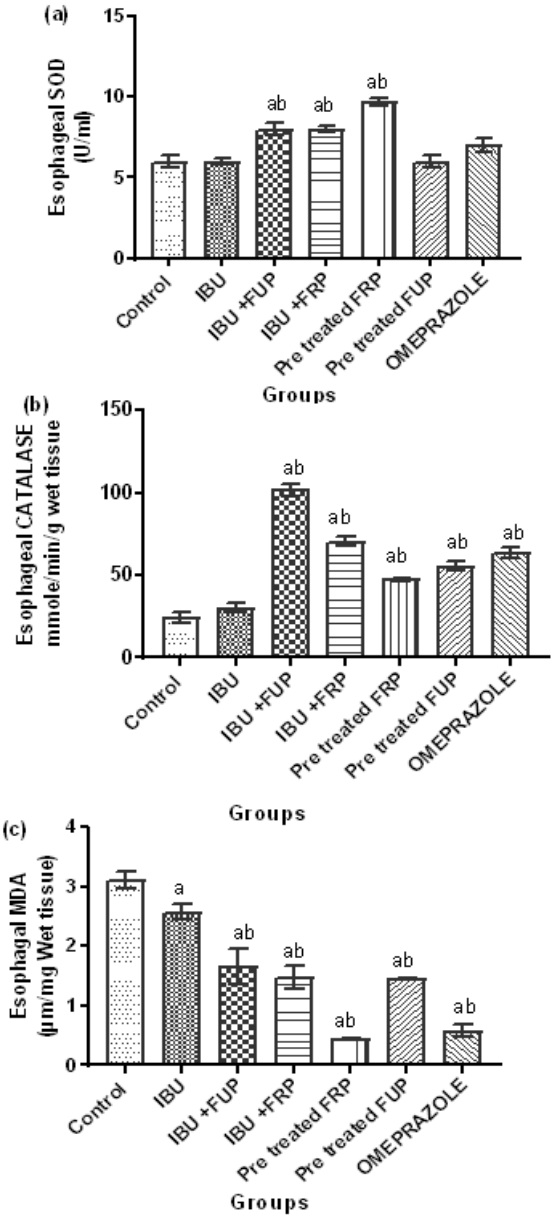

Fig. 2: Esophageal Lipid Peroxidation and Antioxidant Expression. (a) SOD; (b) CATALASE; (c) MDA. Control (G1); IBU (G2); IBU+FUP (G3); IBU+FRP (G4); Pretreated FRP+IBU (G5); Pretreated FUP+IBU (G6); IBU+OME (G7). Note: IBU (Ibuprofen), FUP (fermented unripe *C. papaya*), FRP (fermented ripe *C. papaya*) and OME (Omeprazole). **a**p<0.05 and **b**p<0.05 denote statistically significant when compared with Control and IBU groups respectively.

Oxidative stress is a state that has been implicated in alteration of a series of physiological reactions, and it has a role in both psychological and pathological

onset of ulcer. Findings from this study revealed that IBU-administration significantly stimulated oxidative stress in the esophagus of the rats when compared to the esophagus of rats in other groups, leading to an increase in MDA level. Furthermore, SOD and Catalase have been shown to catalyze the elimination of free radicals, mitigating their reactive potentials and consequently preventing oxidative cytotoxicity. In this study, IBU-administration significantly lessened the activities of antioxidant enzymes in the esophagus of rats when compared to the esophagus of rats in other groups, thereby leading to reduction in the SOD and Catalase levels.

The increased concentration of MDA in the esophagus of Ibuprofen-ulcerated rats, as well as the reduced activity of SOD and Catalase, can be attributed to accelerated lipid peroxidation and overproduction of free radicals, resulting in mucosal injury. Free radicals inhibit the functioning of antioxidant enzymes and consequently result in lipid peroxidation, which is a major step in the toxicity mechanism of ibuprofen. Therefore, the ability of fermented *C. papaya* extracts to act in favour of antioxidant defences was assessed. Treatment with the two extracts in the protective and therapeutic interventions restored the SOD and Catalase activities and reduced MDA level. As a matter of fact, the impacts of the two extracts on Catalase and MDA expressions, compared favourably to both the Control and omeprazole (the standard pharmacological reference drug) employed in this study. These findings indicate that the reduction in oxidative damage and improvement in antioxidant enzymes could be associated with the antioxidant and anti-inflammatory properties of fermented *C. papaya* extract.

Plate 1 shows histological assessment of the esophagus. Esophageal ulcerations seen in infectious etiology are multiple and punctuate, circumferential in distribution and tends to involve the proximal esophagus (Chiejine and Samant, 2021). Studies have shown that ibuprofen could induce apoptosis in gastrointestinal mucosal cells, as a result of increased leukocyte infiltration into the mucosa, which is followed by ROS production (Golbabapour, 2013). Cell damage is attributed to the expression of ROS which is a principal factor in NSAID induced ulceration. Therefore, this study confirmed the features of damages caused by ibuprofen on the esophageal mucosa. Within the esophageal mucosa, IBU administered rats demonstrated severe squamous dysplasia. However, protective and therapeutic interventions with FUR and FRP extracts following IBU treatment, progressively improved the esophageal histological alterations. These groups showed significant protection against the formation of lesions in the mucosa compared to the rats receiving no medicinal intervention after ulcer induction, although, the most potent protection was seen in FUP pre-treated. By comparing the effects of the FUP and FRP extracts to the rats receiving standard medicinal intervention (omeprazole) after ulcer induction, the most potent protection was observed in FUP pre-treated, which is similar to the Control rats. These findings suggest that fermented unripe *C. papaya* could be used as a safe, highly effective and inexpensive herbal remedy in treating esophageal ulcer when compared to some standard medicinal interventions.

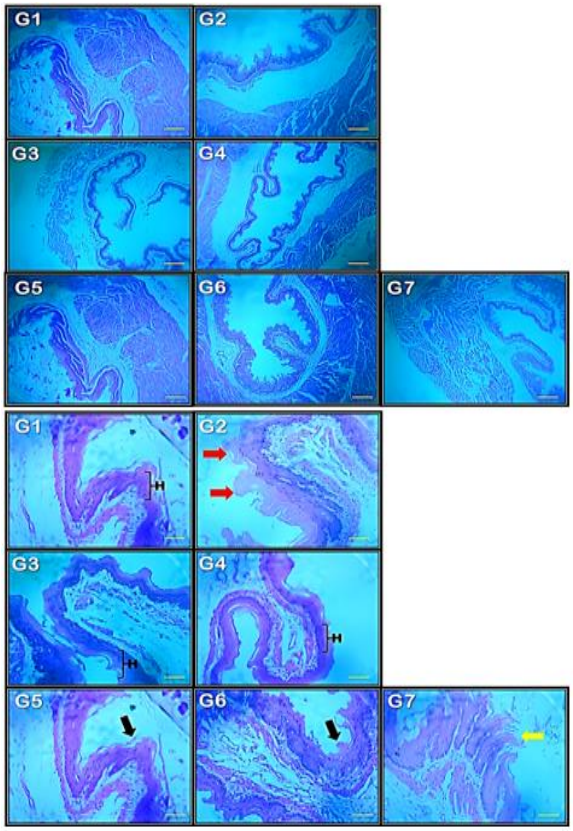

Plate 1: Esophagus: Control (G1); IBU (G2); IBU+FUP (G3); IBU+FRP (G4); Pretreated FRP+IBU (G5); Pretreated FUP+IBU (G6); IBU+OME (G7). Note: IBU (Ibuprofen), FUP (fermented unripe *C. papaya*), FRP (fermented ripe *C. papaya*) and OME (Omeprazole). Hyperkeratosis and squamous hyperplasia (H); severe squamous dysplasia (red arrow); mild squamous dysplasia (black arrow); moderate squamous dysplasia (yellow arrow). (H&E, x100 and x400).

Conclusion: The attenuation of esophageal ibuprofen assaults via oral administration of the fermented ripe and unripe *C. papaya* extracts is reminiscent of their tremendous anti-inflammatory, protective and antioxidative potentials in Wistar rats. Though

adequate to a large extent, this study is inexhaustive following some limitations which were encountered. Consequently, further studies are recommended.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from corresponding author

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