GASTROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT
OF UNRIPE CARICA PAPAYA FRUIT IN RATS

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Running title: Pawpaw fruit and gastroprotection.
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ABSTRACT:
Aqueous extract of unripe Carica papaya fruit (AEUCPF) was investigated for its anti-ulcer, mucus secretion, anti-acid secretory and pepsin binding effects in rats. Ethanol/HCl and Indomethacin were used to induce ulcers while acid and mucus secretion was measured in ulcerated and treated animals. The animals were divided into five groups for each of the anti-ulcer studies and each group was made up of five animals each. These groups included a control and reference groups administered saline and cimetidine (Kg/Kg b.w), while the remaining three groups were administered with 2.5, 3.5 and 4.5ml/Kg of the decoction of the unripe fruits. The extract, cimetidine and saline were all administered orally twice daily for ten days while necrotizing agents were administered (p.o) once daily from day 8 through day 10. The results showed that higher doses of the extract significantly (p<0.05) reduced the ulcer index from 3.6± 0.24 (control) – to 0.70 ± 0.37 (4.5ml/Kg) in the ethanol induced ulcer. The extract also produced similar effects in the indomethacin induced ulcer and in both cases the gastric acidity was significantly reduced. The extract did not increase mucus secretion but it bind substantially with pepsin. In conclusion this study has shown that AEUCPF has beneficial effects on the normal function of the stomach. It has the capacity to ameliorate gastric ulcer as suggested by local traditional medical practitioners.

KEYWORDS: Carica papaya; Extract; Gastric acid; Pepsin; Ulcer; Rats.

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INTRODUCTION:
The stomach is the most distensible and one of the vital parts of the gastrointestinal tract [1]. It is involved in digestion of various foods which it receives from the oesophagus. In addition to food, the stomach is exposed to many potentially injurious agents such as acids, pepsin, bacterial products and drugs [2,3]. There is a continuous effort to find good synthetic or phytochemical agents that will offer gastroprotective effects. Thus many plants and plants derived products have been screened for their anti-ulcer effects. These include: Persea americana, Landolphia owarriensis, Ananas ananassoides, Garcinia kola seeds, a biflavonoid kolaviron isolated from G. Kola seeds, licorice, etc [3-7].

Carica papaya (L) commonly called pawpaw is a large tree like plant with stem growing from 5 to 10 metres tall. It has spirally arranged leaves which are confined to the top of the trunk. It produces fruits which are mainly oval in shape with light green colour in the unripe state but which may turn yellow when it ripens. The unripe fruit can be cooked as parts of salads, jellies and stews while the ripe fruits are usually eaten raw without the skin or seed [8-9]. The plant is employed in the treatment of several ailments by traditional medical practitioners with such uses including but not limited to the treatment of the following: sore throat, asthma, sickle cell anaemia, wound, ulcers, boils, malaria, fever, pain, tonsillitis, indigestion, dyspepsia, jaundice and cancer [8-11]. The unripe fruit have been reported to have anti-sickling, laxative, abortifacient and diuretic properties [11] while the intake of the extract of unripe fruit of the plant has been linked with an anti-ulcer effect [12].

Ezike et al, [12] had investigated the probable beneficial effect of unripe papaya fruit on the treatment of gastric ulcer by administering extract of unripe papaya fruit however; the focus of the present study is to investigate the use of aqueous extract of the unripe fruit on gastric mucosa irritation, mucus secretion and gastric acidity. Therefore this study was designed to specifically mimic the exact practice of the traditional medical practitioners and to see if this practice is effective. Thus we investigated if the aqueous extract of unripe and mature fruit of C. papaya has therapeutic effects in animal models of ulcer and the probable mechanism for such effects.

MATERIALS AND METHODS:
Plant material and preparation of decoction:
The unripe fruit of C. papaya fruits were collected from fruit gardens in Ilorin metropolis and the mini campus of University of Ilorin. The plant had been previously [9] identified at Forestry Research Institute of Nigeria (FRIN) with a voucher specimen number FHI 106933. The unripe fruits (with total weight of 2.9 Kg) were washed with distil water and sliced into small cubed shaped pieces each weighing 50g each. The slices of each of the three fruits were soaked
in 2.5 litres of distil water for 96 hours after which the resulting solution was sieved and immediately used for pharmacological studies.

Animals
Male Wistar rats weighing 180±10.1g were used for these studies. The animals were bred in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin and fed on standard mouse cubes (exotic Feeds, Ilorin, Nigeria). They were kept in clean cages with optimum temperature of about 250°C, humidity of 60-65% and 12 hours light/dark cycle. Animals were provided with water ad libitum. The research was conducted in accordance with the ethical rule for animal experimentation, approved by Ethical Committee, College of Health Sciences University of Ilorin.

The animals were divided into five groups with each group comprising of five animals each. Group A (control) was administered saline (10ml/Kg), group E was administered 11.5g/Kg of cimetidine. The animals in groups B-D were administered 2.5, 3.5 and 4.5ml/Kg of the decoction of unripe papaya fruit extract twice daily for ten days. Ethical approval was obtained from the Ethic Committee of the Department of Physiology, University of Ilorin in accordance with the University of Ilorin guidelines on the care and use of laboratory animals.

Anti-ulcer studies
HCl/Ethanol induced ulcer: Ulceration was induced in experimental animals by the administration of 1 ml of necrotizing solution (150 mm of HCl in 60% ethanol) in accordance with the method used by Mizui and Douteuchi [13]. Animals were orally administered saline, cimetidine or decoction of unripe fruit (2.5, 3.5 or 4.5mg/Kg) of C. papaya twice daily for ten days. However the administration of the necrotizing agent started on the eighth day once daily for three days. The animals were sacrificed 2hrs after the administration of the test substances and saline. The stomachs of the animals were dissected out and an incision was made at the greater curvature in order to collect gastric contents and observation of gastric mucosa for the presence of gastric ulceration. Ulceration was confirmed by using a hand held lens (x10) and the ulcer scores were determined using the arbitrary scale used by Singh et al, [14] as in previous studies [9]. A score of 0 was assigned to no visible lesion; 0.5 for hyperaemia; 1 for one or two slight lesions, 2 for severe lesions; 3 for very severe lesions and 4 for mucosal that is full of many lesions. The ulcer index was also calculated as the means of ulcer scores.

Indomethacin induced ulcer: Ulceration was induced in these groups of animals by administration of 20 mg/Kg of indomethacin as necrotizing agent. Animal grouping and drug administration were as in the HCl-ethanol induced ulcer above. The same ulcer scoring method was also used.

Determination of gastric acidity
Samples of gastric contents from each rats used for antiulcer studies were collected and
centrifuged (2000 rpm) for 10 min. after which 1 ml of the supernatant was analysed for hydrogen ion concentration by titration against 0.1 M NaOH to a pH of 7.0 using phenolphthalein as an indicator.

Mucus secretion
Measurement of mucus production
Gastric mucus production was assessed in rats that were administered HCl/ethanol necrotizing agent immediately after the determination of the ulcer scores of the animals as described previously [7]. Briefly, the mucus layer of the stomach of each rat was scraped using a glass slide into a glass tube containing 1 ml of water whose weight was predetermined. The final weight of the container and the mucus was determined using a digital electronic balance and the difference between the final weight and the predetermined weight was taken as the weight of the mucus.

Pepsin binding activity
Pepsin binding activity of AEUCPF was determined as previously reported, [6,15] 50 ml of the aqueous extract was added to 1 mL of pepsin solution (2 mg/mL) in a test tube followed by the addition of 4 ml of 0.2 N HCl buffered with 1 ml of 0.2 N sodium citrate solution. Thereafter, 1 ml of bovine serum albumin (5 mg/mL) was added to treat the excess pepsin except the control test tubes. All reagents were kept at a temperature 37°C for 30 minutes prior to incubation and at the same temperature for 30 minutes after incubation. The remaining protein in each tube was treated with 1.0 ml of Biuret reagent and 5 ml 0.2 N NaOH solution. The absorbencies were read at 546 nm and the result was expressed as percentage binding of pepsin.

Phytochemical analysis
Preliminary phytochemical analysis of the extract was carried out using standard procedures for alkaloids, reducing sugars, tannins, flavonoids, saponins, steroids, and anthraquinones [16-18].

Statistical analysis
All values are expressed as mean ± standard error of the means (SEM). Statistical significance was determined using the Student’s t-test. Values with P < 0.05 compared with the control group were considered as being significantly different.

RESULTS:
Anti-ulcer studies
The results of the anti-ulcer studies showed that gastric mucosa lesions were significantly (p<0.05) reduced by all the doses of AEUCPF. The ulcer score was reduced by the 4.5ml/Kg from 3.6±0.24 (control) – 0.70±0.37 in the HCl/Ethanol induced lesion (Table 1.). Likewise 4.5ml/Kg of AEUCPF significantly (p<0.05) reduced the ulcer score from 3.8±0.2 (control) - 0.9 ± 0.33 in the indomethacin induced gastric lesion (Table 2.). Fig. 1 shows the percentage protection of the mucosal by AEUCPF in the two models of ulcerogenesis.
Table 1: Effects of aqueous extract of unripe fruit of Carica papaya on HCl/Ethanol induced ulcer in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mL/Kg)</th>
<th>Ulcer index</th>
<th>Total Gastric acidity (μEq/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>-</td>
<td>3.60 ± 0.24</td>
<td>62.2 ± 8.1</td>
</tr>
<tr>
<td>C. papaya 2.5</td>
<td>1.70 ± 0.44*</td>
<td></td>
<td>32.3 ± 7.8*</td>
</tr>
<tr>
<td>C. papaya 3.5</td>
<td>1.10 ± 0.51*</td>
<td></td>
<td>25.8 ± 1.2*</td>
</tr>
<tr>
<td>C. papaya 4.5</td>
<td>0.70 ± 0.37*</td>
<td></td>
<td>24.6 ± 2.5*</td>
</tr>
<tr>
<td>Cimetidine 11.5 (mg/Kg)</td>
<td>0.60 ± 0.19*</td>
<td></td>
<td>24.3 ± 7.2*</td>
</tr>
</tbody>
</table>

*Each value is mean ± S.E.M. for 5 rats. *P < 0.05 compared with control.

Table 2: Effects of aqueous extract of unripe fruit of Carica papaya on indomethacin induced ulcer in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (ml/Kg)</th>
<th>Ulcer index</th>
<th>Mucus secretion</th>
<th>pH</th>
<th>Total Gastric acidity (μEq/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>-</td>
<td>3.81 ± 0.20</td>
<td>11.10±5.51</td>
<td>2.28±0.10</td>
<td>66.0 ± 2.9</td>
</tr>
<tr>
<td>C. papaya 2.5</td>
<td>1.91 ± 0.51*</td>
<td></td>
<td>12.51±4.57</td>
<td>2.21±0.19</td>
<td>24.2 ± 2.1*</td>
</tr>
<tr>
<td>C. papaya 3.5</td>
<td>1.32 ± 0.54*</td>
<td></td>
<td>9.69±5.0</td>
<td>2.42±0.19</td>
<td>12.4 ± 1.1*</td>
</tr>
<tr>
<td>C. papaya 4.5</td>
<td>0.90 ± 0.33*</td>
<td></td>
<td>11.3±6.40</td>
<td>2.59±0.09</td>
<td>22.1 ± 1.4*</td>
</tr>
<tr>
<td>Cimetidine 11.5 (mg/Kg)</td>
<td>0.75 ± 0.19*</td>
<td></td>
<td>54.21±10.70*</td>
<td>2.68±0.14</td>
<td>22.4 ± 0.7*</td>
</tr>
</tbody>
</table>

*Each value is mean ± S.E.M. for 5 rats. *P < 0.05 compared with control.

Fig 1: Percentage inhibition of ulcers by aqueous extract of unripe fruit of Carica papaya. Each value is the mean of 5 rats.
Gastric acidity, Pepsin binding and mucus production

Tables 1 and 2 show the results of the effects of administration of AEUCPF on gastric acid secretion in the HCl/ethanol and indomethacin induced lesions respectively. The acidity was significantly (p <0.05) reduced by all the doses of AEUCPF.

AEUCPF produced 103.25% binding with pepsin. However, the decoction did not produce any significant changes in the mucus production in stomach of animals.

Phytochemical Analysis

The results of the phytochemical screening showed that the extract contains alkaloids, flavonoids, polyphenols, anthraquinones, reducing sugars, saponins and steroids

**DISCUSSION:**

It is indeed amazing why certain plants are used for the treatment of specific ailments. C. papaya is such a useful plant for traditional medical practitioners. The desire to unravel the usefulness of this plant has led us to previously investigate the anti-inflammatory, analgesic and anti-ulcer effects of the leaves based on ethnopharmacological information [9].

The present study investigated the gastroprotective effects of aqueous extract of unripe C. papaya fruit (AEUCPF ) based on its use locally for the treatment of ulcer which included the administration of 96 hours soaked unripe fruit solution to treat ulcer patient for a period of ten days in the first instance. The doses chosen in the study were carefully calibrated to resemble the common doses used by the traditional practitioners where 3.5 ml/kg approximated the doses used twice per day and the 2.5 and 4.5ml/Kg were alternate doses used for comparison with the standard dosage of 3.5 ml/Kg. The two methods used for producing gastric lesion (HCl/Ethanol and indomethacin) are validated models [19-22].

The findings showed that administration of AEUPCF for ten days produced a dose dependent anti-ulcer effects in the HCl/ethanol induced ulcers with the minimum dose producing a percentage inhibition of 52.8% which is relatively high (Fig.1). The highest dose (4.5ml/Kg) of AEUPCF produced comparable anti-ulcer effects with the standard drug cimetidine (11.5mg/Kg). In the indomethacin induced gastric lesion AEUPCF also produced similar dose dependent pattern of anti-ulcer effects with highest dose of the extract producing effects that is comparable with that of cimetidine. The minimum dose also inhibited ulcerogenesis by 50% which is a value similar to what the equivalent dose produced in the HCl/Ethanol induced ulcer model. This showed that the results can probably be replicated in most models of ulcer caused by effects of diverse ulcerogens.
Prior treatment of the animals with AEUPCF before the administration of necrotizing agent was able to strengthen the gastric mucosa against the activities of necrotizing agents. Generally, necrotizing agents may produce gastric lesion by a combination of many factors which includes but not limited to the following: inhibition of prostaglandins (PGE2 and PGI2) synthesis especially with indomethacin a non-steroidal anti-inflammatory agents [23-25], promotion of acid-pepsin aggression on gastric mucosal [26-28] decrease in gastric mucosal barrier/resistance [26] and an increase in lipid peroxidation [26, 29], or the direct increase of gastric acid secretion. Observation in this study showed that AEUPCF use some of these mechanisms to inhibit gastric lesion hence its effectiveness in binding pepsin and reduction of gastric acidity. These help in strengthening mucosal barrier and reducing the direct effects of gastric acid on the mucosa. It can also counteract the effects of NSAIDS on prostaglandins synthesis. Mucus production seems not to be parts of AEUPCF mechanism of protecting gastric mucosal from insults of necrotizing agents as there was no significant changes in the mucus secretion in treated rats compared with the control.

The results of the phytochemical analysis also showed that the AEUPCF might be exerting it effects via its contents of flavonoids, alkaloids, anthocyanides or saponins. Flavonoids have been specifically linked with gastroprotective activities [4,30]. Likewise some alkaloids and saponins have been implicated as active principles responsible for gastroprotective activities of some plants such as Pyrenacantha staudii and Zizyphus sativa.[31-33]. The findings from the phytochemical analysis agree with that of Ezike et al,[12]. They used aqueous and methanol extract of unripe fruit of the plant but the dosages and preparation of the plant material was different from what was used in this study. While they used a single dose of 300 mg/Kg for both aqueous and methanol extract, we used three doses of aqueous decoction which was freshly prepared to simulate the practice by traditional medical practitioners and local users.

Oduola et al, [8] also administered a decoction of the unripe fruit for the treatment of sickle cell disease. The ulcer models by Ezike et al, [12] and in this study were nearly the same with modifications in the dose of ulcerogen in indomethacin induced ulcer; however, they used absolute ethanol as the second ulcerogen while we employed HCl/Ethanol in our studies.

Nevertheless our method of extraction is relatively easier for would be users of this plant product if it is to be consumed raw and Ezike et al, [12] recognized that water extraction is the preferred method by traditional practitioners. Therefore, the present study has thrown more light into the beneficial effect of the use of unripe and mature fruit of C. papaya.
CONCLUSION:
In conclusion, the overall finding of this study is that aqueous extract of unripe fruit of Carica papaya possess antiulcer properties which may be due to its ability to inhibit gastric acid secretion and reduction in pepsin activity and availability. It is a promising material for treatment of gastric mucosal injury and therefore further studies on this plant are encouraged.

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REFERENCES: