Anti-ulcerogenic activity of aqueous extract of Carica papaya seed on indomethacin-induced peptic ulcer in male albino rats

Hussein O. B. Oloyede¹, Matthew C. Adaja¹, Taofeek O. Ajiboye², Musa O. Salawu¹

1. Department of Biochemistry, University of Ilorin, Ilorin 240001, Nigeria
2. Antioxidants, Free Radicals, Functional Foods and Toxicology Research Laboratory, Department of Biological Sciences, Al-Hikmah University, Ilorin 230236, Nigeria

ABSTRACT

OBJECTIVE: Carica papaya is an important fruit with its seeds used in the treatment of ulcer in Nigeria. This study investigated the anti-ulcerogenic and antioxidant activities of aqueous extract of Carica papaya seed against indomethacin-induced peptic ulcer in male rats.

METHODS: Thirty male rats were separated into 6 groups (A–F) of five rats each. For 14 d before ulcer induction with indomethacin, groups received once daily oral doses of vehicle (distilled water), cimetidine 200 mg/kg body weight (BW), or aqueous extract of C. papaya seed at doses of 100, 150 or 200 mg/kg BW (groups A, B, C, D, E and F, respectively). Twenty-four hours after the last treatment, groups B, C, D, E and F were treated with 100 mg/kg BW of indomethacin to induce ulcer formation.

RESULTS: Carica papaya seed extract significantly (P<0.05) increased gastric pH and percentage of ulcer inhibition relative to indomethacin-induced ulcer rats. The extract significantly (P<0.05) decreased gastric acidity, gastric acid output, gastric pepsin secretion, ulcer index and gastric secretion volume relative to group B. These results were similar to that achieved by pretreatment with cimetidine. Specific activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase in the extract-treated groups (D, E and F) were increased significantly over the group B (P<0.05). Pretreatment with the seed extract protected rats from the indomethacin-mediated decrease in enzyme function experienced by the group B. Similarly, indomethacin-mediated decrease in reduced glutathione level and indomethacin-mediated increase in malondialdehyde were reversed by Carica papaya extract.

CONCLUSION: In this study, pretreatment with aqueous extract of Carica papaya seed exhibited anti-ulcerogenic and antioxidant effects, which may be due to the enhanced antioxidant enzymes.

Keywords: Carica papaya; anti-ulcer agents; antioxidants; antioxidant enzymes; indomethacin; anti-ulcerogenic; cimetidine; rats


http://dx.doi.org/10.1016/S2095-4964(15)60160-1
Received July 24, 2014; accepted October 16, 2014.
Correspondence: Taofeek O. Ajiboye; Tel: +234-8035844608; E-mail: ajiboyeyong@yahoo.com
1 Introduction

Peptic ulcer is among the leading causes of morbidity and mortality in Nigeria and many developing countries\(^5\). Its occurrence is largely due to an imbalance in the aggressive (HCl and pepsin) and protective forces (blood supply, mucus, prostaglandins synthesis, etc.)\(^2\) in the stomach. Several factors, such as excessive intake of nonsteroidal anti-inflammatory drugs (NSAIDs), emotional stress, hereditary predisposition and Helicobacter pylori infection, have been found to favor the development of peptic ulcer diseases. Among these contributing factors, NSAIDs lead to gastric ulceration through the production of reactive oxygen species\(^3\). In Nigeria, plants and their extracts have been widely used in the management of gastric ulcers\(^4,5\).

One example is Carica papaya, the seed of which is widely used in the treatment of gastric ulcers. C. papaya Linn, also known as pawpaw, belongs to the family of Caricaceae and is cultivated in tropical regions of the world for its edible melon-like fruit\(^6\). It is one of the most popular and economically important fruit trees, and the fruit is consumed for its nutritional content\(^7\). The fruits and seeds are highly rich in carbohydrate, natural vitamins and minerals, particularly vitamins A and C and potassium. The bioactive constituents of the plant include papain, chymopapain, alkaloids, flavonoids, and phenolic\(^8\).

The plant is traditionally used in treating malarial fever, diabetes mellitus, bacterial infections and eliminating parasitic worms\(^9\). It is also taken to improve digestion. The fresh leaves are efficacious in the treatment of gonorrhea, syphilis and amoebic dysentery\(^9\). Studies have also shown that the aqueous extract of C. papaya seeds has the following activities: diuretic\(^10\), antihyperlipidemic\(^9\), hypoglycemic\(^11\), antioxidant\(^12\) and anti-ulcer\(^13\). Thus, this study investigated the anti-ulcerogenic and antioxidant activities of the aqueous extract of C. papaya seeds against indomethacin-induced peptic ulcer in rats.

2 Materials and methods

2.1 Materials

2.1.1 Collection of plant material

The unripe fruits of C. papaya were collected from the Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria. The plant was identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, with the voucher number UIH001:0971.

2.1.2 Preparation of plant extract

The unripe fruits of C. papaya were cut open and the seeds were collected, air dried on a laboratory bench and pulverized into coarse powder using a hammer mill. Powdered seeds (200 g) of C. papaya were soaked in 1.0 L of distilled water at room temperature with intermittent shaking for 48 h. The extract was filtered and the filtrate was concentrated on a water bath at 100 °C. The yield was calculated to be 5.61% dry material and the extract was stored in a refrigerator at 4 °C.

2.2 Methods

2.2.1 Phytochemical profiling

Phytochemical profile of aqueous extract of Carica papaya seed was analysed using Agilent-7890A gas chromatography (GC) instrument coupled with mass spectrometer (MS) as detector. The GC was equipped with an SE 30 capillary column (30 mm × 0.2 mm) for the present analysis. The initial temperature of the column was set at 150 °C and the maximum of 300 °C. One milliliter of sample was injected with split mode (10:1). Nitrogen gas was used as a carrier at a flow rate of 0.8 mL/min and the total run time was 24 min for each sample. The database of the National Institute Standard and Technology MS Library (NIIST-MS Library) was used for identification of phytocomponents from the chromatograms.

2.2.2 Experimental design

Thirty male rats were randomly distributed into 6 groups of 5 rats each. Group A served as the normal control and was given 1 mL of distilled water. Group B served as ulcer control. Group C was treated with 200 mg/kg body weight (BW) of cimetidine and groups D, E and F were given aqueous extract of C. papaya seed at doses of 100, 150 and 200 mg/kg BW, respectively. Vehicle, extract and drug were administered orally for 2 weeks once per day. After 2 weeks of treatment, all rats were fasted for 24 h; gastric ulceration was induced by oral administration...
of 100 mg/kg BW of indomethacin. Induction was not performed on the normal control rats. Four hours after indomethacin administration all rats were anaesthetized by placing them in a chamber saturated with diethyl ether. The abdomen of each rat was opened to remove the stomach and duodenum. Gastric secretion volume, gastric pH, ulcer index, gastric pepsin secretion, percentage ulcer inhibition, superoxide dismutase (SOD), catalase, GSH-px, GSH-Red, Glc-6-PD, concentration of reduced glutathione and lipid peroxidation (measured by malondialdehyde concentration) were determined.

2.2.3 Gastric acid secretion volume and pH assay

Gastric juice pH and gastric volume were determined using a pH meter and using a standard cylinder as described by Kurasawa et al. Briefly, the stomachs of the sacrificed rats were opened along the greater curvature. Gastric contents from each rat were drained into a centrifuge tube containing 5 mL distilled water and centrifuged at 1 000 × g for 10 min.

2.2.4 Ulcer index and percentage of ulcer inhibition assay

Ulcer index and inhibition (%) were calculated as described by Main et al. Briefly, the stomach of the sacrificed rats was examined under microscope (100 ×) to observe erosions. Scores were made on a scale of 1 to 5. Where 1 = small round hemorrhagic erosion; 2 = hemorrhagic erosion < 1 mm; 3 = hemorrhagic erosion of 2 – 3 mm; 4 = hemorrhagic erosion > 3 mm but < 4 mm and 5 = hemorrhagic erosion > 4 mm.

When the width of erosion was larger than 1 mm, the score was multiplied by 2. Ulcer index and % inhibition were calculated as follows:

\[
\text{Ulcer index (UI)} = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}}
\]

\[
\text{Inhibition (\%)} = \frac{\text{UI}_{\text{model group}} - \text{UI}_{\text{pretreated groups}}}{\text{UI}_{\text{model group}}} \times 100
\]

2.2.5 Determination of gastric acidity

Gastric acidity was determined as described by Shay. Gastric juice (0.2 mL) was titrated against 0.01 N NaOH using an end point of pH 7.0 as determined colorimetrically with phenol red as an indicator. Values were expressed as milliequivalents per liter (mEq/L). In other words mEq/L represent the volume of 1 mol/L NaOH (mL) required to neutralize 1 mL of the gastric juice.

\[
\text{Gastric acidity} = \frac{\text{Volume of 0.01 mol/L NaOH} \times 100}{10}
\]

2.2.6 Determination of gastric acid output

Gastric acid output was calculated as mEq/h by multiplying the volume of gastric secretion (mL/h) of an animal by the gastric acidity (acid concentration) (mEq/L) of the gastric secretion in that animal.

2.2.7 Determination of pepsin in gastric secretion

To quantify pepsin content, 1 mL of supernatant from each gastric secretion centrifugation (section 2.2.3) was added to a tube containing 5 mL of buffer solution (4:1 sodium tartrate and hydrochloric acid) and incubated at 37 °C for 30 min. Then pepsin was allowed to react with bovine serum albumin [BSA (10 mg/mL); 2 mL] and test tubes were incubated again at 37 °C for 30 min. The undigested BSA protein was quantified by adding 1.0 mL of biuret reagent, and reading the absorbance at 546 nm against a reagent blank, after an incubation of exactly 30 min. The pepsin activity was determined from the standard turbidity curve.

\[
\text{Pepsin activity (mg/min)} = \frac{(\text{Unreacted albumin}) \times V_t}{\text{Incubation time} \times V_e}
\]

Where, Unreacted albumin = Concentration of unreacted albumin in mg/mL; Vt = Total reaction volume; Ve = Volume of enzyme used.

2.2.8 Tissue homogenate

Stomach and duodenum homogenates were prepared using the procedure described by Ajiboye et al. Briefly, stomach and duodenum tissue were homogenized in ice-cold sucrose-Tris buffer (0.25 mol/L sucrose, 10 mmol/L Tris–HCl, pH 7.4) and centrifuged at 1 000 × g for 10 min.

2.2.9 Biochemical assay

2.2.9.1 SOD

The activity of SOD in stomach and duodenum of rats was determined as described by Misra et al. Briefly, 0.2 mL of homogenate was added to 2.5 mL of 0.05 mol/L carbonate buffer (pH 10.2) to equilibrate and the reaction was started by addition of 0.3 mL of freshly prepared 0.3 mmol/L epinephrine. An increase in absorbance was recorded at 480 nm every 30 s for 150 s. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of epinephrine as determined by change in absorbance/min at 420 nm.

2.2.9.2 Catalase

Catalase activity was determined using the method described by Beers et al. Fifty microliter of the homogenate was added to a cuvette containing 2 mL of phosphate buffer (pH 7.0) and 1 mL of 30 mmol/L H2O2. Catalase activity is measured at 240 nm for 1 min using a spectrophotometer. The molar extinction coefficient of H2O2, 43.6 Mcm⁻¹, was used to determine the catalase activity.

2.2.9.3 GSH-px

The activity of GSH-px was determined by measuring the oxidation of reduced GSH by cumene hydroperoxide in the presence of GSH-Red and nicotinamide adenine dinucleotide phosphate (NADPH); the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance
was read at 340 nm\textsuperscript{[23]}.  

2.2.9.4 GSH-Red  

The activity of GSH-Red was determined following the reduction of GSH in the presence of NADPH, which is oxidized to NADP\textsuperscript{+}. The decrease in absorbance was read at 340 nm\textsuperscript{[24]}. 

2.2.9.5 Reduced GSH  

The level of GSH in the homogenate was determined using the procedure described by Ellman\textsuperscript{[25]}. Briefly, 1.0 mL of stomach mucosa homogenate was added to 0.1 mL of 25\% TCA and precipitate was removed by centrifuge at 5 000 × g for 10 min. Supernatant (0.1 mL) was added to 2 mL of 0.6 mmol/L DTNB prepared in 0.2 mol/L sodium phosphate buffer (pH 8.0). The absorbance was read at 412 nm. 

2.2.9.6 Malondialdehyde  

The level of malondialdehyde was determined using the procedure described by Reily and Aust\textsuperscript{[26]}. Briefly, homogenate and blank (without lipid) was combined with the TBA/TCA/HCl (0.3\%/15\%/0.2 N) reagent at a reagent/sample ratio of 2:1 (v/v). The samples were mixed thoroughly and placed in a boiling water bath for 15 min and the samples were allowed to cool to room temperature and then centrifuged at 1 000 × g for 10 min at room temperature. The absorbance of the solution was read at 535 nm. 

2.2.10 Histopathological study  

The stomachs were washed thoroughly with saline before tissue samples were collected and stored in 10\% formaline solution. Biopsies were collected from these samples. Sections (5 μm) were taken from these biopsies and stained with hematoxylin and eosin prior to visual inspection for damage under a light microscope (160 ×). 

2.3 Statistical analysis  

Data are expressed as mean ± standard error of mean. One-way analysis of variance (ANOVA) followed by Tukey-Kramer test was used to detect any significant differences among different means in this study using StatPlus (2011). Differences were considered statistically significant at \( P<0.05 \).  

3 Results  

3.1 Gas chromatogram  

GC-MS profiling of aqueous seed extract of \textit{C. papaya} revealed the presence of 7 different phytoconstituents including acacic acid (0.265 3 mg/g), genistein (0.278 2 mg/g), phorbol ester (0.601 9 mg/g), cryptolepine (4.972 2 mg/g), brusatol (1.57 mg/g), α-ionone (1.0625 mg/g) and paclitaxel (0.212 4 mg/g) (Figure 1). 

3.2 Ulcer indices  

There were significant decrease (\( P<0.05 \)) in the gastric pH of indomethacin-induced ulcer rats as compared with the normal control rats. Pretreatment of rats with aqueous extract of \textit{C. papaya} seed significantly increased the gastric pH when compared to the indomethacin-induced ulcerated rats \( (P<0.05) \) and the cimetidine-treated group \( (P<0.05) \) (Table 1). Acidity in the stomach secretions of rats with indomethacin-induced ulcers decreased significantly \( (P<0.05) \) relative to the control rats. Conversely, gastric acidity and concentration of gastric pepsin increased significantly \( (P<0.05) \) relative to the control rats. The ulcer index decreased significantly in the rats pretreated with the aqueous extract of \textit{C. papaya} seed \( (P<0.05) \). Ulcer indices in the treatment groups receiving doses of extract greater than 100 mg/kg BW were not significantly different from those in the cimetidine-treated group \( (P<0.05) \). The concentration of gastric acid and secretion volume decreased significantly \( (P<0.05) \) in the cimetidine and \textit{C. papaya}-pretreated groups when compared to the ulcer group \( (P<0.05) \). The percentage of ulcer inhibition of rats pretreated with aqueous extract of \textit{C. papaya} seed was dose-dependent and at doses of 150 mg/kg and 200 mg/kg BW was not significantly different \( (P>0.05) \) from that of the cimetidine 200 mg/kg BW group (Table 1). 

3.3 Oxidative stress biomarkers  

The activities of reactive oxygen species-detoxifying enzymes, SOD, CAT, GSH-Px, GSH-Red and Glc-6-PD, in the stomach and duodenum decreased significantly \( (P<0.05) \) in rats with indomethacin-induced ulcers (Tables 2 and 3). Group pretreated with \textit{C. papaya} extract at a dose of 200 mg/kg BW had similar enzyme activity to the normal control group (Table 2). Similarly, indomethacin-mediated reduction in the level of GSH in the stomach and duodenum was significantly attenuated by \textit{C. papaya} seed extract (Table 4). 

The level of malondialdehyde, a product of lipid peroxidation, increased significantly \( (P<0.05) \) in the stomach and duodenum of ulcerated rats (Table 4) compared with the normal control rats. However, pretreatment of rats with all doses of \textit{C. papaya} extract reduced indomethacin-mediated increase in malondialdehyde \( (P<0.05, \) Table 4). 

3.4 Visual inspection  

There was severe injury in rats with indomethacin-induced gastric ulcers. While only the dose of 200 mg/kg BW of extract completely protected the mucosa from injury, pretreatment of rats with 100 and 150 mg/kg BW of the extract attenuated indomethacin-mediated injuries relative to the control rats receiving no medicinal intervention. Similarly rats pretreated with 200 mg/kg BW cimetidine displayed same pattern of attenuation in indomethacin-mediated lesion (Figure 2). 

3.5 Histopathological examination of the stomach  

There was severely degenerated stomach tissue, with a total ulceration of the gastric pit, in indomethacin-treated rats, when compared to the control. Similar degeneration was observed in the duodenum. Pretreatments of rats
with extract of *C. papaya* (at all doses investigated) and cimetidine both prevented indomethacin-induced gastric ulceration, as there were normal arrangement of mucosal layers, gastric cells and no evidence of hemorrhage (Figure 3).

4 Discussion

This study demonstrates anti-ulcerogenic and antioxidant effects of pretreatment with aqueous extract of *C. papaya* seed on indomethacin-induced peptic ulcer in rats. The formation of gastric and duodenal ulcers in rats treated with indomethacin may be attributed to the inhibitory effects of indomethacin on prostaglandins synthesis, as the inhibition increases gastric acid secretion, reduces mucosal blood flow and reduces bicarbonate secretion\(^{(27)}\). Paclitaxel enhances the synthesis of prostaglandins through the induction of cyclooxygenase 2 and prostaglandins synthase \(^{2(28)}\). Similarly, anti-ulcer and secretory activities of betulinic acid, one of the components of this extract, have been reported\(^{(29,30)}\). Thus, the presence of these compounds (paclitaxel and betulinic acid) in the extract may be responsible for the elevated gastric pH, acid secretion, acid output, secretion volume and ulcer index in indomethacin-induced ulcer rats.

![Gas chromatograph of aqueous extract of Carica papaya seed](image_url)
### Table 1: Gastric pH, acidity, pepsin, acid output and volume, ulcer index and ulcer inhibition in stomach of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Gastric pH (mEq/L)</th>
<th>Gastric acidity (mg/min)</th>
<th>Gastric pepsin (mg/min)</th>
<th>Ulcer index (mm$^2$)</th>
<th>Gastric acid output (mEq/L)</th>
<th>Gastric volume (mL)</th>
<th>Ulcer inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.81±0.24</td>
<td>6.05±0.46</td>
<td>3.49±0.54</td>
<td>—</td>
<td>8.15±1.70</td>
<td>1.12±0.46</td>
<td>—</td>
</tr>
<tr>
<td>Ulcer (indomethacin)</td>
<td>1.52±0.07*</td>
<td>11.80±0.84*</td>
<td>6.18±0.41*</td>
<td>10.50±0.65</td>
<td>20.95±4.62*</td>
<td>2.33±0.16*</td>
<td>—</td>
</tr>
<tr>
<td>Ulcer + 200 mg/kg BW cimetidine</td>
<td>4.01±0.29* △</td>
<td>6.95±0.41 △</td>
<td>3.76±0.62 △</td>
<td>5.50±0.64 △</td>
<td>9.28±2.02 △</td>
<td>0.83±0.07 △</td>
<td>47.55±6.00</td>
</tr>
<tr>
<td>Ulcer + 100 mg/kg BW extract</td>
<td>4.44±0.34* △</td>
<td>7.50±0.50 △</td>
<td>3.80±0.78 △</td>
<td>8.93±0.42 △</td>
<td>11.28±1.48 △</td>
<td>1.51±0.17 △</td>
<td>18.90±4.83 ▲</td>
</tr>
<tr>
<td>Ulcer + 150 mg/kg BW extract</td>
<td>3.38±0.12* △</td>
<td>7.10±0.53 △</td>
<td>2.89±0.63 △</td>
<td>7.25±1.03 △</td>
<td>10.26±1.53 △</td>
<td>1.43±0.14 △</td>
<td>31.86±6.22</td>
</tr>
<tr>
<td>Ulcer + 200 mg/kg BW extract</td>
<td>3.44±0.21* △</td>
<td>6.50±0.96 △</td>
<td>2.41±0.34 △</td>
<td>6.00±0.41 △</td>
<td>9.20±2.02 △</td>
<td>0.81±0.06 △□</td>
<td>35.89±7.01 □</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean (n=5). *P<0.05, vs control group; △P<0.05, vs ulcer group; ▲P<0.05, vs cimetidine group; □P<0.05, vs ulcer + 100 mg/kg BW extract group. BW: body weight.

### Table 2: Specific activities of superoxide dismutase, catalase and glutathione peroxidase (nmol/min/mg protein) in stomach and duodenum of the rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Superoxide dismutase</th>
<th>Catalase</th>
<th>Glutathione peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
<td>Stomach</td>
</tr>
<tr>
<td>Control</td>
<td>6.15±0.41</td>
<td>3.33±0.11</td>
<td>0.540±0.007</td>
</tr>
<tr>
<td>Ulcer (indomethacin)</td>
<td>1.06±0.15*</td>
<td>1.12±0.03*</td>
<td>0.160±0.038*</td>
</tr>
<tr>
<td>Ulcer + 200 mg/kg BW cimetidine</td>
<td>5.30±0.23△</td>
<td>4.84±0.13△</td>
<td>0.590±0.017△</td>
</tr>
<tr>
<td>Ulcer + 100 mg/kg BW extract</td>
<td>2.22±0.41△ ▲</td>
<td>2.35±0.08* △▲</td>
<td>0.230±0.014* △▲</td>
</tr>
<tr>
<td>Ulcer + 150 mg/kg BW extract</td>
<td>3.54±0.60* △▲</td>
<td>2.05±0.07* △▲</td>
<td>0.410±0.009* △▲</td>
</tr>
<tr>
<td>Ulcer + 200 mg/kg BW extract</td>
<td>6.39±0.29 △ ▲</td>
<td>4.59±0.14* △ ▲</td>
<td>0.560±0.010* △ ▲</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean (n=5). *P<0.05, vs control group; △P<0.05, vs ulcer group; ▲P<0.05, vs cimetidine group; □P<0.05, vs ulcer + 100 mg/kg BW extract group. BW: body weight.

### Table 3: Specific activities of glutathione reductase and glucose 6-phosphate dehydrogenase (nmol/min/mg protein) in stomach and duodenum of the rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutathione reductase</th>
<th>Glucose-6-phosphate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
<td>Duodenum</td>
</tr>
<tr>
<td>Control</td>
<td>56.70±1.44</td>
<td>63.35±0.82</td>
</tr>
<tr>
<td>Ulcer (indomethacin)</td>
<td>31.81±1.06*</td>
<td>36.62±1.32*</td>
</tr>
<tr>
<td>Ulcer + 200 mg/kg BW cimetidine</td>
<td>50.60±0.32△</td>
<td>56.35±0.25△</td>
</tr>
<tr>
<td>Ulcer + 100 mg/kg BW extract</td>
<td>35.23±0.90* △▲</td>
<td>38.34±0.45* △▲</td>
</tr>
<tr>
<td>Ulcer + 150 mg/kg BW extract</td>
<td>42.36±0.63* △ ▲</td>
<td>45.12±0.32* △ ▲</td>
</tr>
<tr>
<td>Ulcer + 200 mg/kg BW extract</td>
<td>51.16±1.96 △ ▲</td>
<td>54.23±0.82 △ ▲</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean (n=5). *P<0.05, vs control group; △P<0.05, vs ulcer group; ▲P<0.05, vs cimetidine group; □P<0.05, vs ulcer + 100 mg/kg BW extract group. BW: body weight.
Oxidative stress has been implicated in the development and pathogenesis of indomethacin-mediated gastric injury [31]. NSAIDs such as indomethacin have been reported to decrease the activities of antioxidant enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc-6-PD) and the level of non-antioxidant enzyme (GSH) in the stomach and duodenum, thereby causing gastric ulceration [32]. The decrease in the activities of these enzymes may lead to an imbalance in the prooxidant and antioxidant status resulting in the generation of free and oxygen-derived radicals, such as hydrogen peroxide, superoxide anion radicals and hydroxyl radicals. Panzarini et al [33] reported that the aqueous extract of Carica papaya seed has antioxidant activity. A similar increase in antioxidant activity was observed in this study and may be due to the presence of betulinic acid and genistein, which have been reported to increase the activities of antioxidant enzymes [34,35]. Cryptolepinone may also contribute to the enhanced activities, as it has been reported to increase the activity of quinone reductase [36]. The augmentation of the activity of these antioxidant enzymes may represent an important mechanism of protection against peroxides, superoxide anion radicals and hydroxyl radicals.

Table 4 Levels of reduced glutathione (nmol/mmg protein) and malondialdehyde (µmol/mg protein) in stomach and duodenum of the rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Reduced glutathione (Stomach)</th>
<th>Malondialdehyde (Stomach)</th>
<th>Malondialdehyde (Duodenum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.29±0.00</td>
<td>5.62±0.13</td>
<td>0.020±0.001</td>
</tr>
<tr>
<td>Ulcer (indomethacin)</td>
<td>0.03±0.00*</td>
<td>0.14±0.22*</td>
<td>0.080±0.010*Δ</td>
</tr>
<tr>
<td>Ulcer+200 mg/kg BW cimetidine</td>
<td>2.38±0.01△</td>
<td>4.38±0.71△</td>
<td>0.020±0.001△</td>
</tr>
<tr>
<td>Ulcer+100 mg/kg BW extract</td>
<td>1.25±0.57△△</td>
<td>1.90±0.19△△</td>
<td>0.050±0.001△△</td>
</tr>
<tr>
<td>Ulcer+150 mg/kg BW extract</td>
<td>1.24±0.19△△</td>
<td>3.05±0.53△△</td>
<td>0.040±0.004△△</td>
</tr>
<tr>
<td>Ulcer+200 mg/kg BW extract</td>
<td>2.31±0.26△△</td>
<td>5.57±0.19△△</td>
<td>0.020±0.003△△</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of mean (n=5). *P<0.05, vs control group; △P<0.05, vs ulcer group; ▲P<0.05, vs cimetidine group; □P<0.05, vs ulcer + 100 mg/kg BW extract group. BW: body weight.

Figure 2 Macroscopic appearance of the gastric mucosa in one rat of each group

A: Rat treated with only distilled water as control (×10); B: Rat treated with 100 mg/kg body weight of indomethacin for inducing ulcer (×10); C: Rat pretreated with 200 mg/kg body weight of cimetidine (×10); D: Rat pretreated with 100 mg/kg body weight of aqueous extract of Carica papaya seed (×10); E: Rat pretreated with 150 mg/kg body weight of aqueous extract of Carica papaya seed (×10); F: Rat pretreated with 200 mg/kg body weight of aqueous extract of Carica papaya seed (×10).
Reduced glutathione complements the enzymatic antioxidant systems, thus reduction in the levels of this antioxidant protein in the stomach and duodenum of indomethacin-treated rats could predispose the tissues to oxidative damage. Reduction of glutathione may also be due to decreased GSH-Red activity, consequently leading to the accumulation of glutathione disulfide. The reversal of indomethacin-mediated decrease in glutathione levels in rats pretreated with aqueous extract of C. papaya seed may be attributed to enhancement of antioxidant enzymes, mostly GSH-Red, which act to replenish GSH.

Previous studies have reported the elevation of MDA, an index of lipid peroxidation, in the stomach and duodenum where ulcers have been induced with indomethacin. The elevation could have resulted from peroxidation of a polyunsaturated fatty acid component of the membrane by generated free and oxygen-derived radicals. The significant attenuation of this increase by pretreatment with aqueous extract of C. papaya seed may also be attributed to the enhanced antioxidant enzymes, which could protect against the peroxidation of membrane lipids by radicals.

Indomethacin produced gross glandular mucosal damage in rats, an indication of ulcerative lesions, which appeared as red streaks (Figure 2B). Pretreatment with aqueous extract of C. papaya seed markedly reduced the visible hemorrhagic lesions induced by indomethacin in rat stomachs.
Changes in the morphological structures of tissues are late manifestations of chemical and inflammatory assault on tissues. The histological examination of indomethacin-treated rats showed severe degenerated architecture of the stomach, indicating total ulceration of the submucosa layer. Pretreatment of rats with aqueous extract of *C. papaya* at doses greater than 150 mg/kg BW resulted in normal arrangement of mucosal layers and gastric cells, similar to the control rats (without ulcer induction) as well as rats receiving cimetidine 200 mg/kg. These groups showed significant protection against the formation of gastric lesions and edema in the submucosa compared to the animals receiving no medicinal intervention after ulcer induction.

**5 Conclusion**

Based on the reduction in gastric pepsin secretion, gastric acidity and gastric secretion volume as well as the increase in gastric pH, it was evident that pretreatment with aqueous extract of *C. papaya* seeds resulted in anti-ulcerogenic activity. This may be due to the capability of the extract to enhance the activities of antioxidant enzymes. The present research supports the use of the extract of *C. papaya* seeds in the treatment of ulcer.

**6 Declaration of conflicting interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**REFERENCES**

23. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman...

Submission Guide

Journal of Integrative Medicine (JIM) is an international, peer-reviewed, PubMed-indexed journal, publishing papers on all aspects of integrative medicine, such as acupuncture and traditional Chinese medicine, Ayurvedic medicine, herbal medicine, homeopathy, nutrition, chiropractic, mind-body medicine, TaiChi, Qigong, meditation, and any other modalities of complementary and alternative medicine (CAM). Article types include reviews, systematic reviews and meta-analyses, randomized controlled and pragmatic trials, translational and patient-centered effectiveness outcome studies, case series and reports, clinical trial protocols, preclinical and basic science studies, papers on methodology and CAM history or education, editorials, global views, commentaries, short communications, book reviews, conference proceedings, and letters to the editor.

● No submission and page charges  ● Quick decision and online first publication

For information on manuscript preparation and submission, please visit JIM website. Send your postal address by e-mail to jcim@163.com, we will send you a complimentary print issue upon receipt.

March 2015, Vol.13, No.2 114 Journal of Integrative Medicine