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Original Research Article

Antiulcer activity of hydroalcoholic extract of unripe fruit of carica papaya in experimental rats

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ABSTRACT

Background: *Carica* papaya has previously reported antioxidant, analgesic and anti-inflammatory, antibacterial and anti-histaminic activity. Peptic ulcer disease involves inflammation and oxidative stress, so hydroalcoholic extract of *Carica* papaya fruits may have strong potential for an antiulcer agent. Aim and objectives of the study were in this study pharmacological evaluation of antiulcer effect of hydroalcoholic extract of *Carica* papaya fruits was performed by pyloric ligation induced gastric ulcers.
Methods: Preliminary phytochemical investigation, estimation of phenolic and flavonoid content, In vitro antioxidant activities, antiulcer activity to find out the efficacy of hydroalcoholic extract of *Carica* papaya fruits.
Results: Treatment with 500 mg/kg of hydroalcoholic extract of *Carica* papaya fruits efficacious in reducing ulcer index in pyloric ligation induced gastric ulcer model. Hydroalcoholic extract *Carica* papaya of showed a dose dependent decrease in ulcer and reduces ulcer index which was supported by morphological and histological studies.
Conclusions: Thus it can be concluded that hydroalcoholic extract of *Carica* papaya fruits have antiulcer activity, which can be attributed to its antioxidant mechanism of action.

Keywords: Antioxidant, Antiulcer, *Carica* papaya

INTRODUCTION

Natural products are source of medicinal agents from many years. These natural medicines which are traditional play very important role in health care because 80% of drugs originated from natural sources.[1]

Ulcers are deep lesions penetrating through entire thickness of gastrointestinal tract mucosa and muscularis mucosa. There are so many types of ulcers and most common are peptic ulcers.[2] Peptic ulcers are defect of the gastrointestinal mucosa that extend through the muscularis mucosa because of the presence of acid and pepsin.[3] Helicobacter pylori infection is responsible for nearly 80% of gastric ulcers and for over 90% of duodenal ulcers. Nonsteroidal anti-inflammatory drugs and aspirin use are other major causes of peptic ulcer, especially for gastric ulcers.[4] Stress either psychological or physical leads to oxidative stress in stomach i.e. production of reactive oxygen species. Oxidative stress

involve in pathogenesis of gastric inflammation, ulcerogenesis and carcinogenesis in *H.pylori* infection.[5] Current peptic ulcer is managed by H_2 receptor antagonist and proton pump inhibitors. Endoscopy therapy is also used now a days.[6] A combination of pharmacological and endoscopic therapy offers the best method of hemostasis to those with active bleeding ulcers. Ulcer due to asprin is more as compare to *H pylori* now a day. An increase in non- *H. pylori* and non- NSAID peptic ulcer bleeding was observed. The reason for non *H. pylori* and non NSAID peptic ulcer may be that the mucosa after NSAID attack or after *H. pylori* infection may not recover properly even after NSAID withdrawal or after antibacterial treatment.[7] Complications in peptic ulcer also arises like bleeding, perforation, obstruction. In bleeding, ulcer erodes the muscles of the stomach or duodenal wall, blood vessels may also be damaged, which causes bleeding. In perforation, ulcer destroys the wall of stomach or duodenum. Bacteria and partially digested food can spill through the opening into the sterile abdominal cavity. The symptom of perforated ulcer include sudden, sharp, severe pain.[8] In obstruction, ulcer located the end of the stomach where the duodenum is attached may causes swelling and scarring. In this endoscopy dilation may be performed. Symptoms of duodenal ulcer are pain that awakens patients from sleep, burning sensation, pain in chest and lower abdomen occurs. Symptoms of gastric ulcer are pain which is less than duodenal ulcer, vomiting, nausea and weight loss.[8] Herbs are used for medicinal, flavouring and aromatic qualities for centuries. Three- quarters of world population relies mainly on plants and plant extracts for health care. 30% of plant species, at one time or other are used for medicinal purposes.[9] Herbal drugs obtained from plant sources are relatively less expensive, safe and possess good tolerability even higher dose .Systems like siddha, Ayurveda, unani and allopathy use plant species to treat health problem. Their uses become popular due to toxicity and side effects of allopathic medicines. The market for herbal medicines is expanding at 20% annually.[10]

METHODS

Qualitative phytochemical screening

Test for detection of alkaloids

Extract were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

a) *Mayer's test:* Filtrates were treated with Mayer's reagent (Potassium mercuriciodide). Yellow cream precipitate indicates the presence of alkaloids.
b) *Wagner's test:* Filtrates were treated with wagner's reagent (Iodine in potassium iodide). Brown or reddish brown precipitate indicates the presence of alkaloids.

Test for detection of saponins:

a) *Foam test:* Small amount of the extract was shaken with little quantity of water. If foam produced persists for ten minutes, it indicates the presence of saponins.
b) *Froth test:* Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. A formation of 1 cm layer of foam indicates the presence of saponins.

Test for detection of phenols

a) Alkaline reagent test: Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.
b) Ferric chloride test: Extracts were treated with a few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Tests for detection of tannins

a) Gelatin test: to the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for detection of flavonoids

a) Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.
b) Shinoda test: To the alcoholic solution of extracts, a few fragments of magnesium ribbion and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

In vitro study

Total flavonoid content

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% AlCl3 solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

Total phenolic contents

Total phenolic content was determined according to the method of Chun *et al*. (2003). Ten milligrams PECL was included in 10 mL volumetric flask, then added with 0.4 mL reagent of Folin-Ciocalteau and incubated for 4-8 min. Furthermore, the solution was added with 4.0 mL of 7% Na2CO3, then added with distilled water. After 2 h of incubation, the solution absorbance was measured in 750 nm wavelength versus a blank consisting distilled water and Folin-Ciocalteau reagent. Total phenolic content was expressed in gallic acid equivalent of each 100 g PECL of dry weight.

Determination of DPPH radical scavenging activity

The free radical scavenging activity of extract was measured *in vitro* by DPPH assay. About 0.3mM solution of DPPH in 100% ethanol was prepared and 1ml of this solution was added to 3ml of the extract dissolved in ethanol at different concentrations (10–50 g/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contains DPPH without any extract using the following formula:

DPPH (%) =A Control-A test/A Control X 100

In this, Acontrol was the absorbance of the control reaction

Attest was the absorbance of the sample of the extracts.

The value of the crude extract was compared with that of ascorbic acid, which was used as the standard.

Nitric oxide radical scavenging assay

Sodium nitroprusside (5μM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25 °C for 5 hr. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula:

100 - [100/blank absorbance × sample absorbance]

The activity was compared with ascorbic acid, which was used as a standard antioxidant.

Experimental protocol

Pyloric ligation induced ulcers

The test samples were administered to animal as oral treatment was carried out 1 and 0.5 hour before pyloric ligature, respectively. After 18 hours of fasting, ulcer induction was undertaken according to Shay et al. The rats were quickly and mildly anaesthetized with diethyl ether and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animals were allowed to recover from anesthesia. After ligation of the pylorus, drinking water was withheld and the gastric examinations were under taken 19 hours after pylorus ligation. The animals were sacrificed with an overdose of diethyl ether and the stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a magnifier lens (x5) to assess the formation of ulcer. Using 3 M transpore surgical tapes assessed ulcer area, which was fixed on a light and transparent sheet and the ulcer area was measured for each stomach. The number of erosions formed on glandular portion of stomach was counted and each was given a severity rating on a 1-3 scale.

Table 1: Protocol for pyloric ligation induced ulcers.

Group	Treatment	Animal used	Dose Mg/kg	Route of administ-ration
I	Exp. Control (vehicle)	6	-	p.o.
II	Standard (Rabeprazole)	6	20	p.o.
III	Hydroalc. Ext of Carica papaya	6	250	p.o.
IV	Hydroalc. Ext of Carica papaya	6	500	p.o.

The overall total divided by a factor of 10 was designated as the ulcer index for that stomach. The gastric contents were collected through the esophagus. The gastric juice was centrifuged and volume was recorded. The mucous was then washed and extent of ulceration was scored as per the method suggested by kunchandy et al.

Swimming induced ulcer model

Stress ulcers were introduced by forcing swimming in the glass cylinder (height 35 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 250C for 3 hours. Animals were fasted for 24 hours prior to the experiment and divided into 5 groups 6 animals in each group. Group 1 received vehicle control, group 2 treated with Swim stress (3 hours) Positive control, group 3 treated with Swim stress (3 hours) + standard and group

4and 5 treated with Swim stress (3 hours) + extract dose respectively after the drug treatment animals were allowed to swim in this water for 3 hours. The stomach of each animal was removed and the extent of gastric damage was assessed. A score of ulcer will be made.

Table 2: Protocol for swimming induced ulcers model.

Group	Treatment	Animal used	Dose mg/kg	Route of administration
I	Control (vehicle)	6	-	p.o.
II	Experimental control (vehicle)	6	-	p.o.
III	Standard (Ranitidine)	6	20	p.o.
IV	Hydroalc. Ext. of Carica papaya	6	250	p.o.
V	Hydroalc. Ext. of Carica papaya	6	500	p.o.

In vivo study

Determination of ulcer index for pylorus ligation and swimming induced ulcer model.

Scoring of ulcer

- 0 = Normal stomach
- 0.5 = Red coloration
- 1 = Spot ulcer
- 1.5 = Hemorrhagic streak
- 2 = Ulcers
- 3 = Perforation

Calculation of ulcer Index

- UI = UN + US + UP x 10-1
- UI = Ulcer Index
- UN = Average of number of ulcer per animal
- US = Average of severity score
- UP = Percentage of animals with ulcer[11]

Determination of pH and free acidity

Gastric juice was collected from the pylorus-ligated rats. The gastric juice thus collected was centrifuged and the volume of gastric juice as well as pH of gastric juice was measured. Free acidity is measured according to the method described by Dushputre et al. Tropfer's reagent is used. 0.01N NaOH used to titrate gastric juice until canary yellow colour observed. Volume of 0.01N NaOH consumed was noted for pylorus ligation and swimming induced ulcer model.[12]

Determination of total acidity

An aliquot of 1ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was established. The volume of 0.01N NaOH consumed was noted, total acidity was calculated and expressed as meq/l for pylorus ligation and swimming induced ulcer model.[13]

The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times N \times 100}{0.1} \text{ mEq/L/100gm}$$

Determination of percentage protection

The percentage protection produced by plant extract against ulcer determined by using a formula for pylorus ligation and swimming induced ulcer model.[12]

$$\frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

(Control- UI in control group; Treatment- UI in treated group).

Estimation of reduced glutathione

Reduced glutathione (GSH) was determined by the method of Ellman for pylorus ligation and swimming induced ulcer model. To 0.1 ml of different tissue homogenate 2.4 ml of 0.02M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50% TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 rpm for 15 min. 1 ml of supernatant was taken and 2ml of Tris-Hcl buffer was added. Then 0.05 ml of DTNB solution (Ellman's reagent) was added and vortexed thoroughly. OD was read (within 2-3min after the addition of DTNB) at 412 nm against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH.

Estimation of catalase

CAT level was measured by the method described by Aebi for pylorus ligation and swimming induced ulcer model. A volume of 0.1 mL of supernatant was added to a cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). A reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of CAT was expressed as µmol H_2O_2 metabolized/mg protein/min.

$$\text{Catalase activity} = \frac{\text{Change in absorbance/min}}{\text{E x volume of sample x mg protein}}$$

Histopathology

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. The tissues were processed according to the standard procedure and sections were cut stained with haematoxylin and eosin Bancroft[12]. The slides were examined microscopically for morphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of this changes.[14]

RESULTS

Qualitative phytochemical screening

Table 3: Qualitative phytochemical constituent of HAECP.

S. no.	Phytoconstituents	Chemical test	HAECP
1	Alkaoids	Mayer's test	+
		Wagner's test	-
		Dragendorff's test	+
		Hager's	+
2	Saponins	Froth	-
		Foam	+
3	Phenols	Alkaline reagent test	-
		Ferric chloride test	+
4	Tannins	Gelatin test	+
5	Flavonoids	Alkaline reagents test	+
		Shinoda test	+

HAECP- Hydroalcoholic extract of *Carica papaya*, (+) Present, (-) Absent

In vitro antioxidant assays

Determination of total flavonoid content

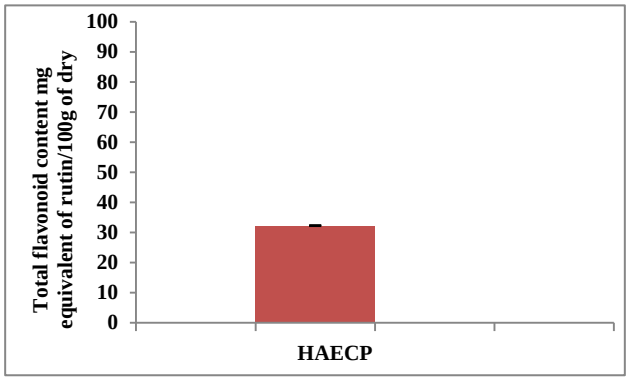


Figure 1: Total flavonoid content.

Amounts of flavonoids present in the plant extracts was estimated as mg equivalents of rutin/g of dry extract. Flavonoids are a group of polyphenolic compounds with known properties that include free-radical scavenging, inhibition of hydrolytic enzymes and anti-inflammatory action .The flavonoid content of hydroalcoholic extract of *Carica papaya* unripe fruits was found to be 32.24±0.14mg equivalent of rutin/100g of dry extract as shown in Figure 1.

Determination of total phenolic content

Amounts of phenols present in the plant extracts was estimated as mg equivalents of gallic acid/ g of dry extract. Phenolic compounds that probably constitute the largest group of plants secondary metabolites. Polyphenols display a number of pharmacological properties in the GIT area, acting as antisecretory, cytoprotective, and antioxidant agents. Figure 2 showed that the total phenolic content of hydroalcoholic extract of *Carica papaya* fruits was found to be 55.46±0.89 mg equivalent of gallic acid/100g of dry extract.

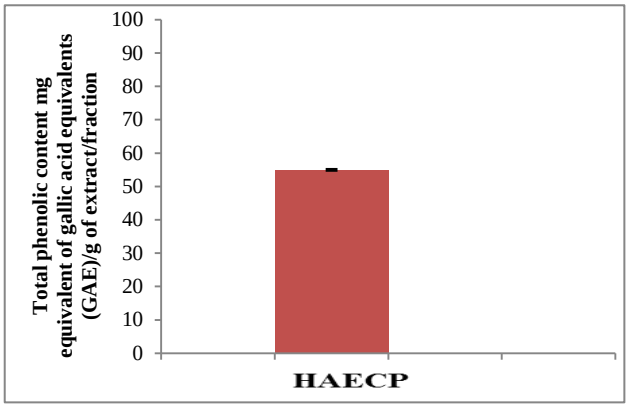


Figure 2: Determination of DPPH radical scavenging activity.

The DPPH radical scavenging assay is based on the ability of DPPH, a stable, organic free radical which gets decolorized in the presence of antioxidant. DPPH contain an odd electron, which is responsible for the absorbance at 517 nm and also for its deep purple colour.

Table 4: DPPH radical scavenging activity.

Concentration (µg/ml)	Percentage scavenging	
	Ascorbic acid (Mean±SD)	HAECP (Mean±SD)
50	71.44±0.025	64.22±0.010**
100	74.09±0.075	67.47±0.013**
200	78.34±0.028	69.02±0.047**
400	82.95±0.028	65.08±0.257**
800	86.24±0.025	72.19±0.133**

The values were expressed as Mean ± SD (n=3) and performed in triplicate, compared with ascorbic acid. The data was analyzed by using unpaired t-test at *P<0.05.

When antioxidant is added to the DPPH solution it accepts the electron donated by the antioxidant and gets yellowish colour which can be measured from the changes in absorbance. In Figure 2, statistically significant difference (P<0.05) to DPPH scavenging activity was observed among the test and reference compounds at different concentrations. HAECP showed significant DPPH radical scavenging activity. It has been suggested that the free radicals are closely related with peptic ulcer disease and gastritis (Demir et al). So the result depict that hydroalcoholic extract of *Carica papaya* has a free radical scavenging activity.

Percentage NO scavenging

This assay was performed according to the method described by Green et al; Marcocci et al. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by Griess reagent. The reaction mixture (3 ml) containing 10 mM sodium nitroprusside in 0.05M phosphate buffered saline, and different plant extracts (at different concentration i.e. 50, 100, 200, 400, 800µg/ml) was incubated at 25°C for 150 min. About 0.5 ml aliquot of the incubated sample is removed at 30 min intervals and 0.5 ml Griess reagent is added. The absorbance of the chromophore formed is measured at 546 nm. Chromophore is formed by diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine. Ascorbic acid used as a standard. Percentage inhibition of nitric oxide was calculated, based on the control reading without any extract using following formula.

NO scavenging activity (%) = 1 - Atest/Acontrol x 100

Where, Acontrol is absorbance of buffer solution.

Free radicals were previously reported as being capable of damaging a lot of cellular components such as proteins, lipids and DNA (Hamilton et al). NO in micromolar concentrations will reversibly inhibit catalase and cytochrome P-450. NO also show toxic property after reaction with oxygen and superoxide radicals. The reaction products are able to cause much cellular damage (Vriesman et al).

Attest is absorbance in presence of plant extract.

Table 5: NO scavenging activity.

Concentration (µg/ml)	Percentage scavenging	
	Ascorbic acid (Mean±SD)	HAECP (Mean±SD)
50	22.27±0.023	65.41±0.01**
100	40.32±0.117	73.64±0.05**
200	47.78±0.010	78.31±0.03**
400	45.58±0.143	81.08±0.07**
800	64.32±0.053	84.46±0.05**

The values were expressed as Mean ± SD (n=3) and performed in triplicate, when compared with ascorbic acid. The data was analyzed by using unpaired t-test at **P<0.01.

Antiulcer activity

Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin, leading to the autodigesion of gastric mucosa (Sofidiya et al). Due to the surgery the stomach gets larger; the pressure on sensitive receptors in the gastric mucosa increases and activates the vagus-vagal reflex, causing increased gastric secretion. Pretreatment with hydroalcoholic extract of *Scindapsus officinalis* fruits in pylorus ligation induced ulcer model, significantly (P<0.001) decreased ulcer index. There was significantly (P<0.001) increase in pH with reduction in volume of gastric contents, free acidity, total acidity in extract treated groups as compared to experimental control group. With pylorus ligation the gastric volume is increased.

Low pH increases free acidity and total acidity as a result ulcer index is increases.

Furthermore, neutrophils migration in the mucosa following pyloric ligation suggests that these cells might be involved in gastric mucosal injury, possibly by releasing free radicals that cause lipid peroxidation and damage to the cell membrane (Andreo et al). The reduced ulcer index, gastric volume, free acidity and total acidity suggested that HAECP fruits have the antisecretory and antiulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since factors such as gastric volume, free acidity and total acidity is directly related to ulcer formation.

Table 6: Effect of hydroalcoholic extract of *Carica papaya* unripe fruits on pylorus ligation induced ulcer.

Groups (n=6)	Dose (mg/kg)	Gastric volume (ml)	pH	Free acidity (meq/l)	Total acidity (meq/l)	Ulcer index	Percentage protection %
Experimental control	–	5.38±0.11	2.14±0.01	48.4±1.02	77.63±0.09	4.62±0.20	_
Standard (Rabeprazole)	20	2.50±0.09	4.53±0.02	24.6±1.35	35.55±0.10	0.29±0.13	93.72
HAECP	250	4.51±0.11	3.04±0.01	44.8±1.26	61.95±0.01	2.65±0.23	42.64
HAECP	500	2.73±0.06	4.04±0.02	32.4±1.12	49.24±0.08	0.51±0.19	88.96

Table 7: Effect of hydroalcoholic extract of *Carica papaya* unripe fruits on swimming induced ulcer.

Groups (n=6)	Dose (mg/kg)	Gastric volume (ml)	pH	Free acidity (meq/l)	Total acidity (meq/l)	Ulcer index	Percentage protection %
Experimental control	–	4.20±0.02	2.43±0.80	49.8±1.24	76.42±0.08	5.02±0.32	_
Standard (Rabeprazole)	20	2.53±0.01	5.04±0.92	25.4±1.56	34.87±0.14	0.34±0.19	93.22
HAECP	250	3.85±0.05	4.04±0.10	46.7±1.38	64.46±0.03	2.94±0.34	41.83
HAECP	500	3.23±0.01	4.84±0.91	34.4±1.24	48.76±0.09	0.88±0.15	82.47

HAECP- Hydroalcoholic extract of *Carica papaya* unripe fruits; Values are expressed in terms of mean±S.E.M (n=6); ***P<0.001, *P<0.05 (ANOVA followed by Dennett's test), compared with the experimental control group.

In vivo antioxidant activity

Estimation of reduced glutathione

The level of GSH was determined from the standard calibration curve of GSH. GSH is a detoxification enzyme capable of catalysing the conjugation of reactive electrophile with the thiol glutathione, providing cellular protection from the highly reactive electrophile (Chung et al). Gastric wall has a high concentration of GSH that provides protection against oxidative damage induced by necrotizing agents, such as ethanol, acetic acid, and NSADs, as well as by ischemia reperfusion and carcinogen agents. However, its depletion in gastric tissue is associated with the increased risk of gastric injury (Robert et al., 1984). GSH plays an important role in maintaining the integrity of the gastric mucosa, maintenance of mucosal integrity and its depletion in the gastric mucosa induces macroscopic mucosal ulceration (Hoppenkamps et al); (Altinkaynak et al). Excessive peroxidation causes increased GSH consumption (Banerjee et al).

Table 8 depicts the GSH level for pylorus ligation method was 73.89±2.02 nmol/g of tissue in rats from the normal control and 54.41±1.89 nmol/g of tissue in experimental control rats. Whereas in standard treated group GSH level found to be 73.96±3.42 nmol/g of tissue. In rats treated with hydro-alcoholic extract at 250 and 500mg/kg dose, showed the GSH level 56.34±1.88 nmol/g and 70.61±1.42 nmol/g of tissue respectively and for swimming induced model was 60.25±3.22 nmol/g of tissue in rats from the normal control and 45.65±1.22 nmol/g of tissue in experimental control rats. Whereas in standard treated group GSH level found to be 60.11±2.95 nmol/g of tissue. In rats treated with hydro-alcoholic extract at 250 and 500mg/kg dose, showed the GSH level 46.72±1.62 nmol/g and 57.01±1.24 nmol/g of tissue respectively.

Table 8: GSH level in stomach tissue.

		Pulorus induced		Swimmimg induced	
Groups	Treatment	GSH nmol/g of tissue (Mean±SD)	% drop in GSH level	GSH nmol/g of tissue (Mean±SD)	% drop in GSH level
Normal control	_	73.89±2.02***		60.25±3.22***	
Experimental control	_	54.41±1.89	24.68	45.65±1.22	26.58
Standard (Rabeprazole)	20mg/kg	73.96±3.42***		60.11±2.95***	
HAECP	250mg/kg	56.34±1.88	20.75	46.72±1.62***	19.64
HAECP	500mg/kg	70.61±1.42***	2.42	57.01±1.24***	2.06

HAECP- Hydroalcoholic extract of *Carica papaya;* GSH- Glutathione; values are expressed in terms of mean±SD (n=6); ***P<0.001, when compared with experimental control. The data was analyzed usinsg One-Way ANOVA followed by Dunnett test.

Estimation of catalase

CAT activity was defined as the amount of enzyme required to decompose 1nmol of H2O2 per minute, at 25^0C and pH 7.8. Results are expressed as millimole per minute per milligram tissue (mmol min/mg/tissue) (Odabasoglu et al). Under normal circumstances, peroxisomally generated H2O2 is quickly degraded to water and oxygen by the primary peroxisomal antioxidant enzyme, catalase (CAT) (Walton and Pizzitelli). In deficient of catalase primary peroxisomal ROS, hydrogen peroxide, is freely diffusible, it elicits oxidative damage. Therefore on treatment with hydroalcoholic extract of *Carica papaya* significant (500 mg/kg, p<0.001), (250 mg/kg, p<0.01) decrease in the ulcer index observed that could be due to increase in catalase levels.

Table 9: Catalase level in stomach tissue.

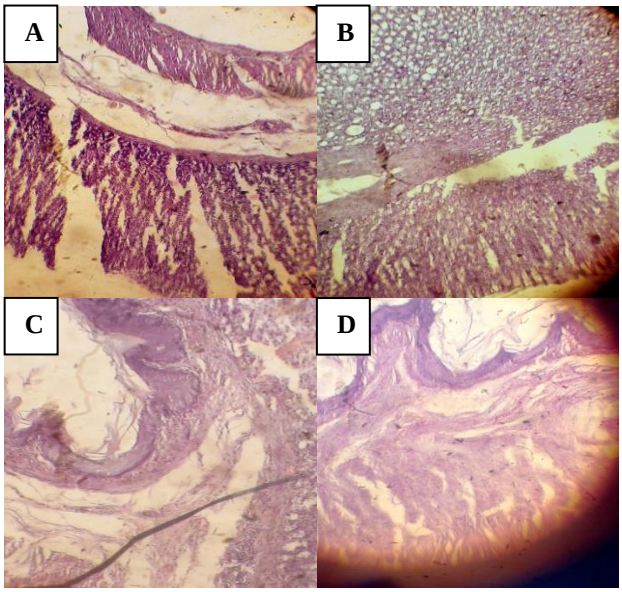
		Pulorus induced		Swimmimg induced	
Groups	**Treatment**	**µmol H_2O_2 /min/100mg of tissue (Mean±SD)**	**% drop in GSH level**	**µmol H_2O_2 /min/100mg of tissue (Mean±SD)**	**% drop in GSH level**
Normal control	-	18.42±2.54***		25.25±1.72***	
Experimental control	-	2.98±1.49	18.21	5.65±1.22	20.73
Standard (Rabeprazole)	20mg/kg	16.62±4.86***	14.64	22.56±1.95***	15.36
HAECP	250mg/kg	12.52±4.01	40.36	14.52±1.69	34.68
HAECP	500mg/kg	15.01±2.02***	22.43	19.98±1.42***	21.78

HAESO- Hydroalcoholic extract of*Carica papaya*; values are expressed in terms of mean±SD (n=6); ***P<0.001, **P<0.01 when compared with experimental control. The data was analyzed using One-Way ANOVA followed by Dunnett test.

In the present study, a significant increase (p<0.001, p<0.01) in the catalase (CAT) activity occurred in treated groups on treatment with hydroalcoholic extract of *Carica papaya* fruits, when compared to the experimental control group. Table 9 depicts treatment with extract at 250 mg/kg and 500 mg/kg showed significant (500mg/kg at p<0.001, 250 mg/kg at p<0.01) increased catalase activity which could be as result of improved gastroprotective effect of extracts. Table 9 showed catalase level was 18.42±2.54 and 25.25±1.72 µmol/min/100mg of tissue in rats from the normal control for pylorus and swimming induced model respectively, 2.98±1.49 and 5.65±1.22µmol/min/100mg of tissue in experimental control for both model. In rats treated with hydroalcoholic extract of *Carica papaya* at 250mg/kg dose, showed increased catalase activity 12.52±4.01 and 14.52±1.69µmol/min/100mg of tissue and for 500 µmol/min/100mg 15.01±2.02 and 19.98±1.42µmol/min/100mg of tissue respectively. Whereas, the standard treated group showed the catalase activity 16.62±4.86 and 22.56±1.95µmol /min/100mg of tissue. The results reviled the catalase activity found to be significantly higher in treatment groups then experimental control group. This increased catalase activity is reason for gastro protective effect of the extract, reduce mucosal damage and ulceration.

Histopathology study

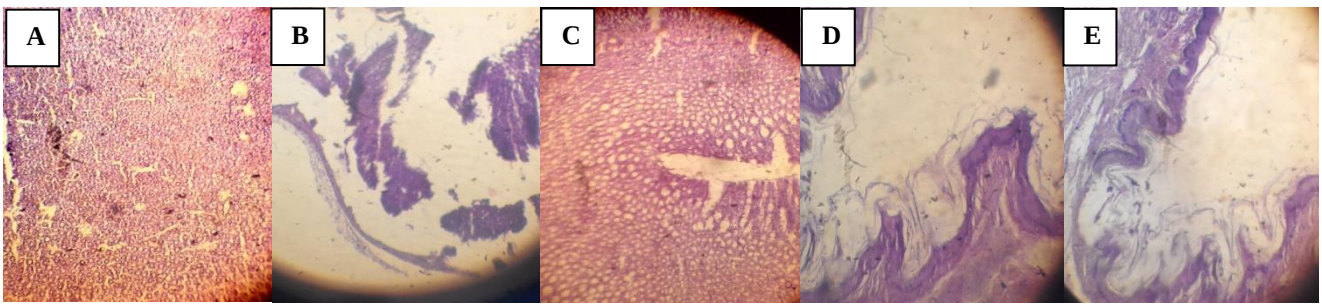
Histopathology of pylorus ligation method



A) Experimental control B) Rabeprazole C) HAECP 250mg/kg D) HAECP 500mg/kg

Figure 3: Histopathology of pylorus ligation method.

Histopathology of swimming induced ulcer model



A) Normal control B) Experimental control C) Rabeprazole D) Extract 250mg/kg E) Extract 500mg/kg

Figure 4: Histopathological examination (haematoxylin and eosin staining) of rat stomach tissue.

DISCUSSION

Peptic ulcers are defect of the gastrointestinal mucosa that extends through the muscularis mucosa because of the presence of acid and pepsin. So, we perform different tests on rat stomach to check the ulcer activity. Normal control gastric mucosa appeared intact and showed a continuous epithelial surface. Experimental control showed mucosal ulceration, damaged mucosal epithelium, disrupt glandular structure and haemorrhage in gastric mucosa. In rabeprazole treated group the gastric mucosa appeared intact and showed a continuous epithelial surface. HAECP, 250 mg/kg showed decreased number of erosions in mucosa but still there is inflammation with areas of haemorrhage. Extent of gastric mucosal rupture was decreased. HAECP, 500mg/kg effectively decreased the epithelial cell lose, gastric lesions and haemorrhage.

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At the start of every major task initially difficulties seem insurmountable. But as we begin treading on the chosen path with honesty and perseverance we find that God is there to help us. Research is an arduous task, which despite a consistent and dedicated mind also requires patience. Towards the completion of my thesis it is a pleasurable aspect that I have now the break to communicate my thankfulness to all those who have been influential in the completion of this task.

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