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PHYTOPHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF *CARICA PAPAYA* LEAVES FOR ANTI-ULCER ACTIVITY

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Abstract:

Peptic disorders like Gastroesophageal reflux disease, gastritis, peptic ulcer, duodenal ulcer, etc., are the common in today's life style. This may be due to stressful life style or improper balance diet. The pathology behind these disorders may be discrepancy between offensive and defensive mechanisms either by excess secretion of acid and pepsin or diminished ability of the gastro-duodenal mucosal barrier to protect against stomach acid-pepsin secretion. Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of the most commonly used medicines and proven to be effective for certain disorders. Some people use NSAIDs on daily basis for preventive purpose. But a variety of severe side effects can be induced by NSAIDs. Studies have shown that edible natural ingredients exhibit preventive benefit of gastric ulcer. Therefore present study was designed to evaluate antiulcer activity of Ethanolic extract of Carica papaya leaves in rats. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics, flavonoids and protein were determined by the well-known test protocol available in the literature. The in vivo anti-ulcer activity of ethanolic extract was assessed against Ethanol induced and Aspirin induced modified pylorus ligated model. Leaves ethanolic extract of Carica papaya exhibited significant protection against Aspirin + Pyloric Ligation Induced ulcer at dose levels of 100-mg/kg and 200-mg/kg, respectively. However, at all these doses, the Leaves ethanolic extract of Carica papaya 100 and 200mg/kg was less effective comparing to that of standard drug used Omeprazole. Therefore, this study validates its anti-ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

Keywords: *Carica papaya, Phytochemical constituents, Antiulcer, Non-steroidal anti-inflammatory drugs, Ethanol induced and Aspirin induced modified pylorus ligated model.*

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INTRODUCTION:

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality. Epidemiological data for this disease and its complications have shown striking geographical variations in incidence and prevalence. Development of ulcer disease and death from it has been associated with the birth of urbanisation and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century. [1-2]

Our understanding of the disease changed greatly with the discovery of Campylobacter pyloridis (renamed *Helicobacter pylori* in 1989 because of a revised taxonomic classifi cation) in 1982 by Warren and Marshall. [3-4]

This discovery switched the notion from an acid-driven disease to an infectious disease, opening a huge area for intensive research that resulted in the reconciliation of previously suggested mechanisms of pathogenesis. The fall of the acid dogma in peptic ulcer disease, which had found its undisputed acceptance during and after the introduction of histamine H2-receptor antagonists, led to the present therapeutic principle. Maintenance acid suppressive therapy for duodenal ulcer, which followed decades of dominance of surgical interventions (subtotal gastric resections, several forms of vagotomy), was replaced with a short-term antibiotic regimen targeting eradication of *H. pylori* infection. [5-6]

The predominant symptom of uncomplicated peptic ulcer is epigastric pain, which can be accompanied by other dyspeptic symptoms such as fullness, bloating, early satiety, and nausea. In patients with duodenal ulcer, epigastric pain occurs typically during the fasting state or even during the night and is usually relieved by food intake or acid-neutralising agents. Roughly a third of these patients also have heartburn, mostly without erosive oesophagitis. Chronic ulcers can be asymptomatic. [7]

In particular, this absence of symptoms is seen in NSAID-induced ulcers, for which upper gastrointestinal bleeding or perforation might be the fi rst clinical manifestation of disease. The most frequent and severe complication of peptic ulcers is bleeding, which is reported in 50–170 per 100 000, with the highest risk in people aged older than 60 years. Perforation is less frequent than is bleeding, with an incidence of around seven to ten per 100 000. Penetration of retroperitoneal organs is characterised by constant severe pain but fortunately is rare.15 Gastric outlet obstruction due to ulcer-induced fibrosis is also rare, and should raise suspicion of underlying malignant disease. [8]

We have selected this topic as most of the synthetic non-steroidal anti -inflammatory drugs produces ulcer as main side effect. Many drugs obtained from the natural source papaya significant role in health care system.

The main reason is the crude from has lesser toxicity or without toxicity. The target is to reduce the side effects as minimum as possible and to discover a new drug from plant kingdom which may provide required pharmacological action and would be free from undesirable effect as well as cheap which would accept by the humans. The study also aims at finding out the phytochemical constituents present in the extract of the plant. The *Carica papaya* L family have shown significant ulcerprotective property. So we have choosen one of its easily available species *Carica Papaya*.

MATERIAL AND METHODS:

Plant material:

Leaves of *Carica papaya*was collected from local area of Bhopal (M.P.) in the month of January, 2022. Following the procurement of plant products, they were thoroughly washed. The cleaning procedure was broken down into the following stages. The decayed or deteriorated plant matter was removed first.

Chemicals and reagents:

All the drugs, solvents and chemicals used in the study were of analytical grade. Omeprazoleand ranitidine was purchased from medical store, Bhopal, MP, India. All other chemicals e.g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India.

Extraction of plant material:

60.7 gram dried powdered of leaves of *Carica papaya*has been extracted with ethanol solvent using maceration for 48 hrs, filtered and dried using vaccum evaporator at 400C[9].

Determination of percentage yield:
The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening:
Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

7. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8.Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and amino acids

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

11. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [10-11].

Quantitative estimation of bioactive constituents:

Estimation of Total Phenolic content:

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [12]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol**.** 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of Phenol. 1 ml of extract or standard was mixed with 5 ml of Folin-

Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content:
Determination of total flavonoids content was based on aluminium chloride method [13]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Pharmacological evaluation of Ethanolic extract of *Carica papaya* leaves for anti-ulcer activity:
Acute toxicity study:
14 Days single dose oral acute toxicity and gross behavioral study [14-15]
Procedure:
In the acute toxicity study, using up and down procedure, Ethanolic leaves extract of *carica papaya* in 0.1% (w/v) aqueous suspension of sodium carboxy methyl cellulose (CMC) was administered orally to female Sprague Dawley rats weighing 160-190 g.

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals for next 14 days). Four sets of healthy female rats (each set of 3 rats) were used for the experiment.

First set of animals were fasted for 18 h deprived from food, water withdrawn before 4 hour of the dosing, body weights were noted before and after dosing. Three rats were dosed at 400 mg/kg and if no mortality or overt toxicity occurred within 48 h another set of three rats were dosed at 800 mg/kg. In the absence of mortality a third set of animals were dosed at 2000 mg/kg and if no evidence of toxicity was observed three additional rats were dosed at this level to nullify the errors. Dosing volumes were fixed at 10 ml/kg.

A control group of 3 rats received only the vehicle. Individually animals were observed for 4 h to see any clinical symptoms, any change in behaviour or mortality. 6 h post dosing again body weights were recorded. From the next day onwards, each day every 1 hour the behavioural change, clinical symptoms or mortality was observed in 49 the same animals for next 14 days and animal body weights were recorded on 8th and 14th day post dosing. The studies were conducted in compliance with OECD Test guidelines 425.

Selection and preparation of dose for pharmacological screening:
The Ethanolic leaves extract of *carica papaya* was suspended in 0.1% CMC solution to prepare two dose levels, 100 and 200 mg/kg b.w. of the animals for acute studies and three dose levels of 100, 200 and 400 mg/kg b.w. for chronic models.

Evaluation of anti ulcer activity in rats:
Ethanol Induced Ulcers in Rats:
Ethanol induced ulcer model [16], in rats was studied for Ethanolic leaves extract of *carica papaya* to determine the ulcer index and ulcer inhibition. Albino rats weighing between 160 - 180 g were divided into 4 groups consisting of six animals each. Experimental design and dosing schedule was as follows.
Group I: Ulcer control - 1ml ethanol/200 g, p.o.
Groups II: Ethanolic leaves extract of *carica papaya* 100 mg/kg b.w, p.o for seven days.
Groups III: Ethanolic leaves extract of *carica papaya* 200 mg/kg b.w, p.o for seven days.
Group IV: Omeprazole, orally at 2 mg/kg b.w.p.o. for seven days.
On the final day of dosing, the animals also received extractives and the standard drug thirty minutes before administration of 1ml of ethanol. Animals were sacrificed after one hour and the contents of the gastric juice in the stomach were aspirated. Later the stomachs were removed and kept immersed in saline for 5 min. Incisions of the stomach were performed along the greater curvature and linear haemorrhagic lesions in the glandular regions were observed. A pair of dividers was used to measure the length of all the lesions in the stomachs. The length (mm) of each lesion was determined at 10 x magnification and summed up per stomach. Ulcer index was the sum of length of all lesions for each stomach. (UI) Stomachs were immersed in 10% formalin for 24 h to study the histopathological changes in treated and ulcerated rats. Photographs of the opened stomachs were taken. The percentage ulcer inhibition was calculated by the following formula and the results were tabulated.

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer index in Test}}{\text{Ulcer Index in Control}}$$

Selection of Extractives:
Based upon the results of ethanol induced ulcer model the Ethanolic leaves extract of *carica papaya* was selected for further anti ulcer evaluation studies using Aspirin + pyloric ligation induced gastric ulceration in rats.

Aspirin Induced Modified Pylorus Ligated Model:
The Ethanolic leaves extract of *carica papaya* were subjected to anti ulcer studies using Aspirin induced modified pylorus ligated model. Adult Wistar albino rats of either sex weighing 180-250 g were fasted for 48 h with free access to water and divided into Four groups of six animals each. They were placed in cages with grating floor to avoid coprophagy. The experimental design and dosing schedule was carried out as follows.

Group 1: Ulcer control (Solvent) (10 ml/kg) + Aspirin (200 mg/kg)
Group 2: Ranitidine (50 mg/kg)
Group 3: Ethanolic leaves extract of *carica papaya* (100 mg/kg)
Group 4: Ethanolic leaves extract of *carica papaya* (100 mg/kg)

In aspirin plus pyloric ligation induced ulcer model, one hour before pyloric ligation, aspirin at a dose of 200 mg/kg was administered orally as a suspension in 0.1% CMC. The animals were orally treated with the Ethanolic leaves extract of *carica papaya*at doses of 100 and 200 mg/kg once daily for seven days and 1 hour before administration of aspirin. The standard group of animals was also treated in the same way.

Pyloric ligations were performed under ether anaesthesia taking care to avoid damage to the pylorus and the blood vessels. After ligation the stomachs were replaced and abdominal wall sutured. Food and water was restricted during the post-operative period of four h. The animals were sacrificed at the end of four hours using excess ether anaesthesia. Thereafter the stomachs were opened and the contents of the gastric juice were collected. The contents were centrifuged and various biochemical estimations were carried out in the collected samples of control and treated groups of animals. The stomach samples were soaked in saline for five minutes and fixed to boards for morphological examinations of ulcer indices. Photographs were taken for further reference.

Evaluation of Ulcer Index and Inhibition:
The ulcer index was calculated by counting the lesions with the aid of hand lens (10 X) and graded as follows.
0 = Normal coloured stomach
0.5 = Red colouration
1 = Spot ulcer
1.5 = Haemorrhagic streaks
2.0 = ulcers > 3 but < 5
3.0 = ulcers > 5
Mean ulcer score for each animal was expressed as ulcer index. Ulcer protection was calculated according to the standard formula [17]:

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer index in Test}}{\text{Ulcer Index in Control}}$$

The volume and pH of the collected gastric juice was recorded. Free acidity and total acidity was calculated. Various bio-chemical estimations like total proteins, total hexoses, hexosamine, fucose, sialic acid, total carbohydrate and carbohydrate/protein ratio of the gastric juice were performed using standard methods.

Bio-Chemical:
Estimations in Gastric Juice:
The various biochemical parameters like carbohydrate content viz. fucose, hexosamine, total hexoses, sialic acid and total carbohydrates were estimated. Gastric volume, pH, free and total acidity and total proteins were also evaluated.
Gastric volume:
The gastric juice was centrifuged, allowed to decant, and taken into a glass syringe of graduation 0.01 ml. The volume of gastric juice was measured.

Determination of pH:
Using the pH meter the pH of the gastric juice was measured.
Determination of free acidity and total acidity.
0.01 N NaOH was standardised using oxalic acid as the primary standard. Free acidity was estimated by titrating 1 ml of gastric juice with 0.01 N Sodium hydroxide using topfer's reagent as indicator until the red colour changes to yellowish orange. The volume of sodium hydroxide consumed was noted which corresponded to free acidity.

Titration with 0.01N NaOH was continued using phenolphthalein as indicator until the yellowish orange colour changed to red. The amount of NaOH consumed was noted and corresponded to total acidity.
Acidity was calculated by using the following formula, and expressed as mEq/l/100g.

$$\text{Acidity} = \frac{\text{Volume of NaOH x Actual Normality of NaOH}}{0.1} x100$$

Estimation of Total Proteins:
An alcoholic precipitate was prepared by adding 9.0 ml of 90% alcohol to 1 ml of gastric juice. To 0.1 ml of this protein mixture, 1 ml of 0.1N NaOH was added. 0.4 ml phenol reagent was added to 0.05 ml of the reaction mixture and was kept for ten minutes to complete the reaction. Absorbance was measured at 610 nm against blank in a spectrophotometer. The amount of protein present in the gastric juice was calculated from standard curve prepared with bovine albumin and was expressed in term of µg/ml of gastric juice.

Estimation of Total Carbohydrates:
An alcoholic precipitate containing the dissolved mucosubstances was prepared by adding 9 ml of 90% alcohol to 1 ml of gastric juice. The mixture was allowed to decant and the supernatant layer was discarded. The precipitate containing the mucosubstances was dissolved in 0.5 ml of 0.1N sodium hydroxide and 1.8 ml of 6N HCl was added. This mixture was boiled on a water bath, neutralised and diluted with distilled water to get a final volume of 4.5 ml. This solution was used for the estimation of carbohydrates like total hexoses, hexosamine, sialic acid and fucose as follows.

Estimation of Fucose:

Reagents:
Cysteine reagent: 600 mg of cysteine hydrochloride was dissolved in 20 ml of distilled water. It was stored in refrigerator until use. D (+) Fucose: Standard

Procedure:
The blank and the sample tubes containing 0.4 ml of distilled water and 0.4 ml of hydrolysate was mixed carefully in a water bath with 1.8 ml sulphuric acid mixture. The reaction was allowed to take place for 3 minutes by heating on a water bath. After cooling the tubes 0.1 ml of cysteine reagent was added to the blank and to one of the tubes containing the hydrolysate (unknown) while cysteine reagent was not added to the test-tube containing the hydrolysate (unknown blank). The reaction was allowed to continue for 90 min. The absorbance was read spectrophotometrically at 396 and 430 nm using distilled water as blank.

The optical density for the fucose in the hydrolysate was calculated. The readings, which were taken at 396 and 430 nm, were noted and the difference calculated. Then the values without cysteine were subtracted from this and evaluated. Standard curve was prepared with D (+) – fucose.

$$\text{True optical density} = \frac{(\text{OD396} - \text{OD430})\text{unknown} - (\text{OD396} - \text{OD430})\text{UnknownBlank}}{(\text{OD396} - \text{OD430})\text{WaterBlank}}$$

The fucose expressed content was in µg/ml of gastric juice.

Estimation of Hexosamine:
Reagents:
Acetyl acetone reagent: 0.3 ml acetyl acetone and 9.7 ml of 1.5N sodium carbonate (anhydrous) were mixed just before use.
Ethanol: It was dehydrated.
Ehrlich's reagent: It was prepared by mixing 1.6 g of p-dimethyl amino benzaldehyde in 30 ml of 90 % ethanol and then adding 30 ml of Conc. HCl. The reagent was then stored in a refrigerator. D (+) Glucosamine hydrochloride was used as standard.

Procedure:
0.5 ml of acetyl-acetone reagent was added to 0.5 ml of the hydrolysate fraction. The reaction was allowed to take place by heating for 20 min in a boiling water bath. The mixture was allowed 58 to cool and 1.5 ml of 90% alcohol and 0.5 ml of Ehrlich's reagent was added. After 30 min the intensity of colour development was measured spectrophotometrically at 530 nm against blank. The amount of hexosamine present in the sample was estimated from the standard curve prepared by using D (+) glucosamine hydrochloride and concentration was expressed in µg/ml of gastric juice.

Estimation of Total Hexoses:
Reagents:
Orcinol reagent: 1.6 g of orcinol was dissolved in 100 ml of distilled water. This was prepared fresh before use. Sulphuric acid: 150 ml of conc. sulphuric acid was mixed with 100 ml of distilled water.
D (+) Galactose- Mannose- Standard

Orcinol- Sulphuric acid reagent: One volume of orcinol was mixed with 7.5 ml of sulphuric acid. This was prepared fresh before use.

Procedure:
The reaction mixture containing 0.4 ml of hydrolysate and 3.4 ml of orcinol reagent was heated for 15 min. in the boiling water bath. The tubes were cooled to room temperature and the colour developed was measured at 540 nm against the blank. The amount of hexoses present in the sample was determined from the standard curve of D (+) galactose - mannose and has been expressed in µg/ml of gastric juice.

Estimation of Sialic Acid:
Reagents:
Sodium arsenite: 0.5 M sodium sulphate was prepared in 0.1N sulphuric acid and to 100 ml of this solution 10 g of sodium arsenite was added and stored in glass stoppered bottle.

Thiobarbituric acid: 0.5 M sodium sulphate was prepared and to 100 ml of this solution 600 mg of thiobarbituric acid was added and stored.
Sialic acid- Standard

Procedure:
A reaction mixture containing 0.5 ml of the hydrolysate in 0.1N H2SO4 and 0.2 ml of sodium periodate was mixed and allowed to stand for 20 min. 1 ml of sodium arsenite solution was added and mixed by shaking. 3 ml of thiobarbituric acid was added and the mixture was heated on a boiling water bath for 15 min. After cooling, 4.5 ml of cyclohexanone was added, thoroughly shaken and centrifuged. The pink colour formed in the supernatant layer was pipetted out and intensity of colour was measured spectrophotometrically at 550 nm. A standard curve was prepared using sialic acid and the amount of sialic acid present in the sample was determined expressed in µg/ml of gastric juice.

RESULTS AND DISCUSSION:

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, phenol, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml.

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The total Flavonoids content (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.040X + 0.012$, $R^2= 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. Total protein content was calculated as Bovine serum albumin equivalent mg/100mg using the equation based on the calibration curve: $Y=0.001X+ 0.001$, $R^2=0.999$, where X is the BSA equivalent (BE) and Y is the absorbance table 2. At 100 mg/kg b.w. the Ethanolic leaves extract of *Carica papaya* showed a protection index of 46.21%, and at 200 mg/kg b.w. the extractives showed protection index of 49 %. The results were comparable to Omeprazole (2 mg/kg) which reduced the ulcer index 78% significantly.

The anti ulcer activity of ethanolic leaves extract of *Carica papaya* was studied at doses of 100 and 200 mg/kg in aspirin induced ulcerogenesis in modified pylorus ligated rat model. The results indicated that ethanolic leaves extract of *Carica papaya* at dose levels of 100 mg/kg and 200 mg/kg significantly decreased the ulcer index ($p<0.01$) which was evident by a significant increase in percentage ulcer protection of 54.88 % & 62.36 % respectively. Ranitidine at 50 mg/kg reduced the ulcer index with a percentage ulcer protection of 86.24%.

Gastric volume in the ethanolic leaves extract of *Carica papaya* treated groups indicated that there was no significant decrease in the volume of the gastric juice at 100 mg/kg (1.78±0.24), but at 200 mg/kg (1.56±0.42) there was a significant decrease in comparison to the control group ($p<0.05$). Ranitidine at 50 mg/kg (1.24±0.07) caused a significant decrease in gastric volume.

The ethanolic leaves extract of *Carica papaya* at 100 mg/kg and 200 mg/kg significantly increased the pH 3.02±0.22 and 3.66±0.44 respectively of gastric juice ($p<0.01$) and was comparable to the standard drug Ranitidine at 50 mg/kg was 5.32±2.55. Estimation of gastric juice of ethanolic leaves extract of *Carica papaya* treated groups indicated that there was a significant decrease in the free acidity and total acidity of the gastric juice. Rats treated with 100 mg/kg and 200 mg/kg of ethanolic leaves extract of *Carica papaya* showed a significant decrease in free acidity and total acidity ($p<0.01$) was (43.32±0.22 & 39.42±2.66) and (58.32±3.56 & 42.42±2.46) was comparable to that of the Ranitidine (50 mg/kg) treated group ($p<0.01$) of rats was 23.13±3.5 and 31.93±3.5 respectively.

The results indicated that the total protein content was significantly decreased in the group treated with Ethanolic leaves extract of *Carica papaya* when compared to the control. A dose of of 100 and 200 mg/kg showed significant decrease in the total protein content ($p<0.01$) 416.03±1.61 and 314.05±1.53 respectively compared to the control group ie 248.50±1.11. Total carbohydrates content (hexose, hexosamine, fucose and sialic acid) and C/P ratio of the Ethanolic leaves extract of *Carica papaya* treated group indicated that there was a significant increase in the total carbohydrate content ($p<0.01$) and C/P ratio and was comparable to the Ranitidine treated group of rats.

Table 1: Phytochemical Screening of *Carica papaya* extract

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Dragendroff's test Wagner's test Mayer's test Hager's test	 -ve -ve -ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenolics** $fecl_3$	 +ve
5.	**Amino acids** Ninhydrin test	 -ve
6.	**Carbohydrates** Molisch's test	 -ve
7.	**Reducing Sugar** Fehling's test	 +ve
8.	**Proteins** Ethanol test	 +ve
9.	**Saponins** Foam test	 +ve

Table 2: Total bioactive constituents content of *Carica papaya*

S. No.	Extract	Total phenol (mg/100mg)	Total Flavonoid (mg/100mg)	Total protein (mg/100mg)
1.	Ethanolic extract	0.435	0.765	0.633

Table 3: Effect of the Ethanolic leaves extract of *Carica papaya* on Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg b.w)	Ulcer Index	% Ulcer Inhibition
Control	10 ml/kg	29 ± 0.46	--
Ethanolic extract of *carica papaya*	100	17.8 ± 0.64**	46.21
Ethanolic extract of *carica papaya*	200	16.5 ± 0.64**	49
Omeprazole	2	8.3 ± 0.5**	78

Data are expressed as mean SEM., n=6 in each group. *p<0.05, **p<0.01 - One way ANOVA followed by Dunnett's test

Table 4: Effect of ethanolic leaves extract of *Carica papaya* on Ulcer Inhibition in Aspirin + Pyloric Ligation Induced Gastric Ulceration in Rats

Treatment	Dose (mg/kg b.w)	Ulcer Index	% Ulcer Inhibition
Control	10 ml/kg	7.20 ± 0.29	---
Ethanolic extract of *carica papaya*	100	3.77 ± 0.25**	54.88
Ethanolic extract of *carica papaya*	200	3.4 ± 0.54**	62.36
Ranitidine	50	0.985 ± 0.3**	86.24

Values are mean ±S.E.M, n=6, *p < 0.05 and **p < 0.01 Vs control- One way ANOVA followed by Dunnett's test

Table 5: Effect of Ethanolic leaves extract of *Carica papaya* on Antisecretory Parameters

Treatment	Dose (mg/kg b.w)	Gastric Volume (ml/100g)	pH	Free Acidity (mEq/l/ 100g)	Total Acidity (mEq/l/ 100g)
Control	10 ml/kg	3.65±0.31	1.93±0.12	63.01±3.1	80.84±3.8
Carica papaya extract	100	1.78±0.24 NS	3.02±0.22**	43.32±0.22**	58.32±3.56**
Carica papaya extract	200	1.56±0.42*	3.66±0.44**	39.42±2.66**	42.42±2.46**
Ranitidine	50	1.24±0.07**	5.32±2.55**	23.13±3.5**	31.93±3.5**

Values are mean ± S.E.M, n=6, NS-not significant, *p < 0.05 and **p < 0.01 Vs control, One way ANOVA followed by Dunnett's test

Table 6: Effect of Ethanolic leaves extract of *Carica papaya* on Total Proteins and C/P

Treatment	Dose (mg/kg)	Total proteins (µg/ml)	C/P
Control	10ml/kg	468.64±0.86	0.84
Carica papaya extract	100	416.03±1.61**	1.6
Carica papaya extract	200	314.05±1.53**	2.20
Ranitidine	50	248.50±1.11**	2.45

Values are expressed in terms of mean ± S.E.M, **p<0.01- One way ANOVA followed by Dunnett's test

Table 7: Effect of Ethanolic leaves extract of *Carica papaya* on Total Carbohydrates

Treatment & Dose (mg/kg)	Total Carbohydrates (µg/ml)			
	Total Hexose	Hexosamine	Fucose	Sialic acid
Control	151.33±0.63	170.42±0.48	70.42±0.22	19.22±0.22
Carica papaya extract (100)	279.42±1.84**	230.80±1.24**	76.42±1.86**	37.42±1.46**
Carica papaya extract (200)	308.14±1.02**	259.22±0.82**	77.22±1.42**	31.64±1.40**
Ranitidine (50)	476.31±0.22**	420.20±0.32**	158.24±0.24**	42.24±0.24**

Values are expressed in terms of mean ± S.E.M, **p<0.01 Vs control- One way ANOVA followed by Dunnett's test

CONCLUSION:

Leaves ethanolic extract of *Carica papaya* exhibited significant protection against Aspirin + Pyloric Ligation Induced ulcer at dose levels of 100-mg/kg and 200-mg/kg, respectively. However, at all these doses, the Leaves ethanolic extract of *Carica papaya* 100 and 200mg/kg was less effective comparing to that of standard drug used Omeprazole. In this model, leaves ethanolic extract of *Carica papaya* in all dose levels showed a significant protection against ethanol induced gastric ulceration.

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