Evaluation of Antiulcer Activity of Ethanolic Extract of *Garcinia Gummi - Gutta* Fruit

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Abstract
Gastric ulcer is a prevalent gastrointestinal disorder caused by excessive gastric acid secretion, impaired mucosal defense and Helicobacter pylori infection. Existing pharmacological therapies such as proton pump inhibitors and H_2-receptor antagonists, face challenges like resistance and adverse effects. Given the increasing need for safer and more effective antiulcer agents, investigating plant-derived compounds offers a promising alternative. The potential of plant extract in modulating gastric acid secretion and enhancing mucosal defense mechanisms warrants systematic evaluation. Garcinia gummi-gutta, a tropical fruit known for its medicinal properties, has shown potential antiulcer activity. This study evaluates the ethanolic extract of Garcinia gummi-gutta (EEG) fruit for its gastroprotective effects.

The study involved the EEG effect on enzyme inhibition assay evaluated by H^+/K^+-ATPase activity. Various assessments including gastric pH, total acidity, gastric wall mucus levels, histamine content in the gastric mucosa and gastric volume, were experimented on ulcer-induced animal models across nine groups. The EEG extract demonstrated potent enzyme inhibition, with inhibition rates increasing from 54.41% at 10 µg/mL to 90.89% at 500 µg/mL, confirming its efficacy in reducing gastric acid secretion. Animal studies revealed dose-dependent ulcer protection, with 500 µg/mL of EEG providing appreciable ulcer prevention supporting histopathological studies. Lower doses (10-250 µg/mL) showed moderate effects. The findings suggested that EEG extract is a promising natural alternative for ulcer management, warranting further clinical investigations to explore its therapeutic potential.

Keywords: *Garcinia gummi-gutta* (Malabar tamarind), Phytochemical evaluation, H^+/K^+-ATPase inhibition, *In vitro enzymatic* Gastric acid secretion inhibition.

Introduction

Peptic ulcer disease (PUD) is a common gastrointestinal disorder impacting millions globally. It is marked by the presence of ulcers or lesions in the stomach lining or the initial part of the small intestine (duodenum), which can result in significant health complications if left untreated. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) and infection with Helicobacter pylori are recognized as primary causes of peptic ulcer formation.[31] Current therapeutic strategies for peptic ulcers primarily focus on reducing gastric acid secretion and eradicating *H. pylori*. H_2 receptor antagonists and proton pump inhibitors (PPIs) play a key role in acid suppression therapy. However, prolonged use of PPIs has been linked to several negative outcomes such as a higher risk of fractures, kidney disease and deficiencies in vitamins and minerals.

The effectiveness of traditional treatments is seriously threatened by the rise of *H. pylori* strains that are resistant to antibiotics. Natural products have garnered considerable attention as potential sources of new therapeutic agents for various diseases including PUD. *Garcinia gummi-gutta* (GGG), commonly known as Malabar tamarind or brindleberry, is one such natural product that has shown promise in various pharmacological studies. This tropical fruit, originating from Southeast Asia, contains bioactive compounds like hydroxy citric acid (HCA), flavonoids and polyphenols, exhibited various biological effects including anti-inflammatory, antioxidant and antimicrobial activities. [29] Previous studies have indicated that various plant extracts can inhibit H+/K+ ATPase activity, leading to reduced gastric acid secretion and offering gastroprotective effects.

However, the specific effects of Garcinia gummi-gutta on this enzyme have not been extensively studied. The H+/K+ ATPase inhibition assay is a well-established *in vitro* method for assessing the antiulcer potential of various compounds and extracts. This assay allows for the direct measurement of the inhibitory effect on the proton pump, providing valuable insights into the mechanism of action of the tested extract. This enzyme, also known as the gastric proton pump, plays a key role in the final phase of gastric acid secretion in the stomach's parietal cells. Inhibiting the H+/K+ ATPase enzyme is a recognized therapeutic strategy to decrease gastric acid production and treat peptic ulcer disease.[25,34] Gastric pH analysis, total acidity measurement, gastric mucus determination and histamine level assessment are fundamental in evaluating the anti-ulcer properties of medicinal agents.

Gastric pH reflects acid secretion balance, while total acidity quantifies the secretory capacity. Mucus, a glycoprotein barrier, protects the epithelium from acid-induced injury. Histamine, acting through H_2 receptors, stimulates acid

secretion, making its regulation critical in ulcer prevention. Several early studies have demonstrated the efficacy of medicinal plants in ulcer therapy. *Glycyrrhiza glabra* enhances gastric mucus secretion and inhibits acid output[32]. *Zingiber officinale* reduces gastric acidity and promotes mucosal healing[13]. *Aloe vera* accelerates ulcer healing by enhancing mucus production[28]. *Ocimum sanctum* and *Cissus quadrangularis* exhibit antioxidant and anti-inflammatory effects in ulcer prevention[13]. Despite these promising findings, gaps remain in research, particularly in understanding long-term efficacy, molecular mechanisms and comparative effectiveness against standard drugs.

The need of the hour is to explore novel plant-based anti-ulcer therapies with enhanced safety and efficacy. *Garcinia gummi-gutta*, known for its hydroxycitric acid (HCA) content, has demonstrated antioxidant, anti-inflammatory and lipid-lowering effects, but its role in gastric protection remains underexplored. Investigating its impact on gastric pH, acidity, mucus secretion and histamine modulation could provide valuable insights into its gastroprotective potential. Filling this research gap will contribute to the development of safer, plant-based alternatives to conventional ulcer treatments, addressing drug resistance and adverse effects.

The aim of this study is to evaluate the anti-ulcer potential of *Garcinia gummi-gutta* by assessing its biochemical, physiological and histopathological effects. To achieve this, we conducted a preliminary phytochemical screening to identify bioactive compounds followed by the quantification of total phenolics and tannins to evaluate their antioxidant and gastroprotective properties. The inhibition of H^+/K^+-ATPase activity was analyzed to determine its role in acid secretion modulation, while gastric pH and total acidity assessments provided insights into acid-base balance. The protective mechanisms of *Garcinia gummi-gutta* were further examined by measuring gastric wall mucus content and histamine levels in the gastric mucosa.

Gastric volume analysis was performed to assess secretory functions and histopathological studies were conducted to evaluate structural and cellular changes in gastric tissues. Through this comprehensive approach, our study aims to establish *Garcinia gummi-gutta* as a potential natural therapeutic agent for ulcer management.

Material and Methods

Instrumentation, Chemicals and Reagents: The High-Performance Thin-Layer Chromatography (HPTLC) equipment employed in this investigation included a CAMAG TLC Scanner 3 and a CAMAG Linomat V sample applicator. The stationary phase for the chromatographic separation was pre-coated silica gel 60 F254 plates (Merck, Germany) and the mobile phase was adjusted based on the chemicals found in the Garcinia gummi-gutta extract. To detect and quantify active components, the chromatograms were examined with a CAMAG TLC Scanner at UV 254 nm and UV 366 nm wavelengths. The extraction of GGG fruits was carried out using a Soxhlet equipment (Labotec, USA) with a heating mantle to maintain the appropriate temperature. The activity of H+/K+-ATPase was assessed using a commercially available assay kit (Sigma-Aldrich, USA), which measures the enzyme's ATP hydrolysis ability.

Absorbance at 540 nm was recorded with a microplate reader (BioTek Instruments, USA) to quantify the enzyme activity in the gastric mucosal homogenates of the experimental ovine. All chemicals and reagents utilized in this study were of analytical grade and sourced from reputable suppliers including Sigma-Aldrich (USA) and Merck (Germany). The solvents utilized for extraction, such as ethanol, chloroform and butanol, were of HPLC grade, whereas the standard anti-ulcer medicines (ranitidine and omeprazole) were bought from local pharmaceutical providers. Filtration during the extraction process was accomplished using Whatmann no. 1 filter paper.

Selection, Collection and Authentication of Plant Material: The dried fruits of Garcinia gummi-gutta were available in the Ayurvedic product stores of Tamil Nadu. The dried fruits were identified and authenticated by botanist Dr. A. Balasubramaniam and the voucher specimen (AUT/VMCP/272/2024) was deposited in the Department of Pharmacology, Vinayaka Missions College of Pharmacy, VMRF (DU0, Salem (TN, India). The authenticated fruit was used to prepare the extracts.

Extraction of the Plant Material: The GGG fruits were initially cleaned to remove dirt and soil and then thoroughly washed with distilled water. After cleaning, the fruits were shade-dried for four weeks to ensure proper moisture removal. Once dried, the fruits were ground into a coarse powder using a mechanical grinder and stored in an airtight container to prevent contamination and to preserve their chemical integrity. For the extraction process, 10 g of powdered GGG was carefully weighed and placed into the thimble of a Soxhlet extractor. Successive solvent extraction was performed using petroleum ether and ethanol, each for 72 hours. Aqueous ethanol solutions at varying concentrations of 20%, 40%, 60% and 80% (v/v) were prepared, with the solvent volumes measured into 250-mL conical flasks, following the feed-to-solvent ratios of 1:10, 1:15, 1:20 and 1:25 (g/mL).

For optimal solvent penetration, the extraction procedure involved refluxing the mixture in the Soxhlet extractor under controlled temperature conditions. After completion, the solutions were allowed to cool to ambient temperature and then filtered through Whatmann No. 1 filter paper to eliminate any remaining plant debris.

The filtrates were subsequently concentrated to dryness under reduced pressure with a rotary evaporator, ensuring the complete removal of the solvent, resulting in the crude petroleum ether and ethanolic extracts. The extractive value

and percentage yield were then calculated based on the weight of the obtained residues.

Preliminary Phytochemical Screening: The pharmacological action and therapeutic potential of any extract obtained from the natural source depend on the nature of its constituents. Thus, it is essential to screen each extract for their chemical constituents, based on which the pharmacological screening study could be assigned and performed. Each extract was subjected to the following preliminary phytochemical screening tests to evaluate the phytoconstituents present in each extract [12,15,16].

(1) Test for Alkaloids - To 1 gm of each extract powder separately, 5 ml of 2M hydrochloric acid was added and to 1 ml of the above reaction mixtures, 1 ml of specific reagent was added and observed for the following changes indicating the presence or absence of alkaloids.

- Dragendroff's reagent - formation of orange brown precipitate
- Hager's reagent - formation of yellow precipitate
- Mayer's reagent - formation of cream precipitate
- Wagner's reagent - formation of reddish brown precipitate

(2) Test for Amino acids (Proteins) - To 1 gm of each extract powder separately, 5 ml of distilled water was added and mixed well. To 1 ml of the above mixtures, 1 ml of specific reagent was added and was observed for the following changes indicating the presence or absence of amino acids and proteins.

- Biuret reagent - appearance of pink or purple colour
- Million's reagent - appearance of red colour
- Ninhydrin reagent - appearance of purple colour

(3) Test for Carbohydrates - To 2 gm of each extract powder separately, 10 ml of distilled water was added and mixed thoroughly, filtered and then concentrated. To 2 ml of the concentrate, 2-5 ml of specific reagent was added and was observed for the following changes indicating the presence or absence of carbohydrates.

- Anthrone reagent - appearance of green or blue colour
- Benedict's reagent - appearance of brick red colour precipitate
- Molisch's reagent - appearance of reddish violet ring
- Fehling's reagent - appearance of brick red colour precipitate

(4) Test for Fats and Fixed Oils - To 1 gm of each extract, 2 ml of 0.5N alcoholic potassium hydroxide solution was added and heated on a water bath for 30-60 min. Drops of phenolphthalein indicator were then added and mixed well. Formation of soap indicates the presence or absence of fats and fixed oils.

(5) Test for Flavonoids (Flavones, Flavanones and Anthocyanins) - To 2 mg of each extract powder, 5 ml of ethanol was added and mixed thoroughly. To the mixture, 2 ml of specific reagent was added and observed for the following changes indicating the presence or absence of flavonoids.

Shinoda's reagent - formation of reddish brown colour.
Sulphuric acid reagent - formation of yellow colour (Anthocyanins); formation of yellowish orange colour (Flavones) and formation of orange to crimson colour (Flavanones).

(6) Test for Glycosides - To 2 g of each extract powder, 10 ml of distilled water was added and mixed thoroughly, filtered and concentrated. To 2 ml of the concentrate, 2-5 ml of specific reagent was addded and observed for the following changes indicating the presence or absence of glycosides.

- Borntrager's reagent - appearance of pink colour
- Legal's reagent - appearance of pink colour

(7) Test for Mucilage and Gums - To 2 gm of each extract powder, 10 ml of absolute alcohol was added with stirring and filtered. The filtrate was air-dried and swelling properties were examined, indicating the presence or absence of mucilage and gums.

(8) Test for Saponins - To 1 gm of each extract powder, 20 ml of distilled water were added in a measuring cylinder and agitated for 15 minutes for the formation of honeycomb-like froth indicating the presence or absence of saponins.

(9) Test for Sterols - To 1 gm of each extract, few ml of acetic anhydride was added and mixed thoroughly and to 1 ml of the above reaction mixtures, 1 ml of specific reagent was added and observed for the following changes indicating the presence or absence of steroids and sterols.

- Liebermann-Burchard's reagent - formation of green precipitate

(10) Test for Tannins and Phenolic compounds - To 1 gm of each extract, 10 ml of distilled water was added and mixed thoroughly. To 1 ml of the above reaction mixtures, 1 ml of specific reagent was added and observed for the following changes indicating the presence or absence of tannins and phenolic compounds.

- Ferric chloride (5%) reagent - formation of violet precipitate
- Gelatin (1%) - NaCl (10%) reagent - formation of white precipitate
- Lead acetate (10%) reagent - formation of white precipitate

(11) Test for Terpenoids - To 1 g of each extract powder, 5 ml of chloroform was added and mixed thoroughly. To 1 ml of the above reaction mixtures, 1 ml of specific reagent was added indicating the presence or absence of steroids and sterols.

- Salkowski reagent - formation of red precipitate

Based on the preliminary phytochemical screening studies, the EEG showed the presence of secondary metabolite classes of phytochemicals such as flavonoids, phytosterols, proanthocyanidins, tannins, phenols (polyphenolics), terpenes etc. Hence, ethanolic extracts were selected for further pharmacological studies.

Quantification of total phenolic and tannins using HPTLC: In this study, a dried crude EEG was produced by Soxhlet extraction and then subjected to systematic fractionation utilizing the liquid-liquid partitioning technique. A precise amount of EEG (100 g) was dissolved in water and then partitioned with solvents of increasing polarity including chloroform, ethyl acetate and n-butanol. Each fraction was filtered and concentrated under reduced pressure to yield the chloroform fraction (GGCH: 6.03 g, w/w), ethyl acetate fraction (GGEA: 38 g, w/w), n-butanol fraction (GGBu: 45 g, w/w) and the residual aqueous fraction (GGAq: 10 g, w/w). The phenolic and tannin contents of each fraction were quantified using a UV-visible spectrophotometer and the Folin-Ciocalteu assay to accurately detect bioactive phytoconstituents.

Phytochemicals were analyzed qualitatively and quantitatively by High-Performance Thin-Layer Chromatography (HPTLC) on a CAMAG HPTLC system, equipped with an automated sample applicator (Linomat 5), a twin-trough developing chamber and a TLC scanner (CAMAG TLC Scanner 4), all controlled by VisionCATS software. Separation was performed on silica gel 60 F254 plates using an optimized mobile phase to ensure the effective resolution of active components. After development, the plates were derivatized with a suitable visualization reagent and scanned to quantify key bioactive compounds in the fractions.

Animals: Healthy ovine of both sexes, with a weight range of 15 to 20 kg, were used. All experimental procedures followed the ethical standards outlined in the NIH Guide for the care and use of Laboratory Animals. The ovine was individually housed in specialized metabolic pens designed to monitor their behavior and physiological parameters during the anti-ulcer trials. Environmental conditions were carefully regulated, with a temperature of 24 ± 2°C, humidity levels between 60% and 70% and a 12-hour light/dark cycle to maintain consistent experimental conditions. The animals were given unrestricted access to clean water and a nutritionally balanced commercial feed (Agricare Ltd., Kumasi, Ghana) for two weeks to help them to acclimatize to the new environment and experimental setup. Prior to the initiation of treatment, the ovine underwent a fasting period of 18 hours, during which they were deprived of both food and water to standardize the physiological state of the animals before the administration of the test treatments.

Ethical statement: The study was authorized by the Institutional Animal Ethical Committee in P.col/149/2024/IAEC/vmcp in accordance with ethical guidelines for animal research.

Dose selection: The gastroprotective effect of the EEG was assessed at various concentrations (10, 50, 100, 250, 500 and 750 µg/mL) in ovine. These oral doses were chosen based on prior research and the percentage yield of the EEG extract fractions (Table 1). The optimal doses for each EEG fraction were then determined. The evaluation of the gastroprotective properties of the bioactive fractions and reference standards was carried out in healthy ovine.

A total of 18 ovine were used, divided into six groups (n = 3 per group) as follows:

- **Group I:** Normal control ovine received distilled water as the vehicle.
- **Group II:** Ulcer-induced control (untreated) ovine received 0.5% carboxymethyl cellulose (CMC) dissolved in distilled water.
- **Group III and IV:** Positive control ovine received standard gastroprotective drugs ranitidine HCl (50 mg/kg, p.o.; Ranititine-50) and omeprazole (20 mg/kg, p.o.; OMZ-20), respectively.
- **Group V to IX:** Ovine received EEG extract at doses of 10, 50, 100, 250, 500 and 750 µg/mL respectively, administered orally.

All reference standards and test drugs were suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally by gavage for seven consecutive days. Before conducting gastric ulcer experiments, all animals underwent 18 hours of fasting, with free access to water *ad libitum*.

***In vitro* Pharmacological Screening for Anti-Ulcer Activity**[3,4,21,23,26]

(1) Gastric pH Analysis: The gastric pH was measured from the gastric mucus to evaluate the acid-neutralizing potential of the *EEG*. After the experimental period of extract incubation, the gastric mucus was carefully collected from the stomach lining of the ovine using a sterile spatula, ensuring minimal contamination. The collected mucus was then homogenized in an appropriate buffer solution to facilitate uniform distribution of the mucus for pH measurement.

The sample was subjected to centrifugation at 3000 rpm for 10 minutes at 4°C to isolate particulate matter from the mucus supernatant, which was then preserved for further analysis. A precise digital pH meter (Orion Star A211,

Thermo Fisher Scientific) was calibrated using standard buffer solutions with pH values of 4.0, 7.0 and 10.0 to ensure the accuracy and consistency of measurements.

Following calibration, the pH electrode was placed in the mucus supernatant and pH readings were taken in triplicate for each sample. The resulting pH values were evaluated and compared with those of the control group to assess the impact of the extract on the acidity of the gastric mucus. This parameter provided insight into the extract's ability to modulate the acid environment in the gastric mucosa, reflecting its potential gastro protective effects.

(2) Total acidity determination: For total acidity estimation, varying concentrations of *EEG* were administered to ovine prior to the excision of the stomach. The experimental groups were treated with different doses of the extract (10, 50, 100, 250 and 500 µg/mL) over 14 days, with a 24-hour fasting period on the 13th day to standardize gastric secretion.

On the 14th day, after euthanizing the ovine, their stomachs were carefully excised. The stomachs were immediately bathed with ice-cold normal saline to eliminate any remaining gastric secretion. The excised stomachs were spread out on a flat surface and the entire stomach surface was traced using a transparency to assess the areas of interest for later analysis.

50% aqueous solution of the gastric juice was collected from the excised stomach and transferred into an Erlenmeyer flask. To this solution, 2–3 drops of freshly prepared 1% phenolphthalein indicator were added. The gastric juice was titrated with 0.01 M NaOH from a burette until the appearance of a persistent pink coloration, signifying neutralization at pH 7. The volume of NaOH consumed was recorded as the titre value.

(3) Measurement of gastric wall mucus: The determination of gastric wall mucus was conducted on excised ovine stomachs that were pre-treated with various concentrations of EEG (10, 50, 100, 250 and 500 µg/mL) over 14 days. On day 13, the animals underwent a 24-hour fasting period to standardize gastric secretions. On day 14, after euthanasia, the stomachs were rinsed with ice-cold normal saline to eliminate any remaining gastric secretions.

The stomachs were then carefully opened along the greater curvature and mucus from the glandular area of the gastric mucosa was scraped off using a sterile spatula. The collected mucus was placed in pre-weighed sterile containers for the determination of its wet weight. To remove excess moisture, the mucus was dried in an oven at 60°C for 24 hours.

After drying, the final weight of the gastric mucus was recorded and the mucus content was expressed as the weight of dried mucus per 100 grams of gastric tissue. The mucus production in each experimental group was compared to the control group to assess the impact of different concentrations of EEG on gastric mucosal defense. An increase in mucus production suggested a potential protective effect, while a decrease indicated a possible harmful effect.

(4) Histamine levels in the gastric mucosa: The estimation of histamine content in the ovine stomach tissue was performed using the spectrofluorimetric method. Following the pre-treatment with varying concentrations of *Garcinia gummi-gutta* ethanolic extract (10, 50, 100, 250 and 500 µg/mL), the ovine stomachs were excised and mucosal scrapings were collected from the glandular portion of the gastric wall after a 4-hour post-treatment period.

The mucosal scrapings were homogenized in an ice-cold buffer and the homogenate was centrifuged at 3000 rpm for 10 minutes to obtain the supernatant. The histamine content in the supernatant was then determined using a spectrofluorimeter at an excitation wavelength of 360 nm and emission at 450 nm.

The concentration of histamine was quantified by comparing the fluorescence intensity with a standard histamine curve. The results were expressed as micrograms of histamine liberated per gram of gastric tissue. Histamine content was analyzed in relation to the varying concentrations of the extract and the data were compared with the control group to assess the potential effects of *EEG* on histamine release in the gastric mucosa.

(5) Gastric volume: Following pre-treatment with EEG (10, 50, 100, 250 and 500 µg/mL), ovine stomachs were excised following a 4-hour post-treatment period. The gastric juice was collected after properly washing the excised stomachs with normal saline to eliminate any remaining gastric contents. The gastric juice was then centrifuged at 3000 rpm for 10 minutes at 25 °C. The amount of gastric juice was measured and represented in milliliters per 100 grams of body weight. The pH of the stomach juice was measured with a calibrated pH meter and the results were recorded. The gastric juice supernatant was kept at 4°C until additional biochemical studies, such as acidity and mucus content.

(6) Histopathology Study: Formalin-fixed specimens of ovine gastric walls were preserved in 10% buffered formalin for 24 hours to facilitate histopathological evaluation for anti-ulcer activity. Subsequently, the tissue samples underwent standard histological processing including paraffin embedding and serial sectioning at a thickness of 3–5 µm using a microtome. The sections were then stained with hematoxylin and eosin and mounted with Canada balsam. Histopathological examination was conducted using a light microscope and representative micrographs of the lesions were captured using an Olympus photomicroscope. The analysis focused on identifying histopathological alterations including congestion, hemorrhage, necrosis, inflammatory cell infiltration, erosion and ulcer formation which were systematically documented and photographed.

H+/K+-ATPase activity

(a) Separation of stomach parietal cells: This study investigated the influence of *Garcinia gummi-gutta* ethanolic extract (EEG) on H^+/K^+-ATPase activity to elucidate its potential inhibitory role in gastric acid secretion. Freshly excised ovine stomachs were immediately immersed in ice-cold physiological saline to preserve tissue integrity. Under sterile conditions, the gastric mucosa was meticulously scraped to isolate parietal cells, which were subsequently suspended in ice-cold Tris-HCl buffer (pH 7.4) containing 250 mM sucrose to maintain osmotic stability and prevent cellular degradation. The suspension was homogenized and initially subjected to centrifugation at 5000 rpm for 10 minutes at 4°C, followed by further centrifugation at 20,000 rpm for 20 minutes to obtain a purified parietal cell fraction.

The isolated cells were immediately resuspended in an oxygenated buffer solution (95% O_2 and 5% CO_2) at 37°C to sustain metabolic activity and membrane integrity throughout the experimental process. For biochemical assays, the cell fraction was stored in an ice-cold homogenization buffer containing 250 mM sucrose to maintain osmotic balance, 20 mM Tris-HCl (pH 7.4) to ensure buffering capacity, 2 mM EDTA to inhibit metalloproteases, 1 mM PMSF (phenylmethylsulfonyl fluoride) to inhibit serine proteases and a protease inhibitor cocktail to prevent enzymatic degradation.

Mucosal scrapings were homogenized using a Potter-Elvehjem homogenizer at 4°C under a nitrogen atmosphere to minimize oxidative damage. The homogenate was then subjected to differential centrifugation, first at 5000 rpm for 10 minutes at 4°C to remove cellular debris and large organelles, followed by centrifugation at 12,000 rpm for 20 minutes at 4°C to pellet the enriched parietal cell fraction. The final parietal cell pellet was resuspended in Tris-HCl buffer and stored at -80°C for subsequent biochemical analysis.

(b) H+/K+-ATPase activity Enzyme inhibition assay: The parietal cells were isolated and categorized into six experimental groups, each pre-incubated with different concentrations EEG for 30 minutes: Group I (10 µg/mL), Group II (50 µg/mL), Group III (100 µg/mL), Group IV (250 µg/mL), Group V (500 µg/mL) and Group VI as the untreated control. The test mixture (1mL) contained 20 mM Tris-HCl (pH 7.4), 2 mM magnesium chloride ($MgCl_2$) and 2 mM potassium chloride (KCl), essential for optimal H+/K+-ATPase function. The mixture was incubated at 37°C with continuous oxygenation for 30 minutes to maintain cell viability. After incubation, 2 mM of adenosine-5'-triphosphate (ATP) with malachite green was introduced to stimulate ATP hydrolysis. The reaction was halted by adding 10% v/v ice-cold trichloroacetic acid, followed by rapid centrifugation at 5000 rpm for 5 minutes to remove cellular debris.

The inorganic phosphate released during ATP hydrolysis, reflecting H+/K+-ATPase activity was quantified spectrophotometrically at 640 nm. The degree of enzyme inhibition was assessed, providing insights into the potential gastroprotective effects of *Garcinia gummi-gutta* through its interaction with the proton pump in gastric parietal cells.

$$\text{Inhibition of Phosphate release (\%)} = \frac{\text{Phosphate levels in Control - Phosphate levels in Test}}{\text{Phosphate levels in Control}} \times 100$$

Statistical analysis: The data are expressed as mean ± S.E.M. (n=6) for the quantification of phytoconstituents and gastric ulcer studies. One-way Anova was used to assess variance, with Tukey's post hoc test applied for comparisons between multiple groups. Statistical significance was considered at a p-value of <0.05. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA).

Results and Discussion

The desiccated fruits of *GGG* were meticulously gathered and subjected to a comprehensive solvent extraction procedure employing a range of solvents including petroleum ether and ethanol (99% v/v). The resultant extract was subsequently utilized for advanced analytical investigations. The extraction yield was quantitatively determined, expressed as a percentage and systematically documented in table 1.

Phytochemical screening: Phytochemical screening constitutes a systematic analytical process aimed at identifying the bioactive constituents of plants that exert a wide spectrum of biological effects. These include, but are not limited to, alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, glycosides and terpenes. The characterization of these phytochemicals facilitates the prediction of a plant's pharmacological properties, thereby contributing to its potential therapeutic applications.

Table 1
Fraction Yield % of GGG obtained with solvents of differing polarity

S. N.	Solvents Used	Colour	Consistency	Shape/Form	Fraction % Yield
1	Petroleum Ether	Dark Brownish green	Sticky	Amorphous	14.29%
2	Ethanol (99% v/v)	Dark Brown	Sticky	Amorphous	15.80%

The extraction using petroleum ether and ethanol (99% v/v) yielded dark brownish-green and dark brown sticky amorphous fractions with percentage yields of 14.29% and 15.80% respectively.

Table 2
Phytoconstituents identified by qualitative chemical analysis of dried fruit extracts of *Garcinia gummi-gutta*

S.N.	Phytoconstituents	Pet. Ether fraction	Ethanolic fraction
1	Alkaloids	++	+
2	Amino acids	+	+
3	Carbohydrates	+	++
4	Terpenoids	--	++
5	Flavonoids	--	+++
6	Glycosides	+	+
7	Phenols	--	+++
8	Tannins	--	--
9	Saponins	--	+
10	Steroids	+	++
11	Tannins/Phenolic compounds	+	+++
12	Tri-terpenoids	+	--

Note: "--" : Absence, "+" : slight Presence "++" : strong Presence

Table 3
Percentage yield and quantification of phytoconstituents (Total phenolic and tannins)

Fraction	Percentage Yield (%)	Color & Appearance	Solubility	Total Phenolic Content (mg /g)	Tannin Content (mg /g)	Major Phytochemicals Identified	Rf Values (HPTLC)	Detection Wavelength (nm)
GGCH (Chloroform Fraction)	6.03	Dark brown, semi-solid	Partially soluble in methanol	24.5 ± 1.2	8.9 ± 0.6	Alkaloids, Flavonoids	0.23, 0.67	254, 366
GGEA (Ethyl Acetate Fraction)	38	Reddish-brown, sticky	Soluble in ethyl acetate	82.3 ± 2.4	45.6 ± 1.8	Phenolic Acids, Tannins, Flavonoids	0.31, 0.78	280, 366
GGBu (n-Butanol Fraction)	45	Yellowish-brown, viscous	Soluble in water and alcohol	97.6 ± 2.9	53.4 ± 2.2	Polyphenols, Xanthones	0.45, 0.82	320, 366
GGAq (Aqueous Residue)	10	Light brown, gummy	Soluble in water	39.2 ± 1.7	18.5 ± 1.0	Saponins, Tannins	0.29, 0.74	280, 366

While contemporary methodologies employing sophisticated analytical techniques have been developed for precise phytochemical determination, traditional qualitative assays continue to serve as foundational tools for preliminary phytochemical screening, as exemplified in table 2.

Based on preliminary phytochemical screening studies, the EEG was identified as a rich source of bioactive secondary metabolites including flavonoids, polyphenols, tannins, alkaloids and other phytochemicals known for their gastro protective, anti-inflammatory and antioxidant properties. These bioactive constituents are reported to play a crucial role in modulating gastric acid secretion, inhibiting H^+/K^+-ATPase activity, enhancing mucosal defense mechanisms and mitigating oxidative stress-induced gastric damage. The presence of these therapeutically relevant compounds strongly justified the selection of the ethanolic extract for further pharmacological investigations. Accordingly, EEG was not only subjected to H^+/K^+-ATPase inhibition assays but also systematically evaluated for its antiulcer potential through comprehensive studies including gastric pH modulation, total acidity determinations, gastric volume measurements, histamine content analysis and gastric wall mucus content assessments. This multifaceted approach ensured a thorough understanding of its mechanism of action and therapeutic efficacy as a gastroprotective agent.

Quantification of Phytoconstituents: The fractionation of the EEG yielded four distinct fractions: GGCH (Chloroform), GGEA (Ethyl Acetate), GGBu (n-Butanol) and GGAq (Aqueous Residue), each exhibiting unique physicochemical properties, solubility and phytochemical profiles. The n-butanol fraction (GGBu) demonstrated the highest percentage yield (45%), appearing yellowish-brown and viscous, with high solubility in water and alcohol. This fraction exhibited the greatest total phenolic content (97.6 ± 2.9 mg GAE/g) and tannin content (53.4 ± 2.2 mg TAE/g), indicating its potential role in antioxidant and antiulcer activities. High-Performance Thin-Layer Chromatography

(HPTLC) revealed major polyphenols and xanthones, with Rf values of 0.45 and 0.82, detected at 320 and 366 nm respectively.

The ethyl acetate fraction (GGEA) accounted for 38% of the yield, with a reddish-brown, sticky consistency, high solubility in ethyl acetate and substantial phenolic acid and flavonoid content (82.3 ± 2.4 mg GAE/g). Tannin concentration (45.6 ± 1.8 mg TAE/g) suggests its potential gastroprotective efficacy. The chloroform fraction (GGCH), though contributing only 6.03% yield, contained alkaloids and flavonoids, with Rf values of 0.23 and 0.67 at 254 and 366 nm. The aqueous fraction (GGAq) (10% yield) exhibited a light brown, gummy consistency, high saponin and tannin content and significant solubility in water. These findings highlight the bioactive diversity of *Garcinia gummi-gutta*, supporting its potential for antiulcer and gastroprotective applications.

Dose optimization: The gastroprotective efficacy of EEG was evaluated using an ulcer-induced animal model across nine experimental groups. Group I (Normal Control), receiving only the vehicle, exhibited no ulcer formation, while group II (Ulcer Control, Untreated) demonstrated severe ulceration, confirming the validity of the ulcer induction model. Positive control groups, group III (Ranitidine HCl, 50 mg/kg) and group IV (Omeprazole, 20 mg/kg), displayed significant ulcer reduction, reinforcing their established antiulcer efficacy. EEG extract was administered at graded concentrations (10–750 µg/mL) to assess dose-dependent gastroprotective effects. Group V (10 µg/mL) exhibited a mild reduction in ulcer severity, indicating partial gastroprotection. Group VI (50 µg/mL) showed moderate ulcer reduction, while group VII (100 µg/mL) demonstrated a significant decrease in ulcer formation, suggesting an enhanced protective effect.

Group VIII (250 µg/mL) exhibited strong gastroprotective activity, with a more pronounced ulcer reduction. Group IX (500 µg/mL) achieved higher ulcer protection than 250 µg/mL which was then considered as an optimized dose since group X (750 µg/mL) showed effect similar to group IX. These findings indicate that EEG extract confers dose-dependent gastroprotection, mostly attributed to its high polyphenolic and tannin content, that would inhibit H^+/K^+-ATPase activity and oxidative stress. The results provide compelling evidence for the therapeutic potential of EEG as a natural antiulcer agent, warranting further pharmacological validation and mechanistic exploration.

The normal control group showed no ulcer protection, while the ulcer control group exhibited significant ulceration. Positive controls, ranitidine (50 mg/kg) and omeprazole (20 mg/kg), demonstrated effective gastroprotection. Lower doses of EEG extract (10–100 µg/mL) provided mild to moderate ulcer protection, with increasing effectiveness at higher doses. 250 µg/mL dose showed a strong gastroprotective effect. 500 µg/mL and 750 µg/mL doses resulted in a marked reduction in ulcer formation with minimal residual ulceration, demonstrating equivalent efficacy.

Table 4
Outcome of different doses as per ten groups

Group	Treatment	Dose (µg/mL)	Dose (mg/kg)	Number of Animals (n)	Observations	Outcome
I	Normal Control (Vehicle)	0	-	3	No gastroprotective effects observed	No changes observed
II	Ulcer Control (Untreated)	0	-	3	Ulceration with no protection	Significant ulcer formation
III	Positive Control (Ranitidine HCl)	50	50 mg/kg	3	Significant reduction in ulcers	Effective gastro protective effect
IV	Positive Control (Omeprazole)	20	20 mg/kg	3	Marked reduction in ulcer formation	Effective gastro protective effect
V	EEG Extract (10 µg/mL)	10	Calculated	3	Mild reduction in ulcer formation	Partial protection
VI	EEG Extract (50 µg/mL)	50	Calculated	3	Moderate reduction in ulcer formation	Partial gastro protective effect
VII	EEG Extract (100 µg/mL)	100	Calculated	3	Significant reduction in ulcer formation	Moderate gastro protective effect
VIII	EEG Extract (250 µg/mL)	250	Calculated	3	More pronounced reduction in ulcer formation	Strong gastro protective effect
IX	EEG Extract (Optimized Dose)	500	Calculated	3	Marked reduction in ulcer formation, with minimal residual ulceration	High gastroprotective effect
X	EEG Extract (Higher Dose)	750	Calculated	3	Marked reduction in ulcer formation, with minimal residual ulceration	High gastroprotective effect

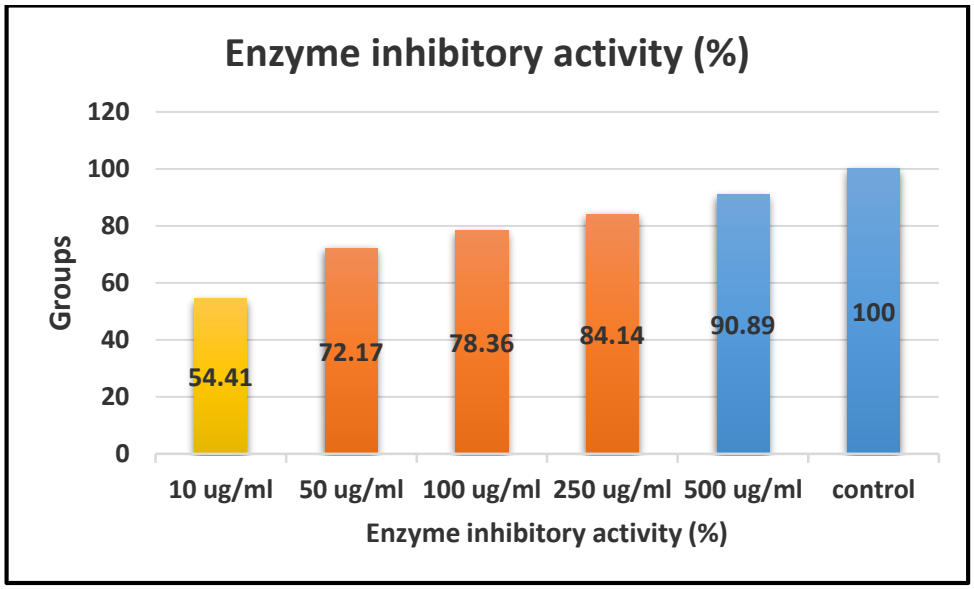
Since the 750 µg/mL dose did not offer additional benefits over 500 µg/mL, we selected 500 µg/mL as the optimal dose for further investigation. This selection ensures effective gastroprotection while maintaining dose efficiency.

H^+/K^+-ATPase Inhibitory Activity: The EEG was assessed for its possible anti-ulcer effects in an *in vitro* study. Gastric acid secretion is regulated by parietal cells. Within these cells, a proton pump facilitates the transport of protons into the stomach, where they undergo hydrolysis in the cytoplasm. The enzyme H+/K+-ATPase plays a crucial role in the production of acidity[18]. Excessive enzyme secretion is a primary contributor to acidity and ulcer formation.

The H+/K+-ATPase enzyme, which resides on the apical secretory membrane of parietal cells, was inhibited by varying concentrations (10, 50, 100, 250 and 500 µg/ml) of EEG. The inhibition followed a dose-dependent pattern with a significant increase in the inhibitory effect as the concentration rose. At the lowest concentration of 10 µg/mL, the extract inhibited enzyme activity by 54.41%, indicating moderate suppression. At 50 µg/mL, inhibition improved to 72.17%, showing enhanced bioactive potential. With a further increase to 100 µg/mL, inhibition rose to 78.36% and at 250 µg/mL, it significantly increased to 84.14% confirming the concentration-dependent inhibition effect.

The maximum inhibition was seen at 500 µg/mL with a remarkable 90.89% reduction in enzyme activity, nearly matching the complete inhibition observed in the control group. These findings indicate that EEG bioactive compounds effectively suppressed the target enzyme, achieving maximum efficacy at higher concentrations. This effect is likely due to the presence of polyphenols, flavonoids and tannins, which are recognized for their strong enzyme-inhibitory activities. These results strongly suggest the extract's potential for therapeutic use, highlighting the need for further mechanistic research and pharmacological evaluations to clarify its exact mechanism of action.

Biochemical Activity: The gastroprotective effects of EEG extract were evaluated by analyzing its impact on total acidity, mucus content, histamine levels, gastric volume and gastric juice pH at varying concentrations (10, 50, 100, 250 and 500 µg/mL).



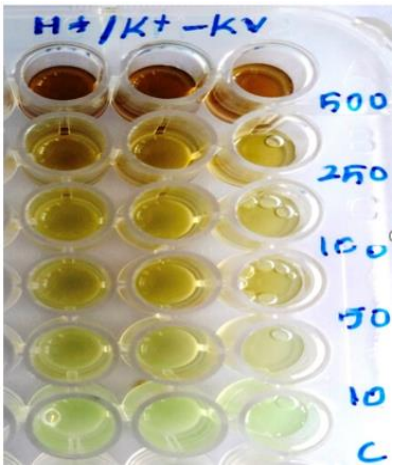


Figure 1: H^+/K^+-ATPase inhibitory activity of ethanolic extract of *Garcinia gummi gutta* fruits using Parietal cells

The study revealed a dose-dependent increase in gastric mucus pH following EEG extract administration. The untreated control group exhibited the lowest pH (2.89 ± 0.07), reflecting a highly acidic environment. However, EEG extract at 10 µg/mL slightly increased the pH to 3.21 ± 0.08, while 50 µg/mL led to a more notable elevation (3.78 ± 0.05). Higher concentrations (100 and 250 µg/mL) significantly increased the pH to 4.12 ± 0.06 and 4.76 ± 0.04 respectively.

The most substantial effect was observed at 500 µg/mL with a pH of 5.32 ± 0.03 suggesting a strong acid-neutralizing capacity and a potential role in ulcer prevention. Similarly, total gastric acidity exhibited a concentration-dependent decline. The control group recorded the highest acidity (85.6 ± 2.4 mEq/L), whereas administration of 10 µg/mL EEG extract reduced acidity to 74.2 ± 2.1 mEq/L.

Increasing concentrations led to further reductions with acidity values of 62.8 ± 1.9, 51.4 ± 1.6, 38.9 ± 1.3 and 26.5 ± 1.1 mEq/L at 50, 100, 250 and 500 µg/mL respectively. This progressive decrease suggests that EEG extract effectively mitigates excessive gastric acid secretion, potentially through phytochemical-mediated mechanisms. Gastric mucus content, a critical factor in mucosal protection, also increased with EEG extract administration. The control group exhibited the lowest mucus content (18.4 ± 1.2 mg/100 g tissue), while incremental doses of EEG extract led to significant increases. At 10 µg/mL, mucus content rose to 22.8 ± 1.4 mg/100 g followed by 27.3 ± 1.6 mg/100 g at 50 µg/mL.

Table 5
Gastric, Histamine and Acidity Content of five groups

Group	EEG Concentration (µg/mL)	Gastric Mucus pH (Mean ± SD)	Total Acidity (mEq/L, Mean ± SD)	Gastric Volume (mL/100g BW, Mean ± SD)	Gastric Juice pH (Mean ± SD)	Gastric Mucus Content (mg/100g Tissue, Mean ± SD)	Histamine Content (µg/g tissue, Mean ± SD)
Control (Untreated)	0	2.89 ± 0.07	85.6 ± 2.4	4.85 ± 0.22	2.1 ± 0.1	18.4 ± 1.2	8.92 ± 0.35
1	10	3.21 ± 0.08	74.2 ± 2.1	4.12 ± 0.18	2.5 ± 0.2	22.8 ± 1.4	7.68 ± 0.29
2	50	3.78 ± 0.05	62.8 ± 1.9	3.56 ± 0.15	3.1 ± 0.2	27.3 ± 1.6	6.35 ± 0.27
3	100	4.12 ± 0.06	51.4 ± 1.6	3.02 ± 0.14	3.8 ± 0.2	33.5 ± 1.8	5.02 ± 0.22
4	250	4.76 ± 0.04	38.9 ± 1.3	2.48 ± 0.11	4.5 ± 0.3	40.7 ± 2.1	3.84 ± 0.18
5	500	5.32 ± 0.03	26.5 ± 1.1	1.95 ± 0.09	5.2 ± 0.3	48.9 ± 2.4	2.49 ± 0.15

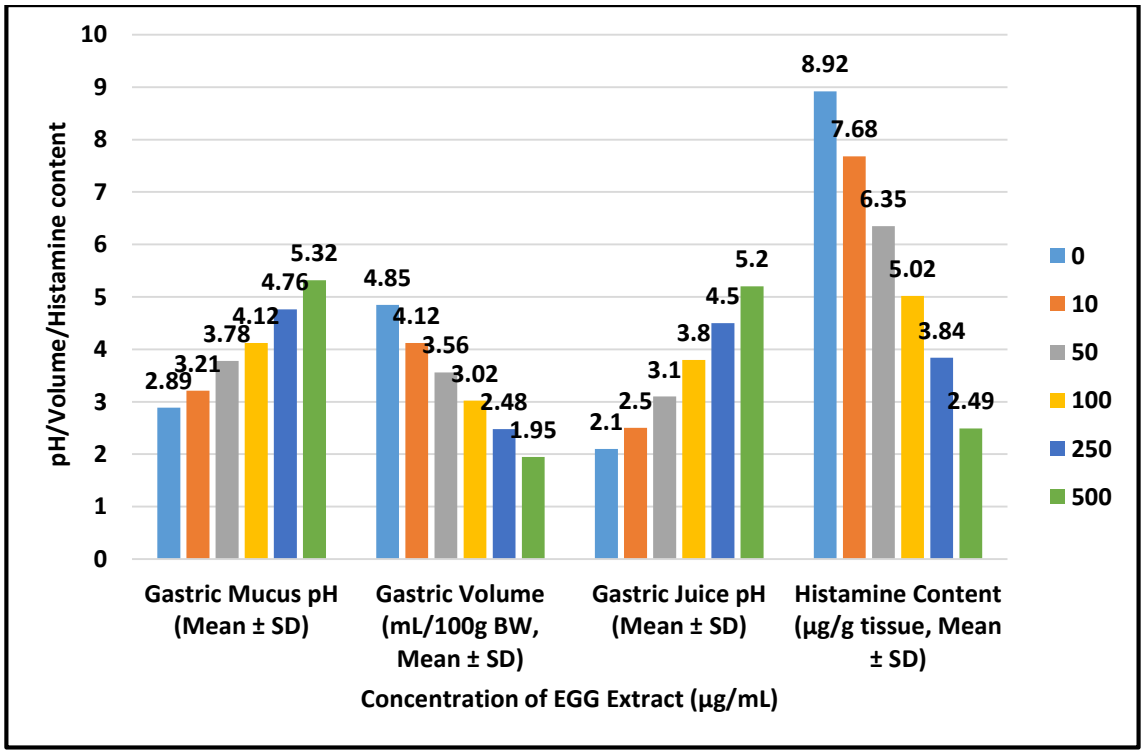


Fig. 2: Concertation of EGG extract vs. pH, volume etc.

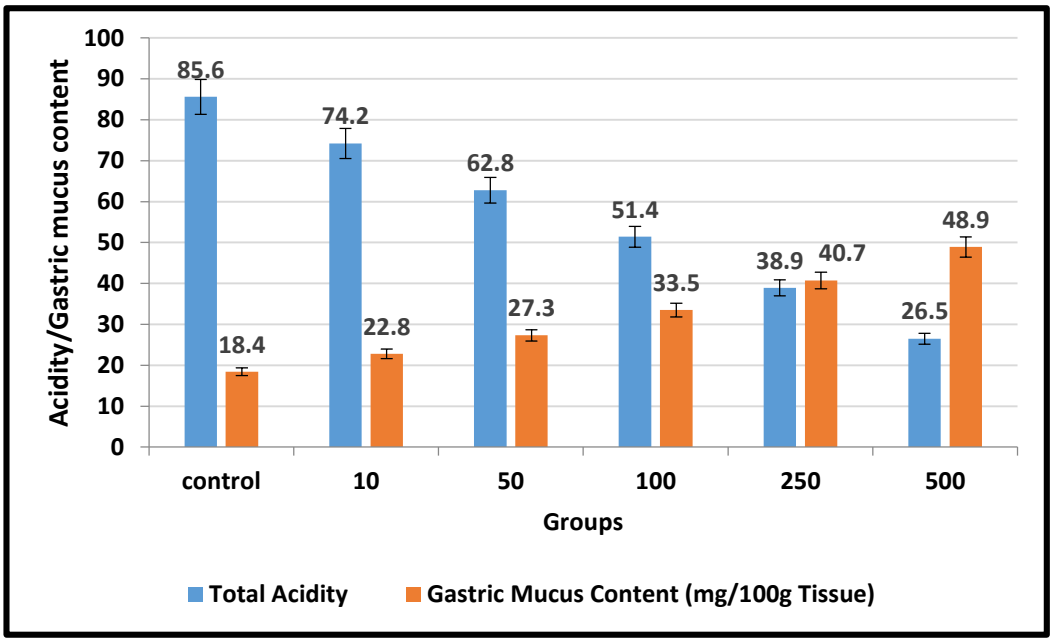


Fig. 3: Gastric Muscus Content vs. Acidity

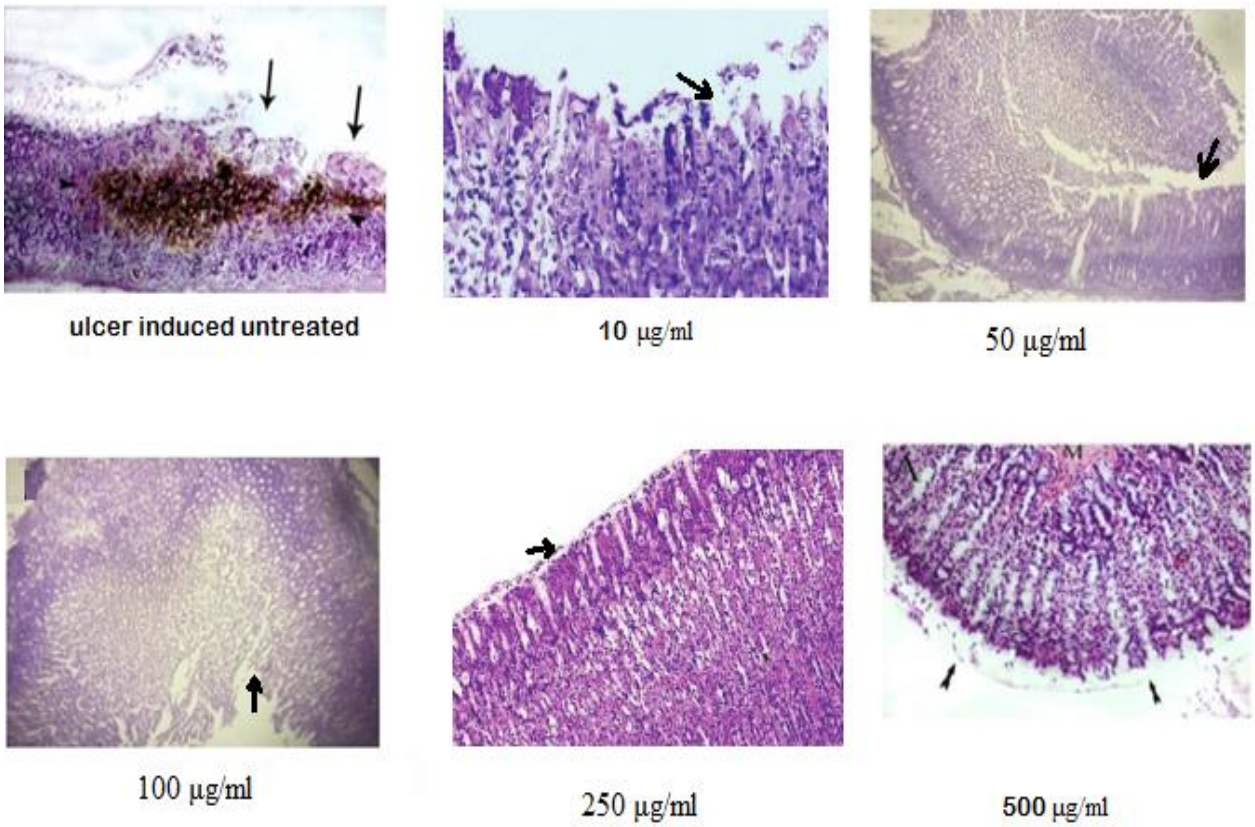


Fig. 4: Effect of EEG on stomach of aspirin induced peptic ucler

Higher doses of 100, 250 and 500 µg/mL resulted in progressively greater mucus secretion (33.5 ± 1.8, 40.7 ± 2.1 and 48.9 ± 2.4 mg/100 g respectively), stressing the extract's potential in enhancing gastric mucosal defense against ulcerative damage. The untreated control group exhibited the highest histamine levels (8.92 ± 0.35 µg/g tissue). Treatment with EEG extract resulted in a dose-dependent reduction, with histamine content declining to 7.68 ± 0.29, 6.35 ± 0.27, 5.02 ± 0.22, 3.84 ± 0.18 and 2.49 ± 0.15 µg/g tissue at 10, 50, 100, 250 and 500 µg/mL respectively. This reduction suggests an anti-inflammatory effect, possibly contributing to the extract's gastroprotective properties. The extract's impact on gastric volume and gastric juice pH was also significant. The control group displayed the highest gastric volume (4.85 ± 0.22 mL/100 g BW) and the lowest gastric juice pH (2.1 ± 0.1), indicating excessive acid secretion.

Table 6
H^+/K^+-ATPase inhibitory activity of EEG fruits using Parietal cells

S.N.	Test Sample Concentrations	OD Absorbance Value* at 640 nm	Enzyme Inhibitory action*(in %)
1	10 µg/ml	0.729	54.41
2	50 µg/ml	0.445	72.17
3	100 µg/ml	0.346	78.36
4	250 µg/ml	0.254	84.14
5	500 µg/ml	0.146	90.89
6	Control	1.599	100.00

* Average of measurement in triplicate

EEG extract administration led to a dose-dependent reduction in gastric volume, reaching its lowest level (1.95 ± 0.09 mL/100 g BW) at 500 µg/mL. Concurrently, gastric juice pH exhibited a progressive increase, with the highest value (5.2 ± 0.3) recorded at 500 µg/mL.

These findings suggest that EEG extract reduces gastric secretions while promoting a less acidic environment, potentially mitigating the risk of gastric ulceration. Thus EEG extract exhibited a dose-dependent gastroprotective effect by increasing gastric mucus pH and content, reducing total acidity and histamine levels and modulating gastric volume and juice pH. These findings suggest that EEG extract holds promise as a natural therapeutic agent for gastric ulcer prevention and treatment.

Histopathological Study: In the Ulcer-Induced Untreated (Control) group, extensive ulceration is evident. Arrows highlight regions of epithelial disruption and loss. The presence of dark, dense material comprising of fibrin, cellular debris, or pigment suggests severe tissue damage and inflammation. Cells with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer. Mucosa of stomach wall showed necropsy, erosion, congestion and hemorrhaging. At 10 µg/ml EEG, only minimal improvement is observed compared to the control. Arrows still indicate areas of surface disruption, albeit with slightly reduced severity. This finding implies that EEG at this low concentration exhibits limited anti-ulcer activity.

Remnants of the necrotic tissue are expressed with arrows to widened openings of the gastric pits in the 50 µg/ml EEG group, a noticeable reduction in ulceration is apparent. The arrow highlights a more intact epithelial layer, suggesting that EEG is beginning to exert a protective and therapeutic effect. At 100 µg/ml EEG, further histological improvement is observed.

The overall tissue architecture appears more preserved, with a marked reduction in epithelial damage, indicating a dose-dependent enhancement of anti-ulcer activity. In the 250 µg/ml EEG group, the gastric mucosa exhibits substantial restoration, with minimal evidence of ulceration. The epithelial layer appears largely intact, demonstrating a pronounced protective effect. It exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer.

At 500 µg/ml EEG, the most significant healing response is evident. The mucosal architecture remains largely preserved, with minimal residual damage and showed mild edema and leucocyte infiltration of the submucosal layer, but no disruption of the surface epithelium. Arrowheads indicate well-defined, normal tissue folds, suggesting that this concentration represents the most effective therapeutic dose in this study. EEG treated group has mucosa of stomach wall showing dose-dependent minimal necrotic changes.

H^+/K^+-ATPase Enzyme inhibition assay*:* The H^+/K^+-ATPase enzyme inhibition assay exhibited a pronounced concentration-dependent effect, underscoring the potential of EEG as a potent H^+/K^+-ATPase inhibitor. This enzymatic inhibition is a pivotal mechanism in the regulation of gastric acid secretion and serves as a key therapeutic target in the management of gastric ulcers. The extract's ability to effectively suppress H^+/K^+-ATPase activity suggests its role in reducing excessive gastric acid production, thereby contributing to ulcer healing and mucosal protection.

The test samples exhibited a progressive increase in inhibitory activity with increasing concentrations. At 10 µg/mL, the extract showed 54.41% inhibition, which steadily increased to 90.89% at 500 µg/mL, highlighting its significant suppressive effect on gastric proton pump activity. The control group, which was assigned 100% enzymatic activity, further validated the extract's effectiveness by demonstrating a notable reduction in enzyme function upon treatment. These findings suggest that the extract may act through a competitive or non-competitive inhibition mechanism, disrupting the H^+/K^+-ATPase-dependent acid secretion pathway in gastric parietal cells. The observed enzyme inhibition profile is comparable to standard antiulcer agents, such as proton pump inhibitors (PPIs), further reinforcing the therapeutic potential of the extract.

The presence of bioactive secondary metabolites such as polyphenols, flavonoids, tannins and alkaloids**,** could contribute to this pharmacological action, possibly through

their ability to interact with the active site of the enzyme, alter membrane permeability, or modulate oxidative stress within gastric mucosa. Furthermore, the observed inhibitory effect aligns with the presence of bioactive secondary metabolites, such as flavonoids and polyphenols, which are known for their gastroprotective properties. The concentration-dependent response not only reinforces the pharmacological relevance of EEG but also provides a strong scientific basis for its potential development as a natural alternative to conventional proton pump inhibitors (PPIs).

Conclusion

The findings of this study suggest that the ethanolic fruit extract of *Garcinia gummi-gutta* could be an effective source for anti-ulcer treatments. The extract demonstrates substantial anti-ulcer activity, primarily through the inhibition of the H^+/K^+-ATPase enzyme, which plays a crucial role in gastric acid production. This activity resembles that of proton pump inhibitors, making it a promising natural alternative for ulcer management. *Garcinia gummi-gutta* exhibits strong antioxidant and anti-inflammatory effects, which may enhance its gastro protective properties by mitigating oxidative stress and inflammation in the gastric mucosa.

Moreover, the extract has shown a favorable safety profile, being non-toxic and ulcer prevention, which positions it as a potentially safer alternative to synthetic anti-ulcer drugs often linked to undesirable side effects. Due to diverse mechanisms of action and low toxicity, GGG warrants further investigation for its potential clinical application in treating gastric ulcers, whether used alone or alongside traditional treatments to improve effectiveness and to reduce side effects.

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