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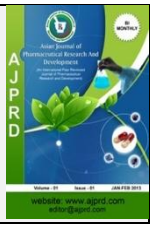

Open Access **Research Article**

Pharmacognostical Standardization, Phytochemical Screening and Antiulcer Activity of *Terminalia bellerica*

Srishti Upadhyay, Jagdish Nagar, Mohammad Mukim

Department of Pharmacology, Kota College of Pharmacy, Kota

A B S T R A C T

Context: Terminalia chebula Retz. (Combretaceae) is a medium-sized tree that grows in the wild throughout India. T. chebula has been extensively used in Ayurveda, Unani, and homoeopathic medicine. The fruit has been used as a traditional medicine for a household remedy against various human ailments. Traditionally T. chebula is used to cure chronic ulcer, gastritis, and stomach cancers.

Objective: The present study is to evaluate the antiulcer effect of hydroalcoholic extract of Terminalia chebula fruit.

Materials and methods: In the pharmacognostical, phytochemical and biological evaluation many chemicals, glassware and instruments were used. Different grades of chemicals were used as per the requirement like laboratory reagent (LR), analytical reagents (AR) and HPLC.

Conclusion : The present study has been done to evaluate the antiulcer effect of METB on indomethacin induced ulcer models at various doses. The results obtained from the present study have shown that METB possesses antiulcer effect on indomethacin induced ulcers. Pre-treatment with METB particularly at a dose of 50mg/kg, 100mg/kg and 200 mg/kg decreases ulcer index, total acidity, total volume of acid secretion and increase in pH when compared with control.

Keywords: Pharmacognostical, Phytochemical, Terminalia chebula.

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***Address for Correspondence:**
Srishti Upadhyay, Department of Pharmacology, Kota College of Pharmacy, Kota

INTRODUCTION:

Peptic ulcer and related acid peptic diseases affect up to 10 percent of the population with sufficient severity to prompt victims to seek medical attention. The most significant disorders requiring medical attention are peptic ulcer and gastro esophageal reflux disease [1]

Peptic ulcers are relapsing lesions that are most often diagnosed in middle-aged to older adults, but they may first become evident in young adult life. They often appear without obvious precipitating conditions and may then, after a period of weeks to months of active disease, heal with or without therapy. Even with healing, however, the tendency to develop peptic ulcers remains, in part because of recurrent infections with *Helicobacter pylori*. Although it is difficult to obtain estimates of the prevalence of active disease, autopsy studies and population surveys indicate a prevalence of 6% to 14% for men and 2% to 6% for women. The male to female ratio for duodenal ulcers is about 3:1, and for gastric ulcers about 1.5 to 2.1[2].

Danger of ulcer

Bleeding: Upper gastrointestinal (UGI) bleeding secondary to peptic ulcer is a common medical condition that results in high patient morbidity UGI bleeding commonly presents with hematemesis (vomiting of blood or coffee-ground like material) and/or melena (black, tarry stools). Hematochezia, usually a sign of a lower GI source, can also be seen with massive UGI bleeding. A nasogastric tube lavage which yields blood or coffee-ground like material confirms this clinical diagnosis; however, lavage may be negative if bleeding has ceased or arises beyond a closed pylorus. Most

patients with bleeding ulcers can be managed with fluid and blood resuscitation, medical therapy, and endoscopic intervention, as appropriate. The mortality from peptic ulcer bleeding has not changed materially in recent years and remains at 7% to 10% despite advances in patient management. Those found to have bled from an ulcer should receive endoscopic hemostatic therapy (eg, with injection sclerotherapy and/or the application of a thermal coagulation device such as the heater probe) if there is active bleeding, a non bleeding visible clot, or possibly an adherent clot in the ulcer base [3-5].

Perforation: Duodenal, antral, and gastric body ulcers account for 60, 20 and 20 percent of perforations due to peptic ulcer, respectively.

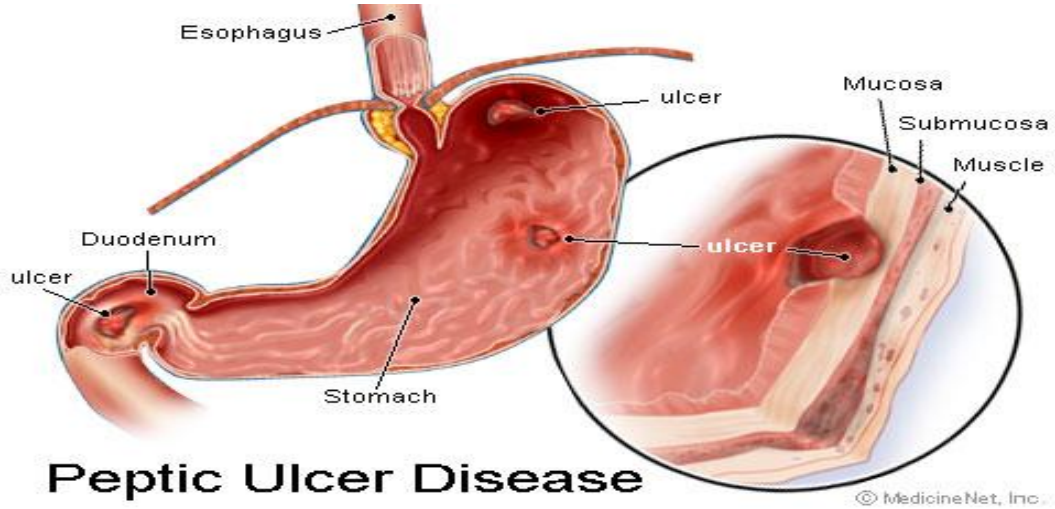


Figure 1: Peptic Ulcer

Penetration: Ulcer penetration refers to penetration of the ulcer through the bowel wall without free perforation and leakage of luminal contents into the peritoneal cavity. Surgical series suggest that penetration occurs in 20 percent of ulcers, but only a small proportion of penetrating ulcers become clinically evident. Penetration occurs in descending order of frequency into the pancreas, gastrohepatic omentum, biliary tract, liver, greater omentum, mesocolon, colon and vascular structures. Antral and duodenal ulcers can penetrate into the pancreas. Penetration can also involve pyloric or pre-pyloric ulcers penetrating the duodenum, eventually leading to a gastroduodenal fistula evident as a "double" pylorus. A long-standing ulcer history is common but not invariable in patients who develop penetration. Penetration often comes to attention because of a change in symptoms or involvement of adjacent structures. The change in symptom pattern may be gradual or sudden; it usually involves a loss of cyclicity of the pain with meals, and loss of food and antacid relief. The pain typically becomes more intense, of longer duration and is frequently referred to the lower thoracic or upper lumbar region. The diagnosis of penetrating ulcer is suspected clinically when an ulcer in the proper region is found. Mild hyperamylasemia can develop with posterior penetration of either gastric or duodenal ulcer, but clinical pancreatitis is uncommon. Penetration can be associated with a wide array of uncommon complications including perivisceral abscess (evident on CT or ultrasonography)], erosion into vascular structures leading to exsanguinating haemorrhage (aortoenteric fistula), or erosion into the cystic artery. Rare biliary tract complications include erosion into the biliary tree with choledochoduodenal fistula, extra hepatic obstruction or hematobilia. Fistulization into the pancreatic duct has also been reported with penetrating duodenal ulcer fistulae are seen with greater curvature gastric ulcers, particularly marginal ulcers. Typical features of this complication include pain, weight loss and diarrhoea; feculent vomiting is an uncommon, but diagnostic symptom. A duodenocolic fistula can also occur. No rigorous studies are available to guide the management of penetrating ulcers [4]

Obstruction: Gastric outlet obstruction is the least frequent ulcer complication. Most cases are associated with duodenal or pyloric channel ulceration, with gastric ulceration accounting for only 5 percent of cases [5].

Changes in lifestyle and dietary:

Aspirin and related drugs (non-steroidal anti-inflammatory drugs), [6] alcohol, [7] coffee [8] (even decaf) and tea can aggravate or interfere with the healing of peptic ulcers. Smoking is also known to slow ulcer healing [11]. People with ulcers have been reported to eat more sugar than people without ulcers, [12] though this link may only occur in those with a genetic susceptibility toward ulcer formation [13].

Sugar has also been reported to increase stomach acidity [14]. Salt is a stomach and intestinal irritant. Higher intakes of salt have been linked to higher risk of stomach ulcer [15]. As a result of these reports, some doctors suggest that people with ulcers should restrict the use of both sugar and salt, although the benefit of such dietary changes remains unknown.

Glutamine, an amino acid, is the principal source of energy for cells that line the small intestine and stomach [16]. Glutamine has also prevented stress ulcers triggered by severe burns in another preliminary study [17].

Herbs helpful in antiulcer therapy

Worldwide interest in natural products as preventive and therapeutic agents has led to a greater appreciation of the rich heritage of traditional systems of medicine. Dietary and lifestyle modifications are the basis of Ayurvedic medicine, with herbal formulas rounding out therapeutic programs. Ayurvedic formulas contain many balancing herbs offering a high degree of safety and efficacy. Peptic ulcer is a major health hazard both in terms of morbidity and mortality. It occurs due to imbalance between offensive (acid-pepsin secretion, *H.Pylori*, bile, increased free radicals and decreased antioxidants) versus impaired mucosal resistance (mucus, bicarbonate secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury). Various reports have shown that commonly used drugs for peptic ulcers such as H_2 blockers (ranitidine, famotidine etc.), M_1 blockers (pirenzepine, telenzepine etc.), Proton pump inhibitors (omeprozole, lanzoprozole etc.), have danger of drug interaction, adverse effect and increased incidence of relapses during ulcer therapy [18].

Various herbal drugs like Shilajit, Ginger, Bael have been tried for their ulcer protective effectsand have promising results. Therefore, the search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their better protection, easy availability, low cost and toxicity [19].

MATERIALS AND METHODS

Chemicals and Reagents

In the pharmacognostical, phytochemical and biological evaluation many chemicals, glassware and instruments were used. Different grades of chemicals were used as per the requirement like laboratory reagent (LR), analytical reagents (AR) and HPLC.

PHARMACOGNOSTIC INVESTIGATIONS

Collection and Authentication of Plant Material

*T. bellerica*barkwere collected from out skirt area of Kota, Rajasthan, in January 2021. The plant was identified, authenticated and certified (HIMCOSTE/HPSBB/7185) by Dr. Pankaj Sharma, Senior Scientist, Himachal Pradesh State Biodiversity Board, Shimla, India.

Organoleptic Evaluation

Bark of *T. bellerica*were evaluated for their impact on various organs of sense that is organoleptic properties. Its color, odor, size (length and width), taste and other diagnostic parameters was observed and noted .

Microscopic Evaluation

Powder characteristics

The coarsely powdered leaf was mounted on a glass slide in glycerin and studied under microscope. Photographs of different magnified cellular structures were taken with Nikon lab photo 2 microscope units .

PHYSIOCHEMICAL ANALYSIS

Physiochemical parameters (ash value, moisture content, foreignmatter, fluorescence analysis, extractive value, foaming index and swelling index) were determined.

Determination of Foreign Organic Matter

The powdered drug sample (100 gm) was weighed accurately and spread out in thin layer. Foreign matter was detected by inspection with the use of lens (6x). The foreign matter was separated and weighed and percentage of the foreign matter was calculated in % w/w .

Moisture Content

The powdered drug (10 gm) was heated at 105ºC in hot air oven to constant weight. Weight loss after drying gave the moisture content of the drug. The moisture content was reported in %w/w .

Determination of Ash Value

The determination of ash value is meant for detecting low grade products, exhausted drugs and sandy or earthy matters. Ash contains inorganic radicals like phosphates, carbonate and silicates of sodium, potassium, magnesium and calcium. Sometimes inorganic variables like calcium oxalate, silica and carbonate contents of the crude drug affect the total ash value. Such variable ash value can be determined by different methods which were measured for total ash, acid insoluble ash and water soluble ash.

Determination oftotal ash value

About 2 gm of the powdered material was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered plant was spread as a fine even layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450˚C until free from carbon. The crucible was cooled and weighed and the procedure was repeated to get the constant weight. The percentage of the total ash was calculated with reference to the air-dried sample. [78].

The total ash value was calculated and reported as % w/w

$$\text{Total ash value of the sample} = \frac{(z-x)}{y} \times 100$$

Where

z = weight of the dish + ash (after complete incineration)

x = weight of the empty dish

y = weight of the drug taken

Determination of acid insoluble ash

The ash obtained as described in the total ash was boiled with 25 ml of 2M hydrochloric acid for five minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited, cooled and weighed and the procedure was repeated to get the constant weight. The % w/w of acid insoluble ash was determined with reference to the air-dried drug [77,78].

$$\%\ \text{Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Determination of water soluble ash

The ash obtained as described in the total ash was boiled in 25 ml of chloroform water for five minutes. The insoluble matter was collected in a crucible or ash less filter paper

and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible, ignited for 15 minutes at a temperature not exceeding 450ºC, cooled and weighed and the procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as the water-soluble ash. The % w/w of water-soluble ash was determined with reference to the air-dried drug.

$$\% \text{ Water solube ash value } = \frac{(\text{Wt. of total ash} - \text{Wt. of water insoluble ash})}{\text{Wt. of crude drug taken}} \times 100$$

Sulphated ash

Heat a silica crucible to redness for 10 minutes, allow to cooling in a desiccator and weighing, transferring to the crucible one gram of sample and weighing the crucible and the contents accurately. Ignite, gently at first, until the substance is thoroughly charred. Cool moisten the residue with 1ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at $800^0C \pm 25^0C$ until all black particles disappear. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before, allow to cooling and weighing. Repeat the operation until two successive weighing do not differ by more than 0.5mg. Cool the silica crucible in a desiccator, weigh accurately, and calculate the percentage of residue.

$$\% \text{ sulphated ash value } = \frac{\text{Wt. of sulphated ash}}{\text{Wt. of crude drug taken}} \times 100$$

Extractive Value

Determination of ethyl acetate soluble extractive

Macerate 5 gm of air dried coarsely powdered drug was mixed with 100 ml of ethyl acetate in a closed flask and kept for 24 hours, shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105ºC and weighed. Calculated the % w/w of ethanol soluble extractive with reference to the air-dried drug.

Determination of methanol soluble extractive

Macerate 5 gm of air dried coarsely powdered drug was mixed with 100 ml of methanol in a closed flask and kept for 24 hours, shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105ºC and weighed. Calculated the % w/w of methanol soluble extractive with reference to the air-dried drug .

Determination of water soluble extractive

Macerate 5 gm of air dried coarsely powdered drug was mixed with 100 ml of water in a closed flask and kept for 24 hours, shaking frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105ºC and weighed. Calculated the % w/w of water soluble extractive with reference to the air-dried drug.

Dried bark was milled to a coarse powder and then passed over sieve No. 14. The obtained dried powdered leaves of *T. bellerica*(20 g) were placed in the tube of Soxhlet apparatus in the form of a thimble and extracted with various solvents, such as ethyl acetate, methanol, and water (300 mL) at 60–65 °C for 3–4 h. The obtained extracts, respectively, ethyl acetate (EAE), methanol (ME) and aqueous (AE) extracts, were filtered while hot and dried by evaporation using a rotary vacuum evaporator and the final dried extract samples were kept at low temperature in the fridge for further study. The residue obtained from each extract was dissolved in the same solvent for further analysis

Determination of Foaming Index

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of foaming index.

Procedure: Accurately weigh 1 gm of the powdered bark was transferred to a 500 ml conical flask containing 100 ml of boiling distilled water. It was boiled moderately for 30 minutes. The filtrate was cooled and filtered in a 100 ml volumetric flask and sufficient distilled water was added through the filter to dilute to volume. The decoction was poured into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and the volume of the liquid was adjusted in each tube with water to 10 ml. the tubes were shaken in a lengthwise motion for 15 seconds, two shakes per second. Allowed to stand for 15 minutes and the height of the foam were measured.

Foaming index = 1000/a

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

Determination of Swelling Index

Many herbal drugs are of specific for the therapeutic use because for their swelling properties. Swelling index is used to find the swelling properties of drug.

Procedure:

Weight accurately about 1gm of drug and transfer it to a 25ml of stopper cylinder. Filled the measuring cylinder up to mark of 20ml with water. Agitate it gently and occasionally and allowed to stand for 24 hours. Measured the volume of the measuring cylinder occupied by the swollen drug.

Fluorescence Analysis of Extracts

The fluorescence analysis can be used for the qualitative examination of herbal drugs. It is one of the techniques to detect adulteration in herbal drugs. All the extracts were examined in the day light, short-wave (254 nm) and long-wave (365 nm) UV light to detect fluorescent compounds.

PHYTOCHEMICAL SCREENING

Preliminary Phytoprofile

The powder of the air dried bark of *T. bellerica*extracted (figure 5a) in soxhlet apparatus with solvents of increasing polarity as follows:

i) Petroleum ether

ii) Ethyl acetate

iii) Methanol

iv) Water

Each time before extracting with the next solvent, the material was dried. Each extract was concentrated by distilling off the used solvent and concentrated extracts were evaporated using rotary evaporator (figure 5b) to dryness and weighed. Colour and percentages of extracts were calculated with reference to air dried plant material.

(a)

(b)

Figure 5.(a) Extraction by soxhlet extractor (b) Evaporation and concentration of extract using rotatory evaporator

Quantitative Phytochemical Analysis

Total phenolics content (TPC)

It was determined by using Folin-Ciocalteu assay [81]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. Volume was then made up to the mark. After incubation for 90 min at room temperature, absorbance against reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Total flavonoid content (TFC)

It was measured by aluminum chloride colorimetric assay. An aliquot (1 ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.3 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and volume was made up to 10 ml with distilled water. Solution was mixed and absorbance was measured against blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QUE).

Development of Chromatographic Profile by HPTLC

CAMAG-HPTLC system having a sample applicator Linomat 5 was used to obtain HPTLC chromatograms using standard methods [83]. HPTLC profile of the various extracts of *T. bellerica*was developed using toluene:ethyl acetate (7:3) as mobile phase to confirm the occurrence of different phytoconstituents. The chromatographic development processes were performed in an air conditioned room where the temperature was maintained at 22^{o}C and relative humidity was maintained at 55%. Ready to use silica gel-G precoated HPTLC aluminum plates were used for chromatographic separation. The extract (10 ml) was spotted as bands of 10mm width with the help of the auto sampler fitted with a 100 ml Hamilton syringe. The solvent system was transferred to CAMAG twin trough plate development chamber lined with filter paper and pre-saturated with mobile phase (25 ml) for 30 min. The resulted plates were air dried and scanned. Developed spots on the places were scanned at the wavelength of 254 and 366 nm respectively. The retention factor (R_f) for each spot found on plate was recorded.

Fourier Transform Infrared Spectroscopy (FTIR)

Functional groups and types of chemical bonds present in phytochemicals are identified by FTIR spectroscopy analysis [84]. Light absorbed wavelength is the prominent aspect of chemicals bonds, which can be seen through interpreted spectrum. Compound chemical bonds can be deduced via absorption infrared spectrum. Each extract (8mg) was loaded to Fourier transform infrared spectrophotometer for functional group analysis. The IR peaks absorbance (wave number, cm^{-1}) was recorded in the range of 4000cm^{-1} to 400cm^{-1}.

Dose Fixation:

Sireeratawong and his co-workers evaluate the acute and chronic toxicities of the water extract from the dried fruits of *Terminalia bellerica* (Gaertn.) Roxb.were assessed in both female and male rats. For the study of acute toxicity, a

single oral administration of the water extract at a dose of 5,000 mg/kg body weight (10 female, 10 male) was performed and the results showed no signs of toxicity such as general behavior changes, morbidity, mortality, changes on gross appearance or histopathological changes of the internal organs of rats .

ANTIULCER STUDY

Induction of gastric ulcer

Gastric ulcer was induced by injection of indomethacin (25 mg/kg, intraperitoneal (i.p.)) to animals that were already fasted for 24 hours [86]. Oral and parenteral (i.p.) administration of the extract was done 2 and 1 hr before ulcer induction, respectively. Six hours later, the animals were sacrificed by inhalation of an overdose of diethyl ether. The abdomens were opened and the stomachs were excised while the both sides (cardiac and pyloric) were ligated appropriately. The contents of stomachs were removed, cut through their greater curvature and deposited on work sheets [87]. Photographs were taken and tissues were evaluated for ulcer surface area and severity as mentioned below [88]. Animals were sacrificed by cervical dislocation, one hour after administration of indomethacin. Rat stomachs were removed, opened along the greater curvature, pinned on the soft board and stomach lesions were evaluated to determine the ulcer score.

Animal Grouping

Group I(Normal control) Normal saline 2 ml/kg p.o.

Group II(Disease Control) Indomethacin 30 mg/kg p.o.

Group III(Standard) Omeprazole 20 mg/kg

Group IV(Test Drug) 50 mg/kg p.o.

Group V (Test Drug) 100 mg/kg p.o.

Group VI(Test Drug) 200 mg/kg p.o.

Ulcer Index

The stomach was removed and fixed on a cork plate and the number of and severity of ulcers was registered with a stereo-microscope using the following scores.

Severity score:

0 = Normal coloured stomach

0.5 = Red colouration

1 = Spot ulcer

1.5 = Hemorrhagic streaks

2 = Ulcers ≥ 3 but ≤5

3 = ulcers > 5

Calculation:

Ulcer index was calculated as:-

$UI = UN+US+ UP X 10^{-1}$

Where,

UI = ulcer index

UN = average of number of ulcers per animal

US = average of severity score

UP = percentage of animals with ulcer

Determination of total acidity.

Principle:

A known amount of gastric residue was titrated with 0.1 N sodium hydroxide to a pH of 3.5. If pH meter is not available, add two drops of Topfer's reagent which changes to a salmon colour when all the free hydrochloric acid is neutralized. The total acidity however was determined by titration using phenolphthalein as indicator.

Reagents:

(a) **Sodium hydroxide solution (0.1N NaOH):** Stock Sodium hydroxide solution (0.1N NaOH) was diluted ten-folds. Alternatively, 4g of NaOH was dissolved in fresh distilled water and made up to 1000 ml.

(b) **Phenolphthalein solution (1% alcoholic):** 1 g of phenolphthalein was dissolved in 100 ml of 95% alcohol.

(c) **Topfer's reagent (Dimethylaminoazobenzene), 0.5% alcoholic solution:** 0.5 g of Topfer's reagent was dissolved in 100 ml of 95% alcohol.

Procedure:

10ml of gastric juice specimen was transferred in a porcelain evaporating dish.

1-2drops of Topfer's reagent is added.

A colour change was observed; a bright red colour appears if free hydrochloric acid is present. 1-2 drops of phenolphthalein was added to the gastric juice with Topfer's reagent. Titrated with 0.1 NaOH from a burette, mixing was done after each addition until the last trace of red colour disappeared and was replaced by a canary yellow colour.

The numbers of milliliters of NaOH used was read from the burette. This represents the amount of free hydrochloric acid.

The titration was continued until the red colour of phenolphthalein appeared (deep pink), titrated to the point at which the further addition of alkali did not deepen the colour.

Reading was taken (ml NaOH) for total acidity.

Calculation:

Y = ml of 0.1 N NaOH x 10

Where,

Y= Total acidity (mEq/L)

Acid volume

The stomach was removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature [90].

The volume of the juice was measured.

pH

Procedure:

Animals will be sacrificed by overdose of ether.

The stomach was removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature.

The tubes were centrifuged at 3000 rpm for 10 minutes and the centrifuged samples were decanted and analyzed for pH (using digital pH meter, Type DPH – 100- Data instruments)

Histopathological evaluation of gastric tissues

The wall of sacrificed rat stomach was fixed in 10% formalin and then paraffin blocks were made. Five micrometer thick section was cut from the paraffin block and stained with hematoxylin and eosin. Histo-pathological evaluations were carried out on the collected gastric tissue samples according to the method as reported elsewhere .

Statistical analysis

One-way ANOVA followed by Dunnett's posthoc by using GraphPad Prism version 6 was used for statistical analysis. Data were presented as mean ± SEM. $p \leq 0.05$ was taken as statistically significant value.

RESULTS

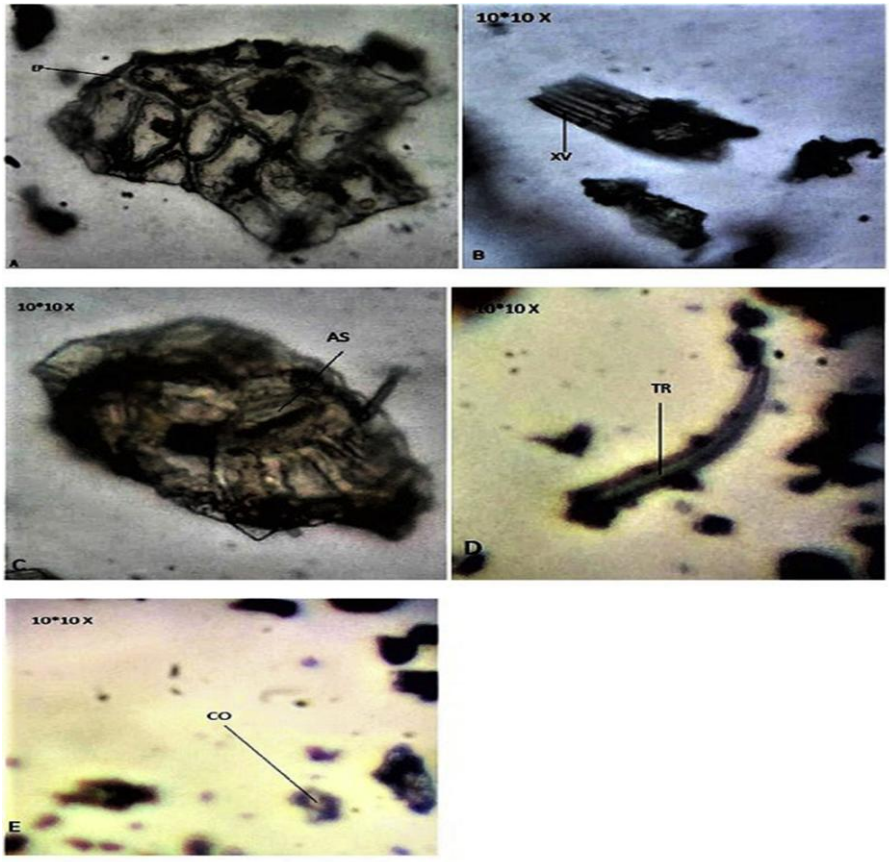
ORGANOLEPTIC FEATURES

Organoleptic characters *T.bellerica*bark is dark brown color. Bark is odorless but having a slight bitter and astringent taste that may be due to presence of tannins.

MICROSCOPIC CHARACTER

Powdered Drug Microscopy

The powder was brown in color, with no distinct odour and an astringent taste. It was easy flowable with a fine to coarse texture. Microscopic powder features revealed presence of crystals of calcium oxalate and starch granules; trichomes were found to be hollow unicellular and uniseriate; parts of epidermis in surface view showing straight-walled polygonal epidermal cells, anomocytic stomata and spiral xylem vessels. The guard cells were shrinking elongating the stomata pore (Fig. 6).



A: Epidermal cells (EP) B: Xylem vessels (XV) C: Anomocytic stomata (AS) D: Trichomes (TR) E: Calcium Oxalate Crystals (CO)

Figure 6: Powdered characteristics of the bark of *T. bellerica*.

PHYSICOCHEMICAL EVALUATION

The results of physicochemical parameters are summarized in Table 8. Sulphated ash value (2.41%) was found lower than the total ash value (2.83%). The acid insoluble ash was found to be 0.52% and water soluble ash value was 0.38%. On further studies it was concluded that the drug contain 8.72% moisture content, foaming index and swelling index were found to be nil, while foreign organic content was reported to be less than 1%. The extractive values for various solvents such as methanol, ethyl acetate and aqueous were found to be 21.2%, 15.7%, 8.72% respectively.

Table 8. Physiochemical parameters of bark of *T. bellerica*

Physiochemical parameters		**Results (%)**
Ash value	Total ash	2.83
	Acid insoluble ash	0.52
	Water soluble ash	0.38
	Sulphated ash	2.41
Extractive value	Methanolic extract	38.51
	Ethyl acetate extract	21.2
	Aqueous extract	15.7
Moisture content		8.72
Foreign organic matter		Less that 1% (Presence of petiole stalks with leaves)
Foaming index		Nil
Swelling index		Nil

Preliminary Phytochemical Screening

Various phytochemical analysis tests supported that the extracts contain alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and glycosides, recorded in Table 9. The aqueous extract was found to be negative for the presence of alkaloids as compared to methanolic and ethyl acetate extract.

Table 9: Results of phytochemical screening of different extracts of *M. esculenta* leaves extracts

Constituent	**Bark extracts**		
	Aqueous extract	**Ethyl acetate extract**	**Methanolic extract**
Alkaloids	-ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve
Proteins & amino acids	-ve	-ve	-ve
Fixed oils & fats	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Phenolic compounds	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Saponins	-ve	-ve	-ve

+ ve: Present; -Ve: Absent

FLUORESCENCE ANALYSIS

The fluorescence characters of powdered drug impart a valuable role in the determination of quality and purity of the drug materials. The powdered drugs when subjected to ultraviolet light and visible light in the presence of various chemical reagents, exhibit characteristic fluorescence. Fluorescence report of *T. bellerica* powdered bark is tabulated in table 10.

Table 10: Fluorescence characteristics of bark extracts.

S. No.	**Treatment**	**Visible light**	**UV Light**	
			254 nm (Short Wavelength)	**366 nm (Long wavelength)**
1	Bark Powder	Brown	Brown	Light Brown
2	Bark Powder rubbed on filter paper	Light Brown	Light Brown	Light Brown
3	Bark Powder + 1N NaOH	Reddish Brown	Black	Brown
4	Bark Powder + 1N HCl	Light Brown	Light Yellow	Light Brown
5	Bark Powder + 1N HNO_3	Light Yellowish brown	Light Brown	Light Yellow
6	Bark Powder + 1N H_2SO_4	Light Brown	Light Brown	Light Brown
7	Methanolic extract of Bark Powder	Dark Brown	Black	Brown
8	Ethyl acetate Extract of Bark Powder	Brown	Light Brown	Light Brown
9	Aqueous extract of Bark powder	Greenish Brown	No Fluorescence	Brown

Development of Chromatographic HPTLC Profile of Different Extracts of *T. Bellerica Bark*

Qualitative HPTLC of ethyl acetate extract, methanolic extract and aqueous extract was carried out by using toluene:ethyl acetate (7:3) solvent system to assure the presence of various phytochemicals. HPTLC chromatogram of ethyl acetate extract (Fig. 7) showed a total of 3 spots at different R_f value at 254 nm, whereas 7 spots were observed at 366 nm (Fig. 5.6). Methanolic extract showed 2 spots having different Rf values at 254 nmwhile 5 spots at 366 nm (Fig. 7). Least spots were observed in aqueous extract. There were a total of 2 spots at 254 nm and 3 spots at 366 nm having different Rf values (Fig. 7).

Table 11: HPTLC profile of *T. bellerica* extracts

Extracts	Wavelength (nm)	R_f Value
Ethyl acetate	254	0.13, 0.5, 0.7
	366	0.10, 0.21, 0.33, 0.51, 0.63, 0.72, 0.83
Methanol	254	0.525, 0.775
	366	0.36, 0.55, 0.71, 0.82, 0.97
Aqueous	254	0.1, 0.63
	366	0.093, 0.65, 0.81

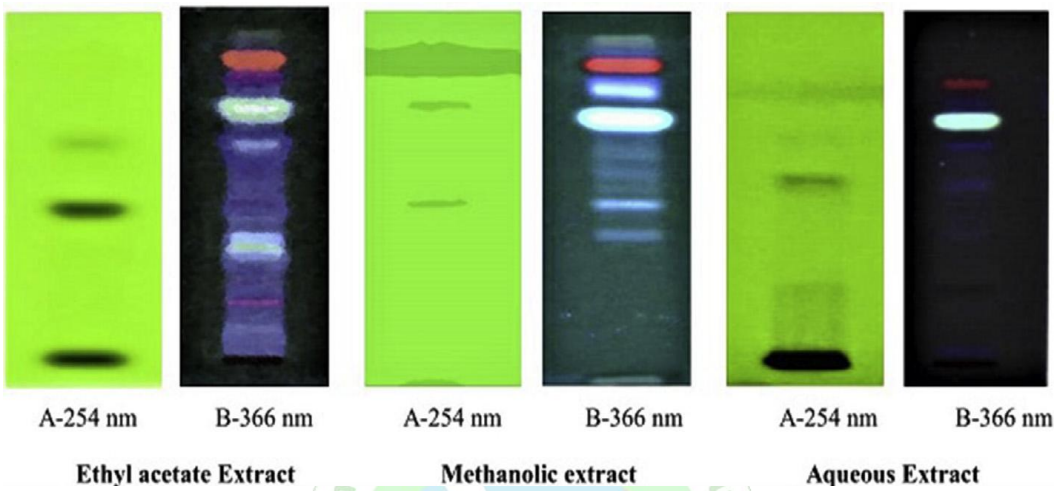


Figure 7: Visualization of ethyl acetate, methanolic and aqueous extract of *T. bellericabark* at 254 and 366 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

Compounds functional groups were examined by Fourier transform infrared spectroscopic studies by their peak values (cm−1). Alkenes, amines, carboxylic acids, amides, esters, alcohols, phenols, ketones, carboxylic acids, and aromatic compounds were identified. Aromtics, sulfons and aliphatic amines showed main peaks 1460, 1365 and 1074cm−1. Different intensity peaks were identified for primary and secondary amines at (3462, 1603, 745 and 780cm−1) carboxylic acids (3300cm−1, 1712cm−1 and 1290 cm−1), alcohols and phenols (3360cm−1, 3307cm−1 and 1290 cm−1), alkenes (2965cm−1 and 1634cm−1) (Tables 12-13).

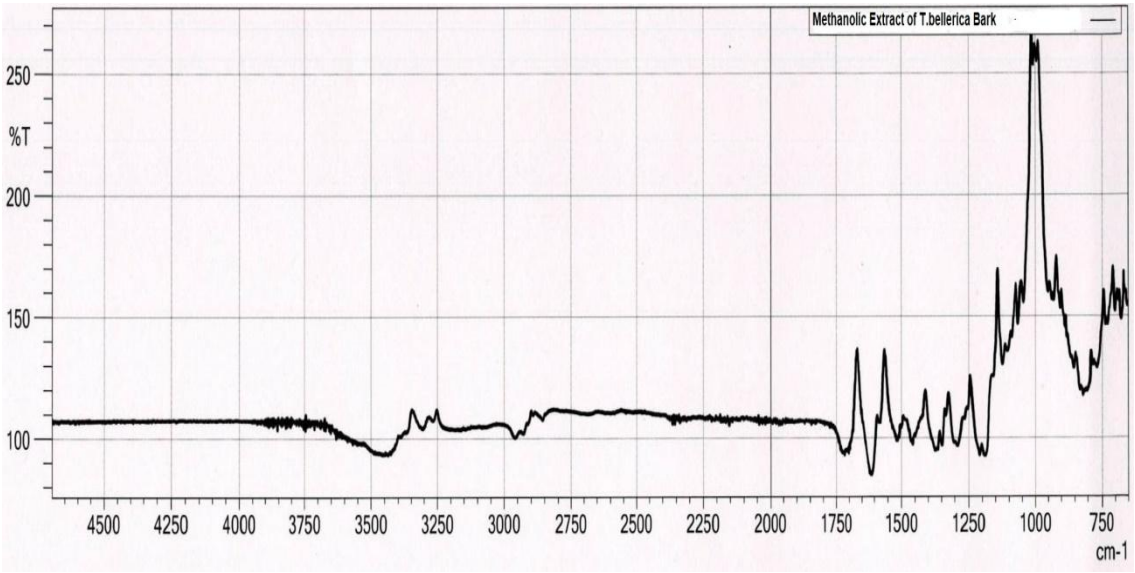


Figure 8: FTIR spectrum of ME of *T. bellerica* bark

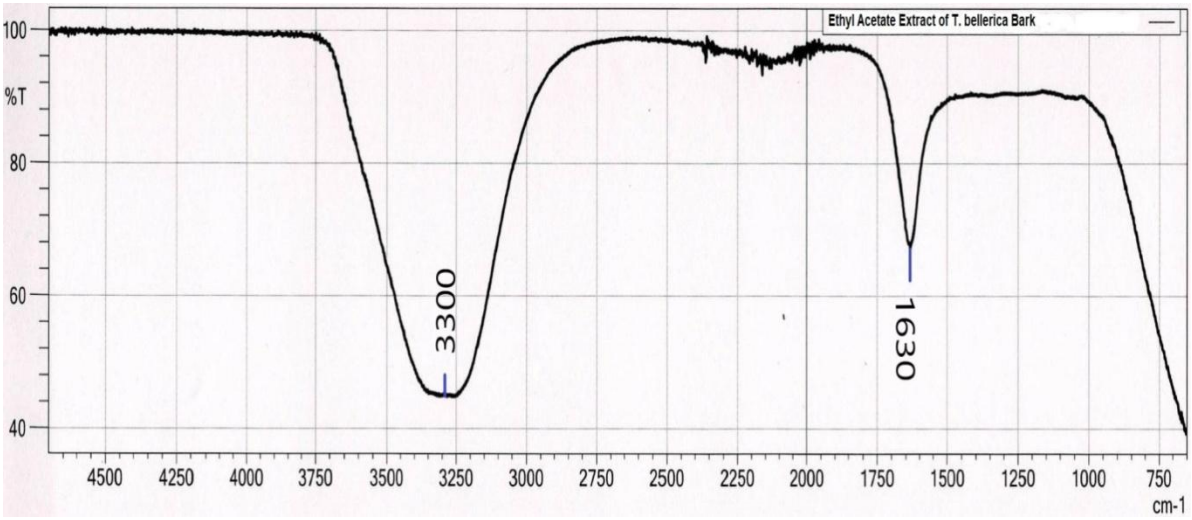


Figure 9: FTIR spectrum of EAE of *T. bellerica* bark

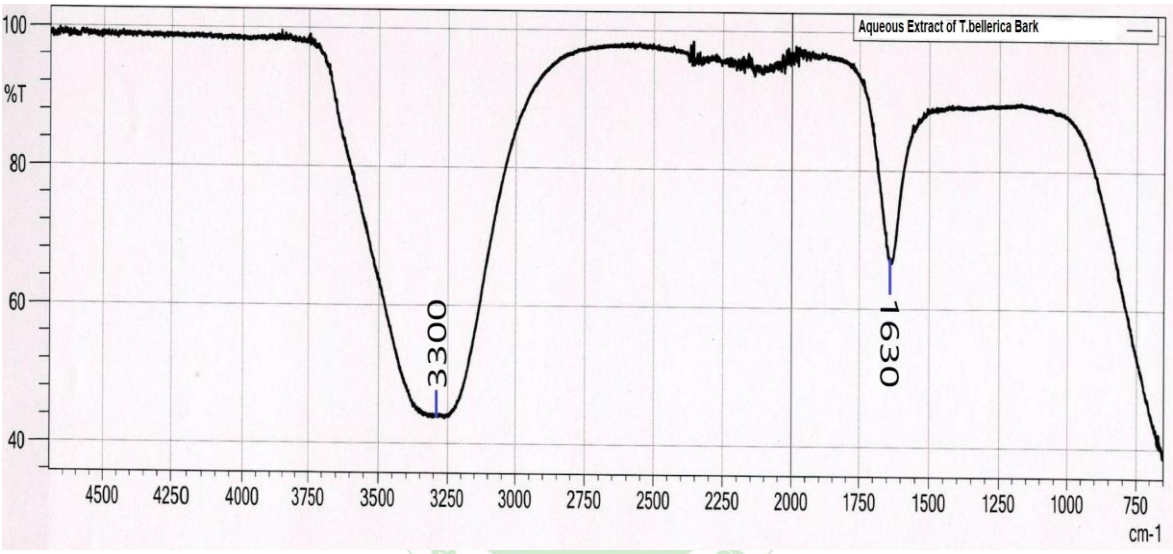


Figure 10: FTIR spectrum of AE of *T. bellerica* bark

Table 12: FTIR spectra of *T. bellerica*bark methanolic extract

S. No.	Peak (Cm^{-1})	Frequency range	Possible functional groups
1	3462	3500-3100	N-H stretching vibration presence of primary and secondary amines and amides
2	3360	3500-3200	O-H stretching vibration presence of alcohols, phenols
3	3300	3300-2500	O-H stretching vibration presence of carboxylic acids
4	2965	3000-2850	C-H stretching vibration presence of alkenes
5	1712	1725-1705 1725-1700	C=O stretching vibration presence of ketone and carboxylic acid
6	1603	1640-1550	N-H bending vibration presence of primary and secondary amines and amides
7	1460	1500-1400	C-C stretching vibration presence of aromatics
8	1365	1375-1300	S=O stretching vibration presence of sulfones, sulfonyl chlorides, sulphates and sulphonamides
9	1290	1320-1000	C-O stretching vibration presence of alcohols, carboxylic acids, esters, ethers and anhydrides
10	1074	1250-1020	C-N stretching vibration presence of aliphatic amines
11	745, 680	910-665	N-H stretching vibration presence of primary and secondary amines

Table 13: FTIR spectra of *T. bellerica*bark ethyl acetate extract

S. No.	Peak (Cm^{-1})	Frequency range	Possible functional groups
1	3307	3500-3200	O-H stretching vibration presence of alcohols, phenols
2	1634	1680-1640(m)	-C=C- stretching vibration presence of alkenes

Table 14: FTIR spectra of *T. bellerica*bark aqueous extract

S. No.	Peak (Cm^{-1})	Frequency range	Possible functional groups
1	3307	3500-3200	O-H stretching vibration presence of alcohols, phenols
2	1634	1680-1640(m)	-C=C- stretching vibration presence of alkenes

Ulcer score, ulcer index and anti-ulcer activity (%) for ethanol- and indomethacin-induced ulcer models are given in Table 15.

Table 15: Ulcer Score

Treatment Group	Treatment	Ulcer Index
Group I (Normal control)	Normal saline 2 mL/kg	-----
Group II (Disease Control)	Indomethacin 30 mg/kg	1.6 ± 0.0
Group III (Standard)	Omeprazole 20 mg/kg	0.4 ± 0.0^{a}
Group IV (Test Drug Min. Dose)	METB 50 mg/kg	0.6 ± 0.1^{a}
Group V (Test Drug Medium Dose)	METB 100 mg/kg	0.5 ± 0.0^{a}
Group VI (Test Drug Max. Dose)	METB 200 mg/kg	0.3 ± 0.1^{a}

In indomethacin-induced ulcer models, there was dose-dependent decrease in ulcer index activity in all treated groups. Results revealed that ulcer index decreases in dose dependent manner. Table 16 shows the effect of extracts on the gastric pH, totalacidity and acid volume. In indomethacin-induced ulcer models, extracts at doses 50, 100, 200 mg/kg, orally, and standard drug 20 mg/kg, orally significantly ($p \leq 0.05$) reduced the free acidity, total acidity and volume of gastric juice but significantly increased the pH of gastric volume in a dose-dependent manner when compared with the disease control group.

Table 16: Effect of extract on the gastric pH, totalacidity and acid volume

Treatment Group	Treatment	Total Acidity(mEq/L)	pH	Acid Volume
Group I (Normal control)	Normal saline 2 mL/kg	51.4 ± 4.5^{a}	2.0 ± 0.1^{ns}	3.5 ± 0.6^{a}
Group II (Disease Control)	Indomethacin 30 mg/kg	68.4 ± 3.5	1.9 ± 0.1	4.1 ± 0.7
Group III (Standard)	Omeprazole 20 mg/kg	28.2 ± 4.6^{a}	4.7 ± 0.2^{a}	2.0 ± 0.1^{a}
Group IV (Test Drug Min. Dose)	METB 50 mg/kg	38.5 ± 1.3^{a}	3.6 ± 0.1^{a}	2.5 ± 0.1^{a}
Group V (Test Drug Medium Dose)	METB 100 mg/kg	32.6 ± 1.4^{a}	3.9 ± 0.1^{a}	2.3 ± 0.1^{a}
Group VI (Test Drug Max. Dose)	METB 200 mg/kg	25.1 ± 2.8^{a}	4.9 ± 0.3^{a}	2.6 ± 0.1^{a}

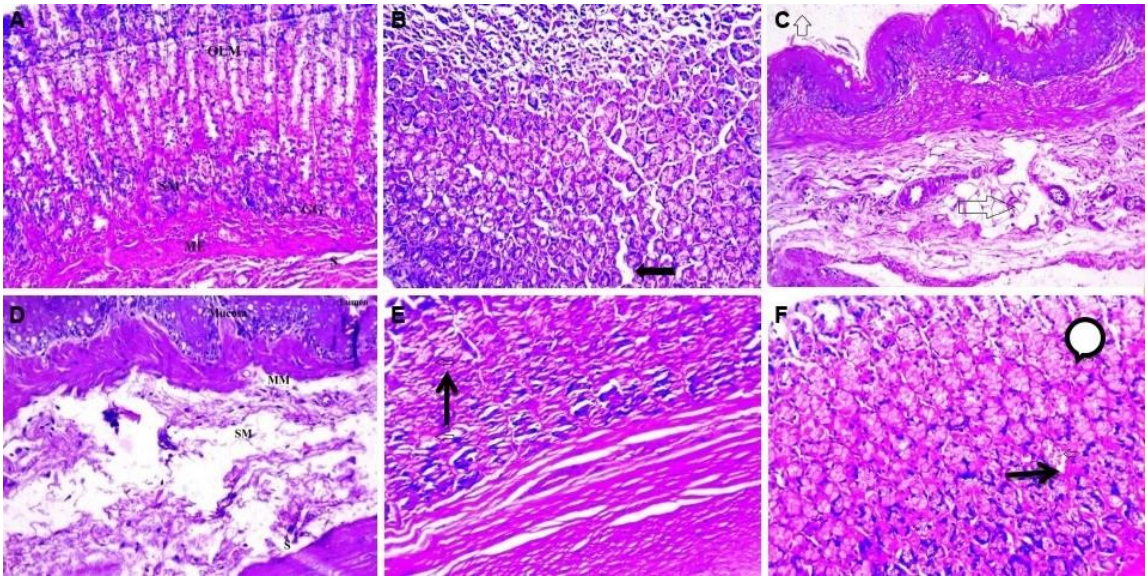


Figure 11: Histological examination of gastric ulcers; A: Photomicrograph of rat stomach section from the control group showing the histological structure of the normal submucosa (SM), gastric gland (GG), serosa (S), muscularis externa (ME), outer longitudi-nal muscle (OLM); B: Standard group (omeprazole) is showing normal appearance of Gland cell and sub mucosal cell; C: Disease control (Indomethacin induce ulcer) is indicating mucosal and sub mucosal ulceration along with loss epithelium cells in gastric mucosa; E: Methanolic extract 200 mg/kg + indomethacin treated group is showing normal muscularis mucosa (MM), Submucosa (SM) and blood in mucosa cells; F: Methanolic extract 100 mg/kg + indomethacin treated group is indicating condensation of mucosa and clustering of RBC's in sub mucosal layer

DISCUSSION

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/ or duodenum leading to a local defect or excavation due to active inflammation. Despite the constant attack on the gastroduodenal mucosa by a host of noxious agents (acid, pepsin, bile acids, pancreatic enzymes, drugs, and bacteria), integrity is maintained by an intricate system that provides mucosal defense and repair. This intricate biologic system consist of mucus bicarbonate layer, surface epithelial cells and a rich submucosal micro-circulatory bed which provides bicarbonate ions to neutralize the acid generated by parietal cell secretion of hydrochloric acid. Moreover, this micro-circulatory bed provides an adequate supply of micronutrients and oxygen while removing toxic metabolic by products.

Various studies suggested that changes in gastric motility may play a role in the development and prevention of experimental gastric lesions.

Relaxation of circular muscle may protect the gastric mucosa through flattening of the folds. This will increase the mucosal surface area exposed to necrotizing agents and reduce the volume of the irritant on the rugal crests. Such an action has been postulated to play a role in the cytoprotective effect of prostaglandins.

The present study has been done to evaluate the antiulcer effect of bark extract of *T. bellerica*on indomethacin induced dose dependent ulcers. The results obtained from the present study have been shown that methanolic extract of *T.bellerica* bark possesses antiulcer effect on indomethacin induced ulcers. In ethanol and stress induced model, there is decrease in ulcer index, total acidity, total volume of gastric secretion, and pH of gastric secretion when compared with control. In the present study Omeprazole has been used as a standard.

CONCLUSION

The present study has been done to evaluate the antiulcer effect of METB on indomethacin induced ulcer models at various doses. The results obtained from the present study have shown that METB possesses antiulcer effect on indomethacin induced ulcers. Pre-treatment with METB particularly at a dose of 50mg/kg, 100mg/kg and 200 mg/kg decreases ulcer index, total acidity, total volume of acid secretion and increase in pH when compared with control. All these observation imply that the METB could be regarded as a favourable antiulcerogen which could be attributed to its content of flavonoids and mucilage.

REFERENCES:

1. Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, Goel KR. Effect of ethanolic extract of *Eugenia Jambolana* seeds on gastric ulceration and secretion in rats. Indian J Physiol Pharmacol 2007; 51(2):131-140.
2. Merseresu WA, Hinchey EJ. Role of gastric mucosal folds in formation of local ulcers in the rat. Surgery 1982; 91:150-155.
3. Takeuchi K, Ueki S, Okabe S. Importance of gastric motility in the pathogenesis of indomethacine-induced gastric lesions in rats. Dig Dis Sci 1986; 32:1114-1122.
4. Garrick T, Buack S, Bass P. Gastric motility is a major factor in cold restraint induced lesion formation in rats. Am J Physiol1986; 250: 6191-6199.
5. Takeuchi K, Nobuhara Y. Inhibition of gastric motor activity by 16, 16-dimethyl prostaglandin E2: a probable explanation of cytoprotective. Dig Dis Sci 1985; 32:1181-1188.
6. Shetty R, Kumar KV, Naidu MUR, Ratnakar KS. Effect of *Ginkgo biloba* extract on ethanol induced gastric mucosal lesions in rats. Indian J Pharmacol 2000; 32:313-7.

7. Al-Harbi MM, Quershi S, Raza M, Ahmed MM, Afzal M, Shah AH. Gastric antiulcer and cytoprotective effect *Commiphora molmol* in rats. J Ethnopharmacol 1997; 55:141-50.
8. Vanisree AJ, Mitra K, Shyamala Devi CS. Antiulcerogenic effect of UL-409 against experimentally induced gastric ulcer in rats. Indian J Pharmacol 1996; 28:265-8.
9. Hollander D, Taranawski A, Gergely H, Zipser KD. Sucralfate protection of the gastric mucosa against alcohol-induced injury: A prostaglandin-mediated process. Scand J Gastroenterol 1984; 101:97-102.
10. MillerTA, Henagan JM. Indomethacin decreases resistance of gastric barrier disruption by alcohol. Dig Dis Sci 1984; 29:141-9.
11. Takeuchi K, Ueshima K, Ohuchi T and Okabe S. The role of *Capsaicin* sensitive sensory neurons in healing of HCl-induced mucosal lesions in rats. Gastroenterology 1994; 104:15-24.
12. Goodman, Gillman. The Pharmacological Basis of Therapeutics. 9thed. New York: McGraw-Hill; 1996. p. 902-903.
13. Sedlak J, Lindsay RH. Estimation of total protein and non protein sulfhydryl in tissue with Ellman's reagent. Anal Biochem1968; 25:192.
14. Ogle CW, Koo MWL, Cho CH, Tong M. The influence of *Verapamil* on the gastric effects of stress in rats. Eur J Pharmacol 1985; 11(2): 399-404.
15. Grossman MI. Control of gastric secretion in gastrointestinal disease, Pathophysiology - diagnosis and management. Sleisenzer MH, Fordtran JS, editors. 2ed. Philadelphia: W B Saunders Co; 1978. p. 640-659.
16. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol1967; 20:169.
17. Olsen CE. Glutathione modulates toxic oxygen metabolite injury of canine chief cell primary culture. Am J Physiol 1988; 254:649-656.
18. Lüllmann H, Mohr K, Ziegler A, Bieger D. Color Atlas of Pharmacology. 2nded. New York: Thieme Stuttgart; 2000. p. 166.
19. Body SC, Sasame HA, Body MR. Gastric glutathione depletion and acute ulcerogenesis by diethylmaleate given subcutaneously to rats. Life Sci1981; 28:2987-92.
20. Lau WY, Leow CK. History of perforated duodenal and gastric ulcers. *World J Surg*. 1997; 21:890–6.