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R E S E A R C H A R T I C L E

Evaluation of Anti-Ulcer and Anti-Inflammatory Activities of Arial Parts of Flemingia Macrophylla

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A B S T R A C T

A major revolution in clinical neuropharmacology and psychopharmacology was herald during the past years. The natural compounds have played a major role in neurological disorders. A number of herbal drugs traditionally employed in the Indian system of medicine Ayurveda. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. The main aim of this study is to estimate the antiulcer &anti inflammatory activity of aerial parts of the plant based on the study of cytoprotective activity of flavanoids from the aerialparts of Flemingia macrophylla. Estimation of the activities will be done by In vitro & in vivo methods. The revealed results of the preliminary phytochemical screening of the ethanolic extract of dried aerial parts of Flemingia macrophylla. The ethanolic extract has given positive results for flavanoids, glycosides. The results of preliminary phytochemical screening of the ethanolic extract of Flemingia macrophylla (FME) revealed that presence of flavonoids, glycosides. From the above studies it has been concluded that Flemingia macrfophylla has both antiulcer & anti inflammatory activity. Further studies are carrying to find out the active ingredients which are responsible for both activites.

Key words: Cytoprotective activity, Flemingia macrophylla, Antiulcer & anti inflammatory

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

A peptic ulcer, also known as PUD or peptic ulcer disease, is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm) of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. As many as 70-90% of ulcers are associated with Helicobacter pylori, a spiral-shaped bacterium that lives in the acidic environment of the stomach; however, only 40% of those cases go to a doctor. Ulcers can also be caused or worsened by drugs such asaspirin, Plavix (clopidogrel), ibuprofen, and other NSAIDs. Contrary to general belief, four times as many peptic ulcers arise in the duodenum (first part of the small intestine, just after the stomach) rather than in the stomach itself. About 4% of stomach ulcers are caused by a malignant tumor, so multiple biopsies are needed to exclude cancer. Duodenal ulcers are generally benign.

ClassificationBy Region/Location:

Stomach (called gastric ulcer)
Duodenum (called duodenal ulcer)
Esophagus (called esophageal ulcer)
Meckel's Diverticulum (called Meckel's Diverticulum ulcer; is very tender with palpation)

Modified Classification of peptic ulcers:

Type I: Ulcer along the body of the stomach, most often along the lesser curve at
Incisura angular is along the locus minors resistentiae.
Type II: Ulcer in the body in combination with duodenal ulcers. Associated with acid over secretion.
Type III: In the pyloric channel within 3 cm of pylorus. Associated with acid over secretion.
Type IV: Proximal gastro esophageal ulcer
Type V: Can occur throughout the stomach. Associated with chronic NSAID use (such as aspirin)

Signs and symptoms

Symptoms of a peptic ulcer can be
Abdominal pain, classically epigastric with severity relating to mealtimes, after around 3 hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it.Bloating and abdominal fullness.Water brash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus - although this is more associated with GERDNausea, and copious vomiting;Loss of appetite and weight loss;Hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting.Melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin);Rarely, an ulcer can lead to a gastric or duodenal perforation, which leads to acute peritonitis. This is extremely painful and requires immediate surgery.

Complications

Gastrointestinal bleeding is the most common complication. Sudden large bleeding can be life-threatening. It occurs when the ulcer erodes one of the blood vessels, such as the gastroduodenal artery.Perforation (a hole in the wall) often leads to catastrophic consequences. Erosion of the gastro-intestinal wall by the ulcer leads to spillage of stomach or intestinal content into the abdominal cavity. Perforation at the anterior surface of the stomach leads to acute peritonitis, initially chemical and later bacterial peritonitis. The first sign is often sudden intense abdominal pain. Posterior wall perforation leads to pancreatitis; pain in this situation often radiates to the back. Penetration is when the ulcer continues into adjacent organs such as the liver and pancreas.

Scarring and swelling due to ulcers causes narrowing in the duodenum and gastric outlet obstruction. Patient often presents with severe vomiting. Cancer is included in the differential diagnosis (elucidated by biopsy), Helicobacter pylori as the etiological factor making it 3 to 6 times more likely to develop stomach cancer from the ulcer.

Causes

A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to *Helicobacter pylori*that colonizes the antralmucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis), resulting in a defect in the regulation of gastrin production by that part of the stomach, and gastrin secretion can either be decreased (most cases) resulting in hypo- or achlorhydria or increased. Gastrin stimulates the production of gastric acid by parietal cells and, in H. pylori colonization responses that increase gastrin, the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation.Another major cause is the use of NSAIDs (see above). The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of cyclooxygenase 1 (cox-1), which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatories (such as celecoxib or the since withdrawn rofecoxib) preferentially inhibit cox-2, which is less essential in the gastric mucosa, and roughly halve the risk of NSAID-related gastric ulceration. As the prevalence of H. pylori-caused ulceration declines in the Western world due to increased medical treatment, a greater proportion of ulcers will be due to increasing NSAID use among individuals with pain syndromes as well as the growth of aging populations that develop arthritis.

The incidence of duodenal ulcers has dropped significantly during the last 30 years, while the incidence of gastric ulcers has shown a small increase, mainly caused by the widespread use of NSAIDs. The drop in incidence is considered to be a cohort-phenomenon independent of the progress in treatment of the disease. The cohort-phenomenon is probably explained by improved standards of living which has lowered the incidence of H. pylori infections. Although some studies have found correlations between smoking and ulcer formation, others have been more specific in exploring the risks involved and have found that smoking by itself may not be much of a risk factor unless associated with H. pylori infection . Some suggested risk factors such as diet, spice consumption and blood type, were hypothesized as ulcerogens (helping cause ulcers) until late in the 20th century, but have been shown to be of relatively minor importance in the development of peptic ulcers. Similarly, while studies have found that alcohol consumption increases risk when associated with H. pylori infection, it does not seem to independently increase

risk, and even when coupled with H. pylori infection, the increase is modest in comparison to the primary risk factor. Gastrinomas (Zollinger Ellison syndrome), rare gastrin-secreting tumors, also cause multiple and difficult to heal ulcers.

Inflammation

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent.

It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues.

Causes

The agents causing inflammation may be as under

Physical agents like heat, cold, radiation, mechanical trauma.

Chemical agents like organic and inorganic poisons.

Infective agents like bacteria, viruses and their toxin.

Immunological agents like cell mediated and antigen-antibody reactions.

Thus inflammation is distinct from infection –the former being a protective response by the body while the latter is invasion into the body by harmful microbes and their resultant ill- effects toxins.

Inflammation involves 2 basic processes with some over lapping, viz. Early inflammatory response and later followed by healing .Though both these processes generally have protective role against injurious agents , inflammation and healing may causes considerable harm to the body as well as e.g.; anaphylaxis to bites by insects or reptiles, drugs , toxins , atherosclerosis, chronic rheumatoid arthritis, fibrous bands and adhesions in intestinal obstruction.

Types

Depending up on the defense capacity of the host and duration of response , inflammation can be classified as acute and chronic .Acute Inflammation is of short duration and represents the early body reaction and is usually followed by repair.

The main features of acute inflammation are:

Accumulation of fluid and plasma at the affected site

Intra vascular activation of platelets and Polymorph nuclear neutrophials as inflammatory cells.Chronic inflammation is of longer duration and occurs either after the causative agent of acute inflammation persists for a long time or the stimulus is such that it induces chronic inflammation from the beginning .The characteristic feature of chronic inflammation is presence of chronic inflammatory such as lymphocyte, plasma cells and macrophages.

Signs: The roman writer celsus in 1st century A.D.named the famous 4 cardinal signs of inflammation as ;Rubor (redness);Tumor (swelling);Calor (heat); andDolor (pain). To these, fifth singn funstio laesa (loss of function) was later added by virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation.

Acute inflammation

Cellular events:

The cellular phase of inflammation consists of two process exudation and phagocytosis

Exudation of leucocytes:

The escape of leucolytes from the lumen of microvasculature to the insterstitial tissues is the most important feature of inflammatory respose. In acute inflammation, polymorphonucler neutrophils (PMNs) comprise the frist line body defense , followed later by monocytes and macrophages. The changes leading to migration of leucocytes are as follows ;

Changes in the formed elements of blood:

In the early stage of inflammation, the rate of flow of blood is increase due to vasodilatation. But subsequently, there is slowing are stasis of blood stream. Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as margination. As a result of this redistribution, the neutrophils of the central column come close to the vessel wall, this is known as pavementing.

Rolling and adhesion:

Peripherally marginated and pavement neutrophils slowly roll over the endothelial cells lining the vessels wall (rolling phase). This is followed by the transient bond between the leucocytes and endothelial cells becoming firmer (adhesion phase) .The following adhesion molecules bring about rolling and adhesion phases.

2. Materials and Methods

Preliminary Phytochemical Analysis

The ethanolic extract of the aerial parts of *Flemingia macrophylla* was subjected to preliminary phytochemical screening [14].

Test for Alkaloids

The extract was treated with diluted HCl and filtered. The filtrate is treated with various alkaloidal agents.

Mayer's Test: Sample was treated with Mayer's reagent, appearance of cream color indicates presence of alkaloids.

**Dragendroff`s Test**: Sample was treated with Dragendroff`s reagent, appearance of reddish brown precipitate indicates presence of alkaloids.

Hager's Test: Sample was treated with Hager's reagent; appearance of yellow color indicates presence of alkaloids.

Wagner's Test: Sample was treated with Wagner's reagent; appearance of brown precipitate indicates presence of alkaloids.

Test for Carbohydrates

The extracts were treated with 3 ml of α- Naphthol in alcohol and Conc.Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates.

**Fehling`s Test:** To the sample Fehling`s solution A and B was added and heated for two minutes. Appearance of reddish brown color indicates presence of reducing sugars.

Benedict's test: To the sample Benedict's solution was added and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.

Barfoed`s Test:

The sample was treated with Barfoed`s reagent and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.

Test for Proteins

Biuret`s Test: To the extracts $CuSo_4$ solution followed by sodium hydroxide solution, a violet color precipitates indicates presence of proteins.

Million's Test: To the extracts million's reagent is added, appearance of pink color indicates presence of proteins.

Test for Steroids

Libermann Burchard`s Test: The extract was treated with Conc.H_2SO_4 and glacial CH_3COOH followed by acetic anhydride, a violet ring appears at the junction of the liquids and appearance of green color in the aqueous layer indicates presence of steroids.

Test for Sterols

The extract was treated with 5%KOH solution, appearance of pink color indicates the presence of sterols.

Test for Phenols

The extract was treated with neutral $FeCl_3$ solution, appearance of violet color indicates presence of phenols.

The extract was treated with 10% NaCl solution, appearance of cream color indicates presence of phenols.

Test for Tannins

The extract was treated with 10% lead acetate solution appearance of white precipitate indicates presence of tannins.The extract was treated with aqueous bromine water; appearance of white precipitate indicates presence of tannins.

Test for Flavanoids

5ml of the extracts solution was hydrolyzed with 10%sulphuric acid and cooled. it was then extracted with diethyl ether and divided in to 3 portions in three separate test tubes .1ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxide and 1 ml of diluted ammonia solutions were added to the first second and third test tube respectively. Development of yellow color in each test tube indicates presence of flavanoids.

Shinoda`s test: The extracts were dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of **C**oncentrated. HCl and heated. Appearance of magenta color indicates presence of flavanoids.

Test for Gums and Mucilage

The extracts are treated with 25 ml absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

Test for Glycosides

A pinch of the extract was dissolved in glacial aceticacid and few drops of $FeCl_3$ solution is added followed by the addition of Conc.Sulphuric acid, formation of red ring at the junction of the two liquids indicates presence of glycosides.

Test for Saponins

Foam test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes indicates the presence of saponins.

Test for Terpenes

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

Pharmacological Screening

Experimental Animals

Swiss albino rats of either sex (125-130 g) were maintained for 7 days in the animal house under standard conditions, temperature (24 ± 10°C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48hr before the start of the experiment[22].

Assessment of antiulcer activity[22]

Pyloric ligation in rats:

Animals are divided into five groups, each consisting of six rats. Control group were received distilled water orally. Second group having pyloric ligated. Fourth& fifth Groups received ethanolic extract of *Flemingia macrophylla* in a dose of 50 and 100 mg/kg. Omeprazole, with the dose of 20 mg/kg was administered orally for third group as a reference drug for ulcer protective studies. After 45 min of EFM and Omeprazole treatment, pyloric ligation was be done by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed.

Aspirin+ pylorus ligation model:

Ulcer was induced by administering Aspirin(10 ml/kg body weight) for 7 days. All the animals were fasted for 36 hours before administration of aspirin .The animals were divided into five groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Group II receive Aspirin (10ml/Kg). Third & Fourth Groups received ethanolic extract of *Flemingia macrophylla* 50 and 100 mg/kg and, Omeprazole, in the dose of 20 mg/kg were administered orally for Fifth group as reference standard drug. The gastric ulcers were induced in rats by administrating Aspirin (10ml/Kg) Orally. All the groups were received ethanolic extract of *Flemingia macrophylla* and Omeprazole treatment for 7days .They were kept in specially constructed cages to prevent coprophagia during and after the experiment. On the 7^{th} day of experiment all the animals were anaesthetized 1h later with anaesthetic ether. Pylorus portion of stomach was lifted out and ligated (Shay et al., 1945). Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Four hours after pylorus ligation the rats were sacrificed and the stomach was removed. The gastric contents were collected. The stomach was then incised along the greater curvature and observed for ulcers. A score for the ulcer was study similar to pyloric ligation induced ulcer model.

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as given below[24,25].

Ethanol induced ulcer model:

Ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol .The animals were divided into five groups, each consisting of six rats. One Group represented the control group, which received distilled water orally. Second group receive ethanol. Third & Fourth Groups received ethanolic extract of *Flemingia macrophylla* 50 and 100 mg/kg and,

Omeprazole, in the dose of 20 mg/kg were administered orally for Fifth group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1ml/200g.) Orally, after 45 min of ethanolic extract and Omeprazole treatment .They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h later with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model[22].

Scoring of ulcer will be made as follows

Normal stomach..... (**0**)
Red coloration..... (**0.5**)
Spot ulcer.....…. **(1)**
Hemorrhagic streak... (**1.5**)
Ulcers.....… **(2)**
Perforation.....…**(3)**

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:-

$$\%\text{protective} = \frac{\text{Control mean ulcer index - test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of acidity:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1 \times 100} \; mEq/L$$

Assessment of anti-inflammatory activity

Animals:

Male and female albino rats weighing 125-130 g were obtained from a random-bed colony of mice which will be maintained on a special diet in the animal house. The animals should be housed in colony rooms with 12/12 h light/dark cycle at 21 ± 2° C and had free access to food and water.

Carrageenan-induced rat paw oedema

The rats weighing 125 - 130 g were divided into four groups, and each group consisting of six animals. Paw oedema was induced by subplantar injection of 0.1 ml of freshly prepared 1% carrageenan suspension into the right hind paw of each rat. The paw volumes were measured using a plethysmometer before as well as 60, 120,180 and 240 min after the injection of carrageenin (Winter et al., 1962). First group had received carrageenan. Second & third Groups received ethanolic extract of *Flemingia macrophylla* in a dose of 50 and 100 mg/kg. Fourth group 10 mgkg-1 indomethacin as drug control respectively, for comparative pharmacological assessment. Test drugs and vehicle were given 1 h before the injection of carrageenin. The relative potency of the drugs under investigations was calculated based upon the percentage inhibition of the inflammation [25,27].

$$VC - VT/ VC \times 100$$

VC = Control (% increase in paw volume in 3rd hour),
VT = Test (% increase in paw volume in 3rd hour).

***In-Vitro* studies for anti-inflammatory activity**

HRBC membrane stabilization method:

The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity. (Gandidasan.R, 1991) Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water) The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug (at various concentration as mentioned in table1), 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Hydrocortisone sodium was used as the reference drug. Instead of hyposaline, 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37°c for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula[26].

Optical density of drug treated sample

$$\%\text{Protection} = \frac{\text{Optical density ofdrug treated sample}}{\text{Optical density of control}} \times 100$$

3. Results and Discussion

Preliminary Phytochemical Investigation:

The revealed results of the preliminary phytochemical screening of the ethanolic extract of dried aerial parts of *Flemingia macrophylla.* The ethanolic extract has given positive results for flavanoids, glycosides.

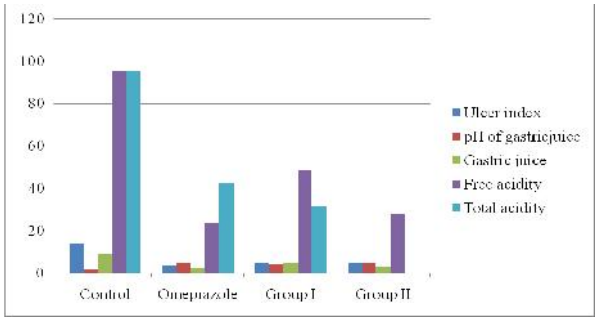

Figure 1: Effect of FME on various parameters of pylorus ligation induced ulcers in rats

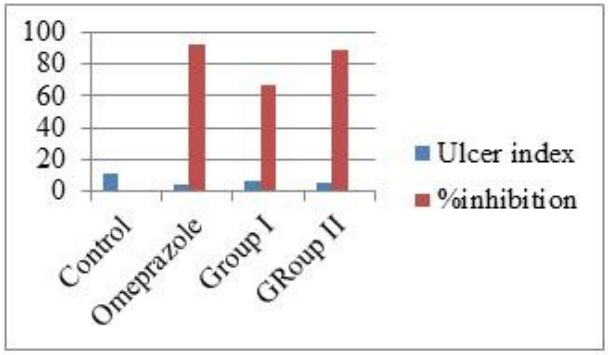

Figure 2: Effect of FME on various parameters in alcohol induced ulcer rats

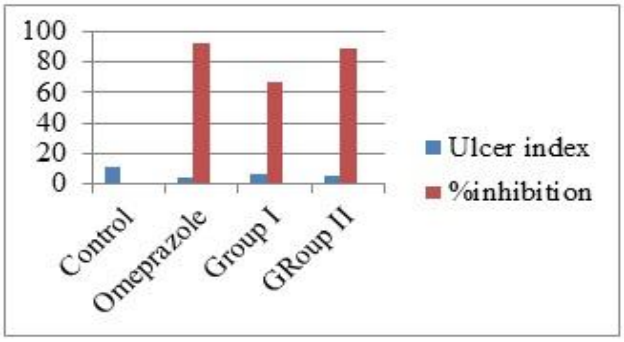

Figure 3: Effect of FME on various parameters in alcohol induced ulcer rats

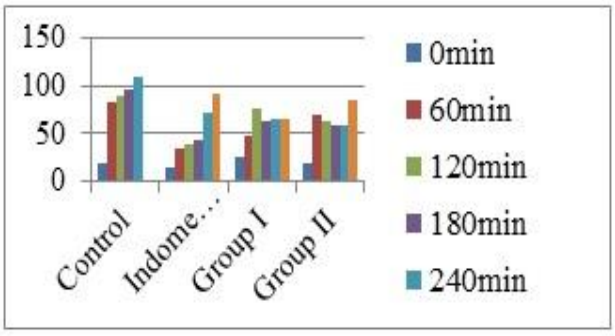

Figure 4: Effect of FME on Carrageenan induced paw edema in rats

Table 1: Experimental Design

Group	Dose	No. of animals
I	Control - treated with distil water.	6
II	Ulcer induced rats	6
III	Standard treated with omeprazole(20mg/Kg)	6
IV	With test drug extract 50mg/kg.	6
V	With test drug extract 100mg/kg**.**	6

Table 2: Assessment of anti-inflammatory activity

Group	Dose	No.of animals
I	Carrageenan induced inflammation	6
II	With test drug extract 50mg/kg.	6
III	With test drug extract 100mg/kg**.**	6
IV	Standard treated with Indomethacin (10mg/Kg)	6

Table 3: Preliminary phytochemical tests

Sr. No.	Phytochemical Tests	Results
1	Test for Alkaloids	-Ve
2	Test for Carbohydrates	-Ve
3	Test for Proteins	-Ve
4	Test for Steroids	-Ve
5	Test for Sterols	-Ve
6	Test for Phenols	-Ve
7	Test for Flavonoids	+Ve
8	Test for Gums and mucilage	-Ve
9	Test for Glycosides	+Ve
10	Test for Saponins	-Ve
11	Test for Terpenes	-Ve

+Ve: indicates the presence of compounds
-Ve: indicates the absence of compounds

Table 4: Effect of FME on various parameters in alcohol induced ulcer rats

Group	Treatment	Ulcer index	Protection (%)
I	Alcohol treated (pyloric ligated)	11.6**±0.06	……
II	Omeprazole (20mg/Kg)	4.5±0.04	93%
III	FME (50mg/Kg)	6.3**±0.03	67%
IV	FME (100mg/Kg)	5.2**±0.01	89%

Values are expressed as mean± SEM of 6 animals.
Comparisons were made between: Group I (control), Group II (Omeprazole20mg/Kg), Group III (FME 50 mg/kg) and Group IV (FME 100mg/kg).
Symbol represents the statistical significance done by ANOVA, followed by Dennett's "t" test. .* P <0.05, **P <0.01.

Table 5: Effect of FME on various parameters of pylorus ligation induced ulcers in rats

Group	Treatment	Ulcer index	protection (%)	pH of gastric juice	Gastric juice (ml)	Free acidity (meq/lt)	Total acidity (meq/lt)
I	Control (pyloric ligated)	13.8**±1.4	…….	1.4**±0.02	8.9**±0.22	95.3**±1.5	95.4**±1.2
II	Omeprazole (20mg/Kg)	3.6±0.01	90%	4.6±0.17	2.2± 0.16	23.7±3.2	42.3±1.6
III	FME (50mg/Kg)	4.7*±0.03	70%	3.9**±.03	4.7**±0.15	48.4**±1.5	31.1**±2.6
IV	FME (100mg/Kg)	4.8*±0.04	88%	4.4*±0.15	3.2**±0.18	28.1**±1.4	38.4**±1.3

Table 6: Effect of FME on various parameters in Aspirin induced ulcer in rats

Group	Treatment	Ulcer index	Protection(%)
I	Aspirin treated (pyloric ligated)	56.2**±0.3	……
II	Omeprazole (20mg/Kg)	18.6±0.21	97%
III	FME (50mg/Kg)	32.1**±0.15	58%
IV	FME (100mg/Kg)	20.3**±0.11	84%

Values are expressed as mean± SEM of 6 animals.

Comparisons were made between: Group I (control), Group II (Omeprazole 20mg/Kg), Group III (FME 50 mg/kg) and Group IV (FME 100mg/kg).

Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test. .* P<0.0, **P<0.01.

Table 7: Effect of FME on Carrageenan induced paw edema in rats

Group	Treatment	% Increase in paw volume Post insult time of assay (min)					% inhibition
		0	**60**	**120**	**180**	**240**	
I	Control (saline treated)	19.53*± 1.20	81.6**±4.22	88.9**±3.92	95.3**±6.7	109.0**±8.1	------
II	Indomethacin (10mg/Kg)	14.2±0.88	33.5*±1.8	38.9±2.3	41.8±3.2	70.9±5.07	92%
III	FME (50mg/Kg)	24.5**±1.81	46.8±2.13	76.32**±3.5	61.8*±4.2	63.9*±5.07	64%
IV	FME (100mg/Kg)	19.28*±0.83	70.3**±4.7	63.2**±2.5	58.6*±3.8	58.8*±3.92	85%

Table 8: Effect of FME on Human Erythrocyte Haemolysis

Treatment	% prevention of lysis
Control (saline treated)	……
Indomethacin (10mg/Kg)	79%
FME (50mg/Kg)	66%
FME (100mg/Kg)	75%

Phyto chemical Screening

The results of preliminary phytochemical screening of the ethanolic extract of *Flemingia macrophylla* (FME) revealed that presence of flavonoids, glycosides.

Effect of FME on various parameters of pylorus ligation induced ulcers in rats:

Gastric acidity in ulcer formation in the mouse may be secondary to gastric distention and interference with circulation[7]. In pylorus ligation induced ulcer model, orally given FME in two different dose had shown their significant action in ulcerindex, pH gastric juice, total acidity, free acidity as compared to standard omeprazole. Percentage inhibition of group III & group IV was 70% &88% respectively where as for standard it is 90%.(Table no :2).

Effect of FME on various parameters in alcohol induced ulcer rats: Alcohol and the caffeine content of coffee act directly on the gastric mucosa to stimulate acid and pepsinogen secretion, so reduction in their use should help the patient. Smoking can also worsen dyspepsia (indigestion) and heartburn, possibly via actions of nicotine on the stomach wall[31]. In control group ethanol had induced characteristic leisions in the glandular portion of stomach which appeared as thick red, black colour leisions. The percentage protection of extract treated in group III & IV was 67%,89% respectively where as 93% standard

treated group. Ulcer index in FME treated groups had shown significant action in comparison with standard(Table no 3).

Effect of FME on various parameters in Aspirin induced ulcers in rats: Aspirin's ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase (PTGS) enzyme required for prostaglandin and thromboxane synthesis[7].Mucosal prostaglandin deficiency increases susceptibility to ulcer formation and that exogenous administration of supplemental prostaglandins reduces ulcer risk. This article reviews the role that mucosal prostaglandins play in defense of the gastric and duodenal mucosa against injury and ulceration[31]. *gasroenterol*In FME(50mg/Kg) group, FME(100mg/Kg) group ulcer index had shown significant action in comparison to standard treated group. Percentage protection of the two extract treated group had shown the same significant action with 58%,84% repectively where as 97% in standard group. In this model aspirin induced leisions were inhibited by the extracts but the action was somewhat less significant than group IV(Table no 4).

Effect of FME on Carrageenan induced paw edema in rats: The ethanolic extract of *Flemingia macrophylla* had shown more significant action in reducing the carrageenan induced paw edema in comparision to standard group. FME had shown the inhibitory action for every interval of time i.e for 0,60, 120,180, 240 min. However the percentage inhibition of group III & IV is 64%,85% respectively where as 92% for standard (Indomethacin 10mg/Kg) treated group(Table no:5).

Effect of FME on Human erythrocyte haemolysis:

The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG). The main action of enzyme is conversion of prostaglandin G2 (PGG2) to PGH2 along with peroxidation which is associated with formation of long channels in membranes. The channel opening occurs due to release of chemical mediations and so arachidonic acid is released from membrane and converted to prostaglandin. The extracellular activity of these enzymes is said to be related to acute and chronic inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) act either by inhibiting these lysosomal enzymes (Cyclooxygenase) or by stabilizing the lysosomal membrane[26].

The anti-inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased[34]. Since HRBC membrane are similar to lysosomal membrane components the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. At concentration of FME 50mg/Kg had shown less significant inhibition where as FME 100mg/Kg had shown significant action in comparision with standard sample. The percentage inhibition is 66%,75% respectively for groupIII & group IV in which inhibition is just near by the percentage of standard (Indomethacin 10mg/Kg) i.e 79%(Table no 6).

4. Conclusion

The present study reveals that *Flemingia macrophylla* exhibit a significant anti ulcer and anti inflammatory agent. Both *In vivo* studies and In *vitro* studies of the extract show a significant activity with the standard drug.FME treated animals had shown significant action in both anti ulcer & anti inflammatory activity. In antiulcer studies ulcer index, percentage protection was more significant in comparison to standard. In the same way anti inflammatory action had shown cytoprotective activity in RBC membrane stabilization methods. From the above studies it has been concluded that *Flemingia macrfophylla* has both antiulcer & anti inflammatory activity. Further studies are carrying to find out the active ingredients which are responsible for both activites.

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