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EVALUATION OF GASTROPROTECTIVE ACTIVITY OF LEAVES EXTRACT OF *ACACIA LONGIFOLIA* ON INDOMETHACIN INDUCED GASTRIC ULCERS IN RATS

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ABSTRACT

The aim of the present investigation is to evaluate the gastroprotective effects of ethanolic extracts of *Acacia Longifolia* leaves against Indomethacin Induced gastric ulcers in rats. Peptic ulcer disease is a major health problem with multifactorial etiology. Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factors. In spite of the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear. Although in most of the cases the etiology of the ulcers is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defence mechanisms. To regain the balance, different therapeutic agents including plant extracts are used. *Acacia Longifolia* leaf extract is one such herbal drug currently undertaken to evaluate its anti-ulcer potential.

KEYWORD: *Acacia Longifolia* **,** Peptic ulcer, Gastroprotective effects

1. INTRODUCTION

An excoriated section of the stomach or intestinal mucosa, primarily brought on by the digesting action of gastric juice or upper small intestine secretions, is known as a peptic ulcer. An imbalance between the pace of gastric juice secretion and the level of protection provided by the gastro-duodenal mucosal barrier, as well as the neutralization of gastric acid by duodenal fluids, is the typical cause of peptic ulceration. Peptic ulcers usually develop near the lower portion of the oesophagus, where stomach acids

commonly reflux, or along the smaller curve of the stomach's antral end. Peptic ulcers are persistent, usually isolated lesions that develop in any part of the gastrointestinal tract that is subjected to the harsh action of peptic fluids and acid. Gastric ulcers, another name for stomach ulcers, are inconvenient lesions in the lining of the stomach. Peptic ulcer disease includes stomach ulcers as a subtype. Any ulcers that impact the small intestine as well as the stomach are classified as peptic ulcers. Reduced thickness of the thick mucus layer that shields your stomach from digestive juices might lead to stomach ulcers. This makes it possible for the stomach's lining tissues to be eroded by the digestive acids, leading to ulcer development. Although stomach ulcers are easily treatable, if left untreated, they can worsen.

1.1 Causes stomach ulcers

Stomach ulcers are almost always caused by one of the following:

a. An infection with the bacterium *Helicobacter pylori (H. pylori)*

b. Long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, or naproxen

Rarely, a condition known as Zollinger-Ellison syndrome can cause stomach and intestinal ulcers by increasing the body's production of acid. This syndrome is suspected to cause less than 1 percent of all peptic ulcers.

1.2 Symptoms of stomach ulcers

Stomach ulcers are linked to several symptoms. The degree of the ulcer determines how severe the symptoms. A burning feeling or discomfort in the middle of your abdomen, between your chest and belly button, is the most typical symptom. When your stomach is empty, the pain usually gets worse and lasts anywhere from a few minutes to several hours.

2. Material and Method

2.1 Collection of plant material

The plant material was collected from Vindhya herbal nursery Bhopal. Drying of fresh leaves was carried out in sun but under the shade. Dried Leaves were preserved in plastic bags and closed tightly and powdered as per the requirements.

2.2 physio-chemical parameters

Based on standard procedures, physicochemical parameters such as percentage of total ash, acid insoluble ash, water soluble ash and loss on drying were calculated (Pradhan *et al.*, 2010; Ansari, 2001).

2.2.1 Determination of ash values: As a result, the collected ash was cooled, weighed, and the air-dried product was used to determine the proportion of ash. Ash Values of extract were calculated by using following formula

$$\textbf{Ash Value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \text{x } 100$$

2.2.2 Moisture contentLoss on drying

The leaves of *Acacia longifolia* was ground into a 1 gram powder and put into a petridish. After one hour of heating at 105 ^{0}C in a hot air oven, the loaded plate was cooled in desiccators. Moisture content was measured as weight loss. The samples' respective moisture content percentage was computed.

2.2.3 Extraction by maceration process

75 gram leaves of *Acacia longifolia* was exhaustively extracted with ethanol solvent and using drug – solvent ratios (1:2) using maceration process (24 hrs). The extracts were evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

The percentage yields of each extract were calculated by using following formula:

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

2.3 Phytochemical Screening

The *Acacia longifolia* extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Terpenoids, Saponins, Steroids.

2.4 Estimation of total Phenolic, flavonoid and alkaloid Content

2.4.1 Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols. 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.4.2 Total flavonoids content estimation

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

2.5 *In Vivo* antiulcer activity

Wistar rats (1–180 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. Preliminary experiments were carried out on rats (n=6). A Ethanolic leaves extract of *Acacia longifolia* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2001). Animals were kept fasting providing only water, amoxicillin microspheres were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

Table 2.1 Experimental designs of Indomethacin induced gastric ulcer

Group	Treatment	Dose
Group-1	Normal control	1% CMC
Group-2	Positive control	1% CMC+ Indomethacin 40 mg/ kg
Group-3	Test group	ELEAL (100 mg/kg) + Indomethacin 40 mg/ kg
Group-4	Test group	ELEAL 200 mg/kg + Indomethacin 40 mg/ kg
Group-5	Standard group	Cimetidine (10 mg/kg) +Indomethacin 40 mg/kg

The ulcer index was determined using the formula:

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

3. RESULTS AND DISCUSSION

3.1 Result of physicochemical parameters of ethanolic extract of *Acacia longifolia*

Table 3.1: Results of Physiocochemical parameters of ethanolic extract of *Acacia longifolia*

S.N.	Parameters	*Acacia longifolia* extract
1	Color	Color varies from Greenwish to brown
2	Odour	Pungent
3	Loss on drying	4.915
4	Taste	Bitter
5.	Total ash % w/w	9.58

3.2 Results of percentage yield of *Acacia longifolia*

The fresh leaves of *Acacia longifolia* were evaluated for its anti ulcer activity. The various observation and results obtained from evaluation are discusses in this chapter. The ethanol solvents used for extraction of bioactive compounds from *Acacia longifolia* (75gm). The percentage yield of ethanol extracts of *Acacia longifolia* was calculated and is shown in Table 3.2.

Table 3.2: Results of percentage yield of *Acacia longifolia*

S.N.	Plant Name	Percentage Yield (%)
1	*Acacia longifolia* ethanolic extract	2.28

3.3 Results of Preliminary phytochemical investigation of the extract

Table No. 3.3: Result of Phytochemical screening of *Acacia longifolia* ethanolic extract

S. no.	Constituents	Ethanolic extracts
1.	**Alkaloids**	
	Dragendroff's test	**-ve**
	Wagner's test	**-ve**
	Mayer's test	**-ve**
	Hager's test	**-ve**
2.	**Glycosides**	**+ve**
	Legal's Test	
3.	**Flavonoids**	
	Lead acetate test	**+ve**
	Shinoda test	**+ve**
	Phenolics	**+ve**
	Ferric Chloride Test	
	Saponin	**+ve**
	Froth Test	
4.	**Tannins**	**+ve**
	5% $fecl_3$ test	
5.	**Proteins and Amino acids**	
	Xanthoproteic Test	**-ve**
	Ninhydrin test	**-ve**
6.	**Cabohydrates**	
	Fehling's Test	**+ve**
7.	**Diterpines**	
	Copper acetate Test	**+ve**

3.4 Result of Estimation of Total phenolic and Total flavonoids content

Table -3.4: Estimation of total phenolics and total flavonoids content

S. No	Extract	Total phenolic content (mg/ml)	Total flavonoids Content (mg/ml)
1	**Ethanolic leaves extract *Acacia longifolia***	1.27	0.93

3.5 Results of *In Vivo* antiulcer activity

Table -3.5: Anti-ulcerogenic effect of Ethanolic leaves extract *Acacia longifolia* against ulcerogenic agents in rats (Ulcer index)

S.N.	Animal (n=6)	Dose and Treatment	Ulcer index
1	**Group 1** Normal control	CMC	0.78±0.25
2	**Group 2** Positive control	CMC +IND	5.7±1.6*
3	**Group 3** Test group	ELEAL (100 mg/kg, p.o.) +IND	4.31±0.21**
4	**Group 4** Test group	ELEAL (200 mg/kg, p.o.)+IND	2.58±0.11**
5	**Group 5** Standard group	Cimetidine (10 mg/kg, p.o.) +IND	1.76±0.11**

CMC-Carboxy methyl cellulose, ELECG-Ethanolic leaves extract *Acacia longifolia* IND-Indomethacin, Data expressed as Mean±SEM (n=6), *p <0.05-when compared with normal control, **p <0.01-when compared with the positive control.

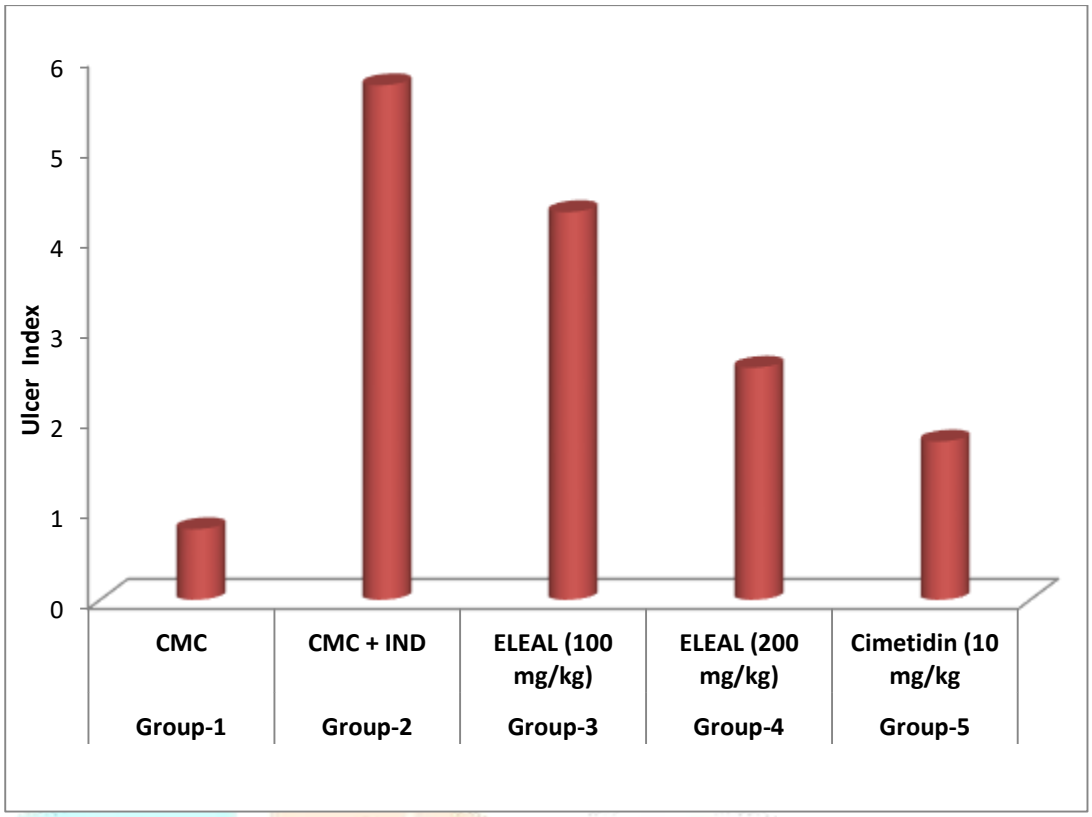

Fig: 3.1 Anti-ulcerogenic effect of ethanolic leaves extract *Acacia longifolia* against ulcerogenic agents in rats (Ulcer index) ethanol induced gastric ulcers model

The positive control group (group-2) treated with indomethacin alone produced significant mucosal erosions with occasional bleeding resulting in increased ulcer index score. The group pre-treated (group-5) with standard drug Cimetidine and the group pre-treated with Ethanolic leaves extract *Acacia longifolia* (group 3and group-4) showed a significant decrease ($p < 0.001$) in ulcer index when compared to the positive control group (group-2). Ulcer index for group pre-treated with extract of Ethanolic leaves extract *Acacia longifolia* (group-3 and group-4) was comparable with that of the standard group.

The present study investigated the effect of Ethanolic leaves extract *Acacia longifolia* on the gastric ulcers. Ethanolic leaves extract *Acacia longifolia* showed effect on the healing of gastric ulcers induced by indomethacin. It acts through the inhibition of cell wall biosynthesis that leads to the death of the bacteria. Ethanolic leaves extract *Acacia longifolia* was effective in reducing the ulcer area and the ulcer score. *Acacia longifolia* has an antiulcer effect. It increased healing of indomethacin induced ulcer.

The study showed the antiulcer activity of Ethanolic leaves extract of *Acacia longifolia* against indomethacin-induced gastric ulcer models. NSAID's are the most common OTC drugs which are used extensively for all types of pain. Indomethacin is a non-selective COX inhibitor, inhibits the arachidonic acid pathway and the formation of protective prostaglandins. Prostaglandins especially PGE-2 and PGI-2 are antiulcerogenic and they play a crucial role in gastric mucus secretion and mucosal blood flow. Apart from that NSAID's also inhibit mucosal cell proliferation and bicarbonate secretion thereby greatly reducing the integrity of the gastric mucosa. Administration of indomethacin in test groups produced significant visible gastric

erosions, bleeding and necrosis thereby increasing the ulcer index score. Groups pre-treated with Ethanolic leaves extract of *Acacia longifolia* showed a significant reduction in the intensity of gastric lesions, and ulcer index score.

CONCLUSION

The analysis and characterization of bioactive compounds from plants is important to ascertain their medicinal value. The phytochemical analysis showed that the ethanolic extract of *Acacia longifolia* plant contains a mixture of phytochemicals as Glycosides, Carbohydrates, Saponins, Tannin, Diterpenes, Phenols and Flavonoids. The total phenolics and total flavonoids content for the ethanolic extract was found to be 1.27 and 0.93 mg/ml in leaves extract of Acacia longifolia. The present study investigated the effect of ethanolic leaves extract of *Acacia longifolia* on the ulcers. ethanolic leaves extract of *Acacia longifolia* showed effect on the healing of gastric ulcers induced by indomethacin. It acts through the inhibition of cell wall biosynthesis that leads to the death of the bacteria. Ethanolic leaves extract of *Acacia longifolia* was effective in reducing the ulcer area and the ulcer score. *Acacia longifolia* has an antiulcer effect. It increased healing of indomethacin induced ulcer.

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