Evaluation aqueous bark and pods extracts of Antiulcer activity of *Acacia concinna*

Syeda Shaheen Sulthana[1]*, Dr K. Appanna Chowdary[2] , Dr Sumer Singh[3]

1.School of pharmacy and medical sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, India- 333515

2.St. Ann's College of Pharmacy, Contonment, Vizianagaram, Andhra Pradesh, India-535003

3. School of Life sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, India- 333515

***Corresponding author: Syeda shaheen sulthana, School of pharmacy and medical sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, India,**

E-mail:-sha_sss1984@yahoo.co.in

ABSTRACT

Preliminary phytochemical investigations of Aqueous extracts of bark and pods of *Acacia concinna* revealed the presence of flavonoids and saponins. The aqueous bark extract showed significant reduction in the pylorus ligation induced ulcers in rats. These results emphasize on the need to diversify in to alternative therapeutic approaches pertaining to herbal medicine. Where in a single easily available plant may provide answers to several therapeutic challenges as observed in the antiulcer activity shown by aqueous extract of *Acacia concinna*

Keywords: Antiulcer activity, Aqueous extract, Methanolic extract, Pyloric ligation, *Acacia concinna*

INTRODUCTION

Gastric hyperacidity and ulceration of the stomach mucosa due to various factors are serious health problems of global concern. Peptic ulcer disease (encompassing gastric ulcer and duodenal ulcer) affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs[1] . The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer[2]. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward in minimizing tissue injury in human disease[3] . Oxygen radical absorbance capacity assay (ORAC), is one of the most popular and best standardized chemical in vitro antioxidant assay[4-7]. It is widely used for evaluation and comparison of the antioxidant capacity in natural products[8] . Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with reenforcing gastric mucosal protection[9, 10]. Although a number of anti-ulcer drugs such as H_2 receptor antagonists, proton pump inhibitors and cytoprotectants are available, all these drugs have side

effects and limitations[11]. There is, thus, a need to search for natural alternatives having anti-ulcer properties. This has been the basis for the development of new anti-ulcer agents, which include herbal substances. In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers[12]. The first drug effective against gastric ulcer was carbenoxolone, discovered as a result of research on a commonly used indigenous plant, *Glycyrrhiza glabra*[13]. Studies on cabbage previously employed as an anti-ulcer agent in folk medicine, has led to the development of Gefarnate[14]. Banana fruit has also been found to inhibit peptic ulceration[15] . Liquorice, the roots and rhizomes of *Glycyrrhiza glabra* Linn. (Family: *Leguminosae*) is one of that hypothesis As there have been no studies conducted earlier to evaluate the antiulcerogenic activity of pods and bark of tree *Acacia concinna*

In this study, we tend to hypothesize that, pods of tree *Acacia concinna* have antiulcerogenic activity, attributable to the presence of saponins and flavonoids they possess antiulcer activity. Therefore the studies were undertaken to judge the attainable antiulcer activity isolated compounds of liquid extract of pods and bark of tree *Acacia concinna*

MATERIALS AND METHODS

1.Collection and extraction

The bark and pods of *Acacia concinna* freed from extraneous matter, shade dried for 10 days and then powdered. The powders were passed through a 40-mesh sieve and then subjected to extraction. 900 gms of the powders were extracted separately using distilled water as a solvent in a Soxhlet apparatus. The residues obtained were dried on a hot water bath at a temperature of 50^0C. The weight of the extracts on drying was 98 g. On calculation, the percentage yields of bark and pods were found to be 12.25% and 23.78g respectively. Further, the dried extracts were subjected separately for preliminary phytochemical screening for the identification of various phytochemical constituents in bark and pods[16]..

2. Experimental Animals:

Albino Wister rats weighing 150-200 g which were procured from the Animal house of PES College Of Pharmacy, were used throughout the experiment. The experimental animals were maintained under standard laboratory conditions with a 12-h light/dark cycle under controlled temperature. All the animals were acclimatized to the laboratory conditions for at least one week before the commencement of the experiment. All the experiments were performed in accordance with the CPSCEA guidelines and approved by Animal ethics committee, PES College Of Pharmacy, Bangalore 560050.**Ref No:-*PESCP/2018/IAEC/0212***

3. Acute Oral Toxicity Studies:

Acute oral toxicity studies of ABE of AC and APE of AC were performed on Albino rats in accordance with the OECD guidelines 425. The animals were kept for fasting for 24 hours. Firstly they were treated with an oral dose of 1000 mg/kg body weight with the extracts dissolved in water. The

animals were continuously observed for 3-4 hours for any changes in their general behavior, morbidity and finally death during a period of 24 hours.

There were no signs of toxicity at a dose of 1000 mg/kg, then a higher dose of 2000 mg/kg body weight of the aqueous extracts of ABE of AC and APE of AC was administered for the assessment of all the biological profiles. Three dose levels were chosen in such a way that, the middle dose (200 mg/kg) was approximately one-tenth of the maximum dose during acute toxicity studies and a low dose (100 mg/kg) which was half of the one-tenth of the maximum dose and a high dose (400 mg/kg) which was twice that of the one-tenth dose of the maximum dose [17].

3. IN VIVO ANTIULCER ACTIVITY

1. Pylorus ligation induced ulcer model[18]

Pyloric ligation (PL) induced ulcers were caused due to an imbalance between offensive and defensive mucosal factors. It is an ideal model to infer the mechanism by which a drug works as an anti-ulcerogenic agent. PL-induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion. Hence, the estimation of acid secretion is a valuable part of the study to clarify the mechanism of action of the drug under trial. Anti Ulceric agents such as omeprazole prevent acid secretion and ulcers.

i. Experimental design:

Fifty-four rats of either sex were randomly allotted to 9 groups each consists of six animals. Group, I served as the vehicle control (10 ml/kg orally); Group II served as the pylorus ligation control (4 h); Group III served as standard control (Omeprazole – 10 mg/kg); Group IV, V and VI – Aqueous bark extract of *Acacia concinna (ABE of AC)* p.o (at the dose levels of 100,200 and 400 mg/kg b.w); and Group VII, VIII, and IX – Aqueous pods extract of *Acacia concinna (APE of AC)* p.o (at the dose levels of 100,200 and 400 mg/kg b.w); were administered orally respectively by gavage using feeding needle. The animals were fasted for 48 h before the test substance administration but had free access to water. After 1 h of administration of test doses, under light ether anesthesia, the abdomen was opened by a small midline incision below the xiphoid process. Pylorus ligation was done without causing any damage to the blood supply of the stomach. The stomach was placed inside carefully and the abdominal wall was closed by interrupted sutures. Animals were allowed to recover and stabilize in an individual cage and were deprived of water during the post-operative period.

Animals were sacrificed after 4 h and the stomachs were removed. Gastric content was centrifuged at 2000 rpm for 10 min. The volume of total gastric juices were measured. 1 ml of gastric content was diluted with distilled water to make it up to 10 ml, and the pH of this solution was determined with the help of the pH meter. To it, 2-3 drops of phenolphthalein indicator were added and titrated against 0.01N NaOH. The total volume of 0.01N NaOH consumed was noted and total acidity in term of meq/L/100 g was calculated. Stomachs were incised along the greater curvature and kept overnight in 10% formalin solution. Next day, the ulcers were examined under a magnifying lens. The ulcers were measured with the help of vernier caliper using the following arbitrary scale:

0 = No ulcers,

1 = Petechial hemorrhages,

2 = Ulcers < 2 mm,

3 = Ulcers > 2 < 4 mm,

4 = Ulcers > 4 mm

Total ulcer index was calculated by addition of the arbitrary scale of individual groups.

2. INDOMETHACIN INDUCED ULCER MODEL[19]

Anti-inflammatory drugs like Indomethacin when administered produce visible gastric ulcers in animals. Ulcers are observed as perforations, petechial hemorrhages, pH of gastric juice and finally calculating them as total ulcer score. Indomethacin is a potent inhibitor of prostaglandin biosynthesis and prostaglandins are known to play an important role in maintaining mucosal integrity. Anti-ulcer drugs like Omeprazole, Ranitidine etc., were known to inhibit the formation of ulcers.

i. Experimental design:

Fifty-four rats of either sex were randomly allotted to 9 groups each consists of six animals. Group, I served as the vehicle control (distilled water 10 ml/kg) orally; Group II served as the Indomethacin control (40 mg/kg b.w); Group III served as standard control (Omeprazole – 10 mg/kg); Group IV, V and VI – Aqueous bark extract of *Acacia concinna (ABE of AC)* p.o (at the dose levels of 100,200 and 400 mg/kg b.w); and Group VII, VIII, and IX – Aqueous pods extract of *Acacia concinna (APE of AC)* p.o (at the dose levels of 100,200 and 400 mg/kg b.w); were administered orally respectively by gavage using feeding needle. The animals were fasted for 48 h before the test substance administration but had free access to water.

Animals were sacrificed 6 h after administration of indomethacin, the stomachs were removed and pH was determined. Stomachs were incised along the greater curvature and kept overnight in 10% formalin solution. Next day, the ulcers were examined under a magnifying lens. The ulcers were measured with the help of vernier caliper using the following arbitrary scale:

0 = No ulcers,

1 = Petechial hemorrhages,

2 = Ulcers < 2 mm,

3 = Ulcers > 2 < 4 mm,

4 = Ulcers > 4 mm

Total ulcer index was calculated by addition of the arbitrary scale of individual groups

3. COLD STRESS INDUCED ULCER MODEL[19]

Cold stress ulcer (CSU) is a well-accepted model for the induction of gastric ulcer in which peripheral sympathetic activation plays an important role in the induction of ulcers. Stress has been reported to play an important role in gastro - duodenal ulceration etiopathology, increased gastric motility, vagal overactivity, degranulation of mast cells ; decreased gastric mucosal blood flow and decreased prostaglandin synthesis. Any of these factors could play a role in the genesis of stress-induced ulcers.

Significantly, when the source of stress is cold as in CSU, the incidence of ulcers is mainly due to increased acid secretion and generation of free radicals etc. Hence worth, the significant decrease in the ulcer index in CSU model suggests its involvement in decreasing the acid secretion. An anti-ulcer drug like Omeprazole is known to inhibit the formation of ulcers.

i. Experimental design:

Fifty-four rats of either sex were randomly allotted to 9 groups each consists of six animals. Group, I served as the vehicle control (distilled water 10 ml/kg) orally; Group II served as the CSU control (kept in the refrigerator for 4 h at 4±1°C). Group III served as standard control (Omeprazole – 10 mg/kg); Group IV, V and VI – Aqueous bark extract of *Acacia concinna (ABE of AC)* p.o (at the dose levels of 100, 200 and 4000 mg/kg b.w); and Group VII, VIII, and IX – Aqueous pods extract of *Acacia concinna(APE of AC)* p.o (at the dose levels of 100, 200 and 4000 mg/kg b.w); were administered orally respectively by gavage using feeding needle. The animals were fasted for 48 h before the test substance administration but had free access to water.

After 1 h of administration of test doses, all the four limbs of the rats were stuck on a wooden plank with the help of leucoplast. A shoulder collar was kept to restrain their head. The remaining body was also restrained with tapes. The animals were completely restrained. The temperature of the refrigerator was fixed at 4 ± 1º C. The restrained animals were then placed in the refrigerator for 4 h respectively.

Animals were sacrificed 4 h after exposure to cold stress, the stomachs were removed and pH was determined. Stomachs were incised along the greater curvature and kept overnight in 10% formalin solution. Next day, the ulcers were examined under a magnifying lens. The ulcers were measured with the help of vernier caliper using the following arbitrary scale:

0 = No ulcers,
1 = Petechial hemorrhages,
2 = Ulcers < 2 mm,
3 = Ulcers > 2 < 4 mm,
4 = Ulcers > 4 mm

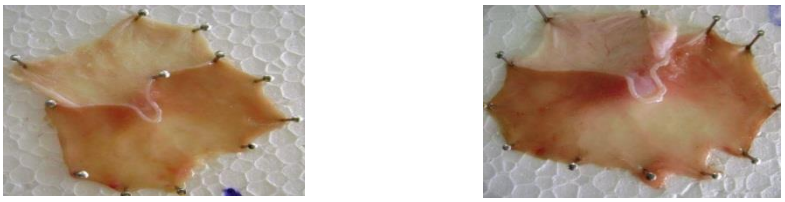
Total ulcer index was calculated by addition of the arbitrary scale of individual group

RESULTS

1.Pylorus ligation induced ulcer

Fig:- 1 Anti-ulcer activity of aqueous bark and pods extract of A*cacia concinna* in pylorus ligation induced ulcer model using albino Wistar rats.

NORMAL CONTROL OMEPRAZOLE MG/KG



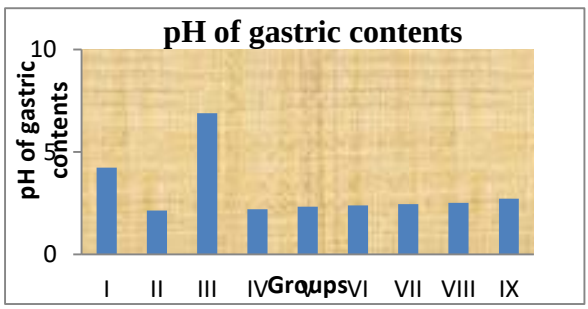
ABE OF AC 400MG/KG APE OF AC 400MG/KG

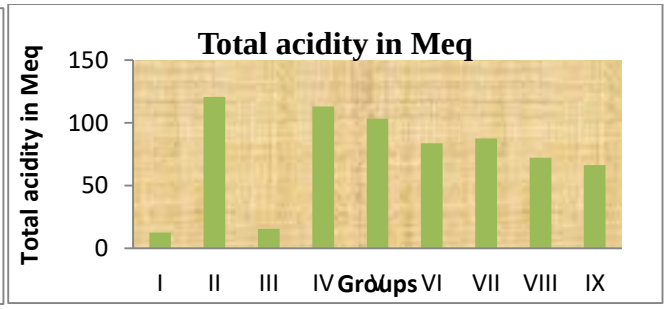
Table1: Anti-ulcer activity of aqueous bark and pods extract of *Acacia concinna* in Pylorus Ligation induced ulcer model using albino Wistar rats.

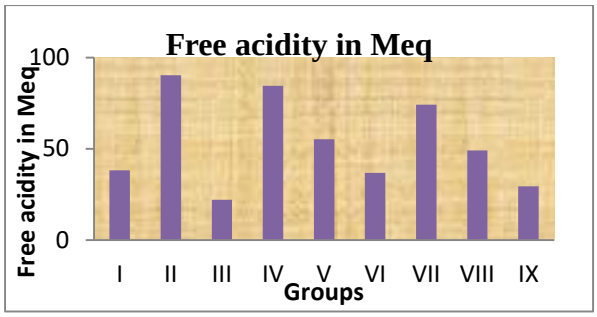
GPS	pH of gastric contents	Gastric Content in ml	Total acidity in meq	Free acidity in meq	Ulcer index Mean±SEM	% Protection
1	4.23±3.10	3.21±4.43	12.32±4.09	38.21±2.33	3.09±2.19	---
2	2.14±0.05	6.33±0.3	120.66±5.55	90.23±2.34	30.41±2.16	---
3	6.89± 0.13*	2.41±0.23**	15.33±0.91**	22.12±3.49**	3.33±1.38**	88.92±0.21
4	2.20±0.06	5.41±0.35	113.03±0.21	84.59±2.01	18.66±0.21	37.80±2.18
5	2.33±0.12	4.58±0.53*	103.36±9.36*	55.21±6.33*	7.66±2.11*	74.46±0.32
6	2.39±0.07**	4.83±0.33**	83.66±8.91*	36.77±3.88**	6.66±3.10**	77.80±3.12
7	2.45±0.12	4.25±0.47*	87.33±1.97*	74.10±2.89*	8.52±3.21**	71.66±2.19
8	2.52±0.17**	3.83±0.43**	72.11±2.23**	49.12±2.33**	5.66±2.17**	81.66±0.34
9	2.72±0.21**	3.33±0.35**	66.19±3.16**	29.44±2.30**	3.52±2.09***	88.33±1.59

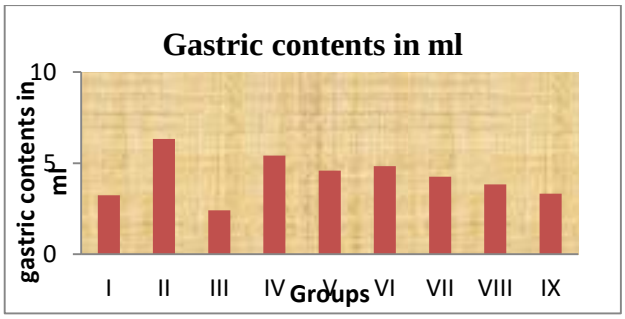
Results are expressed as Mean ± SEM; (n=6). Significance at P<0.05*, P<0.01** as compared to control ANOVA followed by Dunnett's multiple comparison test.

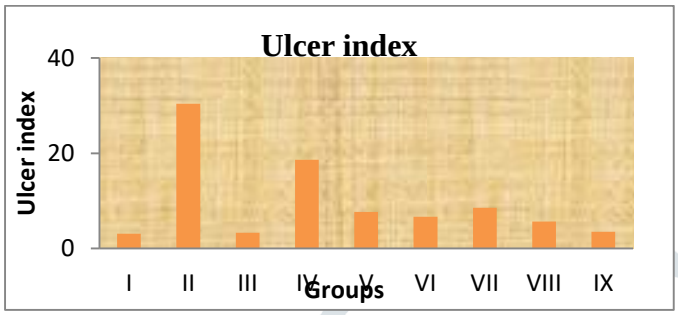
Graph 1 Effect of pH, total acidity, free acidity, vol gastric contents and ulcer index paranmeters on pylorus ligation induced ulcer model in albino Wistar rats











In the present study, the meanof pH, gastric content, total acidity and ulcer index at various doses of test substances is presented in Pylorus ligation at 6th h showed potent ulcerogenic activity. Reference standard used here was omeprazole (Dose 10 mg/kg) which showed potent antiulcer activity significantly at all parameters. Volume of gastric content was reduced significantly by Aqueous bark extract of *Acacia concinna* at the dose level of 200 mg/kg and 400 mg/kg. Total acidity was inhibited by Aqueous bark extract of *Acacia concinna* at all the dose levels. Total acidity was reduced by 45.30% and 30.66% by Aqueous bark extract of Acacia concinna and Aqueous pods extract of *Acacia concinna* respectivelyat their highest dose. Aqueous bark extract of *Acacia concinna* at the dose level of 200 mg/kg and 400 mg/kg. also inhibited ulcerogenic activity of pylorus ligation significantly. Aqueous bark extract of *Acacia concinna* at a dose of 400 mg/kg b.w inhibited ulcer index by 88.33% compared to Aqueous pods extract of *Acacia concinna*, which at the same dose level inhibited ulcer index by 77.8%

2.NSAID 'S Induced ulcers

Fig:-2 Anti-ulcer activity of Aqueous bark and pods extract of *Acacia concinna* in indometheacin induced ulcer model using albino Wistar rats.

NORMAL CONTROL OMEPRAZOLE

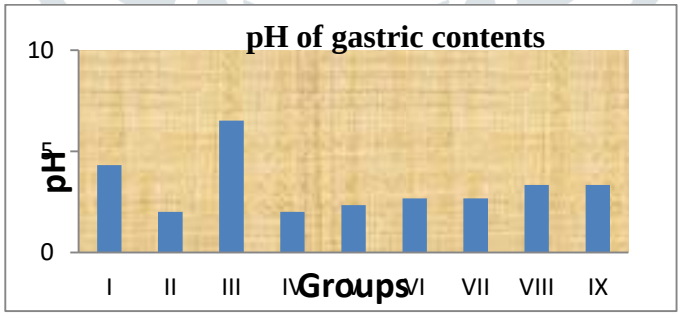
ABE OF AC 400MG/KG APE OF AC 400MG/KG

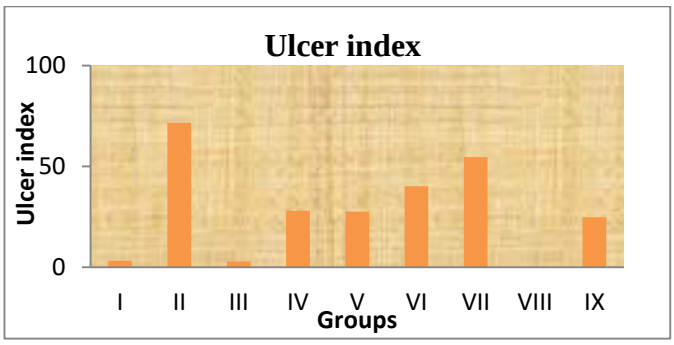
Table:-2 Anti-ulcer activity of Aqueous barks and pods extract of *Acacia concinna* in Indomethacin induced ulcer model using albino Wistar rats.

GPS	Treatment	pH mean ± sem	Ulcer Index Mean ±SEM	% Inhibition Of ulcer index
I	Normal vehicle control 10ml/kg	4.32±3.21	3.09±2.19	--
II	Indomethacine induced ulcers	2.00±0.08	71.5±3.87	-
III	Omeprazole (10 mg/kg)	6.52±2.11*	2.83±1.45***	96.04
IV	Aqueous pods extract of *Acacia concinna* (100 mg/kg)	2.00±1.89	27.83±8.02**	61.07
V	Aqueous pods extract of *Acacia concinna* (200 mg/kg)	2.33±2.89	27.66±5.54**	61.31
VI	Aqueous pods extract of *Acacia concinna* (400 mg/kg)	2.66±0.42*	40.16±1.06	44.05
VII	Aqueous bark extract of *Acacia concinna* (100 mg/kg)	2.66±0.42*	54.50±4.02	23.77
VIII	Aqueous bark extract of *Acacia concinna* (200 mg/kg)	3.33±0.77*	32.16±6.23**	55.02
IX	Aqueous bark extract of *Acacia concinna* (400 mg/kg)	3.33±0.68*	24.66±2.13**	65.51

Results are expressed as Mean ± SEM; (n=6). Significance at P<0.05*, P<0.01** as compared to control ANOVA followed by Dunnett's multiple comparison test

Graph 2 pH and Ulcer index of gastric content of treated groups in Indomethacin induced ulcer modelusing albino Wistar rats

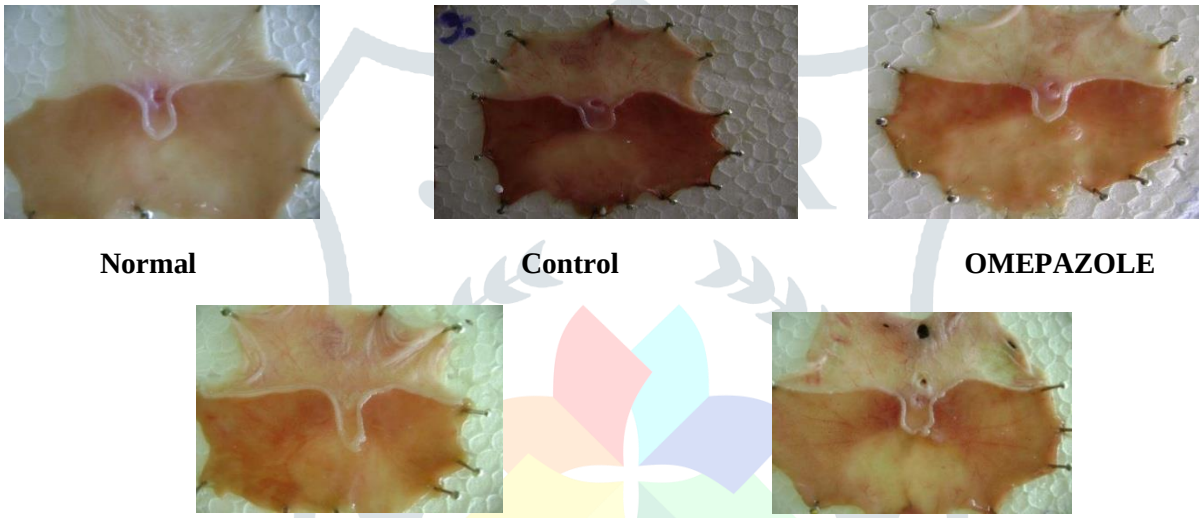




In the present study, the meanof pH andulcer index at various doses of test substances and Indomethacin at the dose levels of 400 mg/kg b.w. showed potent ulcerogenic activity. Reference standard used here was omeprazole (dose - 10 mg/kg) showed potent anti ulcer activity significantly. Aqueous pods extract of Acacia concinna at the dose level of 100 mg/kg and 200 mg/kg significantly inhibited ulcer inductionAqueous bark extract of Acacia concinna at the dose level of 200 mg/kg and 400 mg/kg inhibited ulcerogenic activity of indomethacin significantly.Aqueous bark extract of Acacia concinnaat a dose of 400 mg/kg b.winhibited ulcer index by 65.51% compared to Aqueouspods extract of Acacia concinna, which at the same dose level inhibited ulcer index by44.05%.

3.Cold stress induced ulcerd

Fig:-3 Anti-ulcer activity Aqueous pods and bark extract of *Acacia concinna* Cold stress induced ulcer (CSU) model using albino Wistar rats.



Normal **Control** **OMEPAZOLE**

ABE OF AC 400mg/Kg **APE OFAC 400mg/Kg**

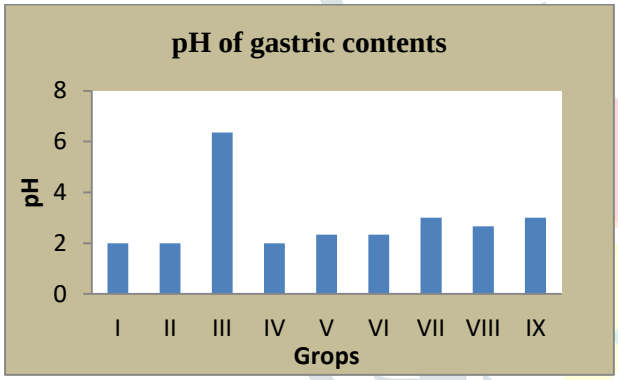
Table :-3.Anti-ulcer activity Aqueous pods and bark extract of *Acacia concinna* cold stress induced ulcer (CSU) model using albino Wistar rats.

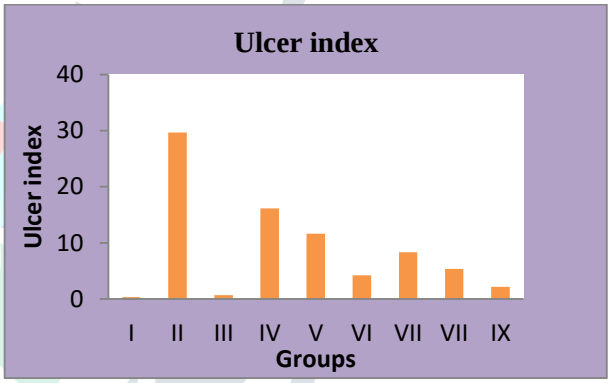
GPS	Treatment	pH Mean ±SEM	Ulcer index Mean ± SEM	% inhibition Of Ulcer index
I	Vehicle control (10 ml/kg)	2.00 ±0.00	0.33±0.21	-
II	Cold restraint induced (4 h)	2.00 ±0.00	29.66±3.62	-
III	Omeprazole (10 mg/kg)	6.35±0.22**	0.66±0.49 *	97.77
IV	Aqueous pods extract of *Acacia concinna* (100 mg/kg)	2.00±0.00	16.16±1.77*	45.51

V	**Aqueous pods extract of *Acacia concinna* (200 mg/kg)**	**2.33±0.33**	**11.66±1.8****	**60.68**
VI	**Aqueous pods extract of *Acacia concinna* (400 mg/kg)**	**2.33±0.33**	**4.21±2.35*****	**86.51**
VII	**Aqueous bark extract of *Acacia concinna* (100 mg/kg)**	**3.00±0.44***	**8.33±1.99*****	**71.91**
VIII	**Aqueous bark extract of *Acacia concinna* (200 mg/kg)**	**2.66±0.42***	**5.33±1.99*****	**82.02**
IX	**Aqueous bark extract of *Acacia concinna* (400 mg/kg)**	**3.00±0.44***	**2.16±0.87*****	**92.71**

Results are expressed as Mean ± SEM; (n=6). Significance at P<0.05*, P<0.01** as compared to control ANOVA followed by Dunnett's multiple comparison test.

Graph 3 pH and Ulcer index of treated groups in Cold stress induced ulcer model using albino Wistar rats





In the present study, the pH and mean total ulcer score at various doses of test substances is presented in Table-3 and Graph 3. Cold stress at 4th h showed potent ulcerogenic activity. Reference standard used here was omeprazole (dose - 10 mg/kg) showed potent antiulcer activity significantly. Aqueous bark extract of *Acacia concinna* at all dose levels inhibited ulcerogenic activity of cold stress significantly.Aqueous bark extract of Acacia concinna at a dose of 400 mg/kg b.winhibited ulcer index by 92.71% compared toAqueouspods extract of Acacia concinna, which at the same dose level inhibited ulcer index by 86.51%.

DISCUSSION

Gastric ulcer is open scores developed inside the lining of the stomach characterized by burning pain. However, the underlying mechanism for gastric ulcer development still poorly understood. One common factor contributes to the development of gastric ulcer is infected by bacterial helicobacter pylori1. Some other factors, such as long-term using of aspirin and certain painkillers, and habits and customs including smoking, alcohol drinking, dietary habits are contributors for gastric ulcer development. Major causes mentioned above interrupt the balance Researchers have focused on drug discovery from herbal or botanical sources, an important group of complementary and alternative medicine (CAM) therapy, over

the past few decades. With a long history of herbal use for the clinical management of a variety of diseases in indigenous cultures, the theoretical success rate of developing a new herbal medicine should be, Higher from chemical sequencing than just that. While "experience-driven" is the attempt to discover herbal medicines, the search for a pharmacologically useful pharmaceutical drug such as "trying to find a needle in a haystack[20]

Herbal medicines have existed worldwide with long recorded history and they were used in ancient Chinese, Greek, Egyptian and Indian medicine for various therapies purposes. World Health Organization estimated that 80% of the world's inhabitants still rely mainly on traditional medicines for their health care. The subcontinent of India is well-known to be one of the major biodiversity centers with about 45,000 plant species. In India, about 15,000 medicinal plants have been recorded, in which the communities used 7,000-7,500 plants for curing different diseases[21]

The global scenario is focusing their face on the use of herbal medicine due to lesser side effects. The folk medicinal system has a deep-rooted history in India of the rural population. This special system of knowledge has evolved from a harmonious living of aboriginal people with nature. The most majority of people on earth still depend on their traditional Material Medicine for daily health care. Medicinal plants properties lie in the active constituents[22] which they contain. Therefore, in many cases, the principal aim of the phytochemical analysis is to detect, isolate and identify the active substances.

current research, an effort was an antiulcer activity of *Acacia concinna* (aqueous extract) by *In vitro* antioxidant and *In vitro* antiulcer study and *In vivo* antiulcer models using various induced ulcer models (pylorus ligation, NSAID and Cold restraint induced ulcers). It was executed in five phases to find out the antiulcer potential of the plant selected for this study and also to find out the plant constituents responsible for the antiulcer activity produced.

In vivo antiulcer activity

Antiulcer activity of ABE of AC and APE of AC was assessed pylorus ligation, Indomethacin and cold restraint stress-induced ulcer model in rats. And the efficacy of the extract was evaluated by treating the animals with three dose levels of treatment and dosing sing.

At the end of 2 weeks treatment, Volume of gastric contents pH of gastric contents, total acidity and ulcer index were parameters were estimated.

.Presences of active plant constituents like Phenolic compounds; saponins; tannins in ABE of AC are responsible for *In Vivo* antiulcer activity potential, several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids, and saponin were responsible for gastroprotective agents [23]. Tannins possess as an antiulcer agent by its astringency property and vasoconstriction effects. Due to precipitation of microproteins on the ulcer site, a protective layer was formed which hinders gut secretions and protects the mucosa from toxins and other irritants. Previous studies have recommended that these above active compounds had the ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen[88] Therefore, it possess antiulcer activity, may be due to the presence of tannins, flavonoids, and terpenoids

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