

Anti-inflammatory and ulcer protective activities of chloroform extract of *Albizzia chinensis* bark and its phytochemical studies

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ABSTRACT

In endoscopic clinical research studies of patients who take non steroidal anti inflammatory drugs (NSAIDS) use, the incidence of new gastric ulcer in stomach. The NSAIDS produced ulcer healing is not widely appreciated. The present study was undertaken to investigate anti inflammatory cum ulcer protective property of chloroform extract of Albizzia chinensis bark. The phytochemical studies were carried out chloroform extract of Albizzia chinensis bark. Carrageenan was used to induced rats paw oedema and diclofenac sodium (100 mg/kg, i.p route), which has been served for positive control. The ethanol induced ulcer was conducted in rats and omeprazole oral dose of 4mg/kg, which has been used for positive control. Ethanol administered 1hr later stomach was isolated from rat and histopathological studies were carried out chloroform extract of Albizzia chinensis bark. The phytochemical studies were indicated in the presence of carbohydrates, proteins, saponin glycosides, flavonoids, tannins, terpenoids, gums and mucilage. The results revealed that the chloroform extract of Albizzia chinensis bark 200 mg/kg and 400mg/kg dose levels have been showed significant decrease in the paw volume ($P<0.001$) when compared to control and positive control. The chloroform extract of Albizzia chinensis bark 200 and 400mg/kg dose levels were showed significantly reduced in the ulcer index ($P<0.001$) when compared to control and positive control. The histopathological studies have been indicated that the chloroform extract of Albizzia chinensis bark 200 and 400mg/kg were significantly increased in the ulcer protective effect when compared with control group.

Key words: *Albizzia chinensis*, anti inflammatory, carrageenan, diclofenac sodium, ulcer and omeprazole.

INTRODUCTION

The literatures have been showed that the nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed medications. As NSAIDs relieve pain and reduce inflammation, most NSAID prescriptions are written for patients with arthritis. In 1991 it was estimated that 13 million americans used NSAIDs as long-term therapy, 5 million with rheumatoid arthritis (RA) and the remainder with osteoarthritis (OA) or other inflammatory states.[1] Approximately half of all NSAIDs prescriptions are written for patients older than 60 years and this increase parallels the age-related prevalences of RA, OA, gout, and bursitis[2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce gastro duodenal prostaglandin mucosal concentrations, resulting in the loss of a major mechanism for protection against mucosal injury.[3] The present study is attempted to develop the novel plant based drugs which have anti inflammatory cum ulcer protective property. The *Albizia chinensis* is a medium sized armed deciduous tree belonging to the family *Fabiaceae*. The plant was collected from the eastern part of Kerala and authenticated from Botanical survey of India, Coimbatore. The *Albizia chinensis* plant portions of bark, flowers, seeds and fruits were used for several therapeutic purposes such as anti-allergic, anti-fungal, anti-inflammatory, anti-microbial, cardiogenic, hypoglycemic and hypolipidemic [4]. Our present study is aimed to justify the traditional claim of the bark as anti inflammatory cum ulcer protective.

MATERIALS AND METHODS

Preparation of extract (5)

The *Albizia chinensis* entire fruit powder 1000g mixed with 2000ml of 50% ethanol in round bottom flask which was kept under 15 days and shaken regularly 2 times per day. The collected extract was dried under reduced pressure and stored in a desiccator.

Phytochemical Studies [6-7]

a. Chemical test for Carbohydrates: Two ml of extract solution is placed in a test tube. Two drops of the Molisch reagent (a solution of α -naphthol in 95% ethanol) is added. The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form.

b. Chemical test for Alkaloids: The little quantity of extract was taken in a beaker and warmed with 2% sulphuric acid at 2 minutes. After 2 minutes the warmed extract was filtered. The collected few drops of filtrate were treated with required quantity of Dragendorff's reagent in a test tube.

c. Chemical test for Saponin Glycosides: A few quantity of the extract was taken and shaken with little amount of water in a test tube.

d. Chemical test for Steroids:

1. Liebermann Burchard Reaction: The extract was dissolved in chloroform and few ml of acetic anhydride + few drops of concentrated sulphuric acid were added.

2. Salkowski Reaction: The extract was dissolved in chloroform and few drops of concentrated sulphuric acid were added.

e. Chemical test for Tannins:

1. The extract was treated with ferric chloride.
2. The extract was treated with bromine water.

f. Chemical test for Terpenoids: The extract were treated with vanillin and few drops of sulphuric acid.

g. Chemical test for Mucilages and Gums: The little quantity of extract was treated with few drops of ruthenium red solution in a test tube.

h. Chemical test for Flavonoids: The little quantity of extract was taken in a test tube and add few ml of dilute sodium hydroxide solution.

Animals

Inbred adult male Sprague-Dawley rats (160-200 g) were obtained from the animal house Vinyaga of College of Pharmacy. The animals were maintained in a well ventilated room at a temperature of $35\pm 2^{\circ}\text{C}$ with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan lever, Bangalore) and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory conditions 10 days prior to initiation of experiments. The project proposal was approved by Padmavathi College of Pharmacy IAEC (Institutional Animal Ethical Committee) and the approval number being (IAEC No: 365\143\1\2005).

Acute Toxicity Studies[8-10]

The animals were fasted over night prior to the (OECD Guidelines 432) experimental procedure. The Up and Down or 'Staircase, method was adopted, and according to doses of chloroform extract of *Albizzia chinensis* bark was fixed to 200 and 400 mg/kg body weight.

Experimental Design

Carrageenan Induced Paw Oedema Method in Rats [8&11-12]

Male sprague-dawley rats about weight range from 160 to 180 gms were used. The animals were starved overnight. To ensure uniform hydration, the rats received water and *libitum*. The rats were divided into four groups and each group consists of six animals. Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Diclofenac Sodium (100mg/kg), Group III served as chloroform extract of *Albizzia chinensis* bark – (200mg/kg), Group IV served as chloroform extract of *Albizzia chinensis* bark– (400mg/kg). Making a mark on left hind paw just beyond the tibia tarsal junction, so that every time the paw was dipped in the mercury column upto fixed mark to ensure constant paw volume. All the groups of rats initial paw volume were measured by mercury displacement method using plethysmograph apparatus. The extracts and standard drug were administered orally by using oral feeding needle. After one hour inject 0.1ml of 1% (w/v) carrageenan in sub plantar region of the left hind paw. After the drug administration paw volume was again measured similarly different time intervals at 1, 2, 3, 4 & 6 h, respectively.

Ethanol Induced Ulcer[13-16]

Male sprague-dawley rats about weight range from 200 to 250 gms were used. The rats were divided into four groups and each group consists of six animals. Groupings were done as

follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Omeprazole (4mg/kg), Group III served as chloroform extract of *Albizzia chinensis* bark – (200mg/kg), Group IV served as chloroform extract of *Albizzia chinensis* bark– (400mg/kg). Before the experiments all rats were starved for 48 h, but had free access to tap water until the beginning of treatment. All the animals, were housed in single cages that had wire-net bottoms to avoid coprophagy and cannibalism. The rats were administered either the appropriate vehicle or the cytoprotective drugs through intragastrically 30 minutes prior to the administration of 1ml absolute ethanol. Rats were euthanized with CO₂ inhalation 1 h after 50% ethanol exposure, and the stomachs were removed, opened along the lesser curvature, rinsed with saline, and examined for the severity and number of mucosal gastric lesions. The stomachs were stretched on a piece of foam core mat with mucosal site up and the ulcer scoring were observed under a microscope.

score the ulcers as below:

0 = normal coloured stomach, 0.5 =red colouration, 1= spot ulcer, 1.5= hemorrhagic streaks, 2 = ulcer lesions, 3 = Perforation.

Statistical Analysis

The values were expressed as Mean±SEM. Statistical Analysis were performed by one way ANOVA followed by Dunnett t test has indicated P<0.001 and P<0.05 were considered for significant activity.

Histopatological Study[13]

Histological evaluation was performed on the glandular stomach of two rats randomly selected from representative experiments. In brief, stomachs were fixed by immersion in 4% buffered formalin, correctly oriented, embedded in paraffin, and cut. Serial paraffin sections (2–4 µm) were hydrated and stained with hematoxylin and eosin. The mucosal injury evaluation was performed under light microscopy. First, the extension of the mucosal lesion was measured and expressed as a percentage of the entire mucosa investigated. Accordingly a partial score from 0–3 was assigned (0 = no lesion; 1 = lesion involving 1–10% of the mucosa; 2 = lesion involving 11–20% of the mucosa; 3 = lesion involving >20% of the mucosa). Second, the deepest mucosal lesion was identified per stomach sample and scored from 0–3 (0 = no change; 0.5 = superficial erosion; 1 = ulcer involving one internal third of the mucosa; 2 = ulcer involving the two internal thirds of the mucosa; 3 = ulcer involving almost the entire mucosal thickness). The total microscopic score resulted by the sum of the two partial scores and ranged from 0–6.

RESULTS AND DISCUSSION

Pytochemical Studies

The preliminary phytochemicals screenings were performed the formation of a purple product at the interface of the two layers which was indicated the presence of carbohydrates. Orange red colour precipitate was formed and it was indicated in the presence of alkaloids. The formation of 1cm layer of foam which was indicated the presence of saponin. Yellow precipitate was formed and it was indicated the presence of tannins. The formation of reddish violet colour indicated the presence of terpenoids. The formation of pink colour which indicated the presence of terpenoids. The yellow colour solution was turns colourless which indicated the presence of

flavonoids. Whereas aromatic acids, steroids, fats, oils, proteins and amino acids absent in the chloroform extract of *Albizzia chinensis* bark. The phytochemical screening results have shown Table. No-I.

Table – I : Details of qualitative phytochemical studies

Phytoconstituents	Chloroform extract of <i>Albizzia chinensis</i> bark
Carbohydrates	+
Flavonoids	+
Saponin glycoside	+
Alkaloids	+
Steroids	-
Proteins & AA's	-
Tannins	+
Terpenoids	+
Gums and mucillages	+

+ = Present; - = Absent

Antiinflammatory Activity

The results have been showed that the aqueous ethanolic extract of chloroform extract of *Albizzia chinensis* bark dose levels 200 and 400mg/kg body weight were significant decrease (*P<0.05) and (**P<.001) in the paw oedema volume when compared with control group. The chloroform extract of *Albizzia chinensis* bark at dose level 400mg/kg body weight was significantly dose dependent manner decreased in the paw oedema volume at 3hr (**P<.001) when compared with (*P<0.05) 200mg/kg body weight. The diclofenac sodium treated group has shown more anti inflammatory activity when compared to each other groups. The anti inflammatory activity results have shown Table No- II.

Table – II : Antiinflammatory activity of Chloroform Extract Of *Albizzia Chinensis* Bark

Treatment	Dose mg/kg	Mean Paw Volume (ml)					
		0h	1 h	2h	3 h	4 h	6 h
CMC	0.5 %	0.35±.03	0.73±.07	1.1±.07	1.3±.10	2.6 ±.11	3.2 ±.12
DC	100	0.36±.05	0.93±.09	0.83±.08**	0.81±.2**	1.3±.06**	1.3±.08**
CEAC	200	0.33±.05	1±.14	0.95±.14	0.93±.13*	1.9 ±.08*	2.1 ±.16*
CEAC	400	0.35±.06	0.97±.10	0.86±.09**	0.82±.07**	1.3±.14** ††	1.3±.18** ††

CMC- Carboxy Methyl Cellulose(control), DC- Diclofenac Sodium (positive control), CEAC- Chloroform Extract Of *Albizzia Chinensis* Bark

The results of rats paw volume were expressed as Mean ± Standard Error (N=6), (P<0.05)* and (P<0.001) ** when compared to control and positive control groups and evaluated by using one way ANOVA followed by Dunnet 't' test and †- is indicated comparison between 200vs 400mg/kg body weight.

Anti Ulcer Activity

The ethanol induced ulcer results have been expressed that the chloroform extract of *Albizzia chinensis* bark at dose levels 200 and 400mg/kg body ulcer index were significantly reduced

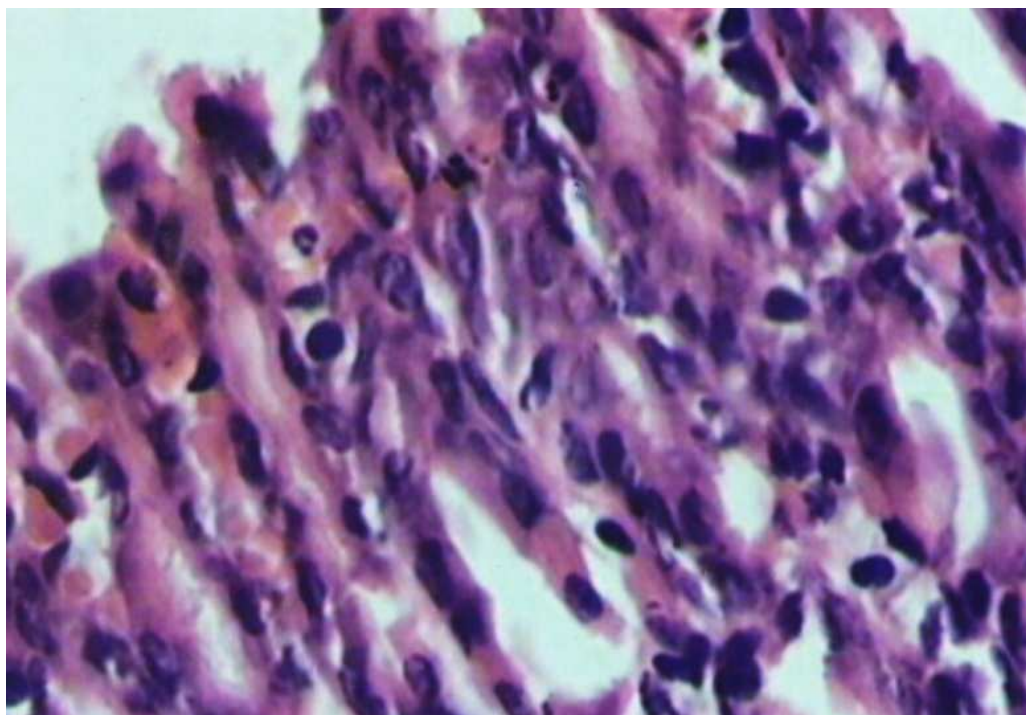
(***P<0.0001) and (**0P<.001) when compared with control group. The chloroform extract of *Albizzia chinensis* bark 400mg/kg body weight was significantly reduced (***P<.0001) in the ulcer index when compared with (**P<0.001) 200mg/kg body weight group and this results indicated a significant dose dependant anti ulcer activity for the chloroform extract of *Albizzia chinensis* bark 400 and 200mg/kg. The omeprazole treated group has shown more anti ulcer activity when compared to each other groups. The anti ulcer activity results have shown Table.No- III.

Table – III: Anti ulcer activity of Chloroform Extract Of *Albizzia Chinensis* Bark

TREATMENT	DOSE mg/kg	ULCER INDEX`
CMC	0.5 %	3.8 ± 0.11
OMEPRAZOLE	4	0.85 ± 0.11***
CEAC	200	1.26 ± 0.13**
CEAC	400	0.87 ± 0.08***££

The results of rats ulcer index were expressed as Mean ± Standard Error (N=6), (P<0.05)*, (P<0.001) ** and (P<0.001) *** when compared to control and positive control groups and evaluated by using one way ANOVA followed by Dunnet ‘t’ test and £- is indicated comparison between 200 vs 400mg/kg body weight.

Figure-I : Control group



Histopathological Studies

Histological assessment of the oxyntic mucosa after EtOH acute challenge in rats treated with Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat) which indicate the more lesions in the stomach borders when compared to each other groups. The chloroform extract of *Albizzia*

chinensis bark at dose levels 200 and 400mg/kg body lesions and cell necrosis were significantly reduced when compared with control group. The chloroform extract of *Albizzia chinensis* bark 400mg/kg body weight was significantly reduced in the coagulative necrosis when compared with 200mg/kg body weight group. The omeprazole treated group has shown superficial ulcer. This indicated that the omeprazole was more anti ulcer activity when compared to each other groups. The anti ulcer activity results have shown Figures.No-.I-IV.

Figure-II : Positive control



Figure-III : Chloroform Extract Of *Albizzia Chinensis* Bark 200mg/Kg

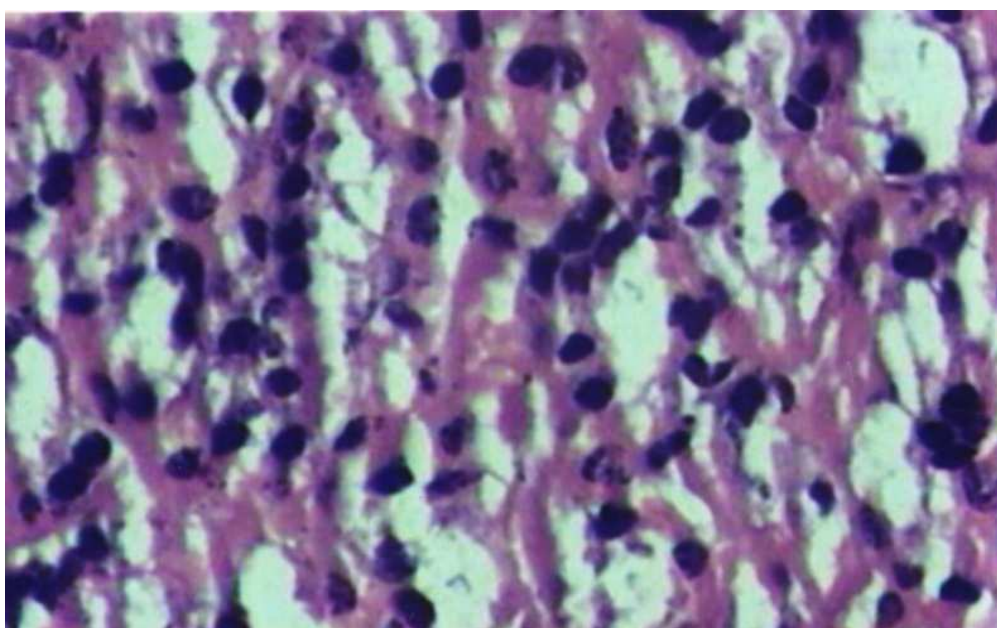
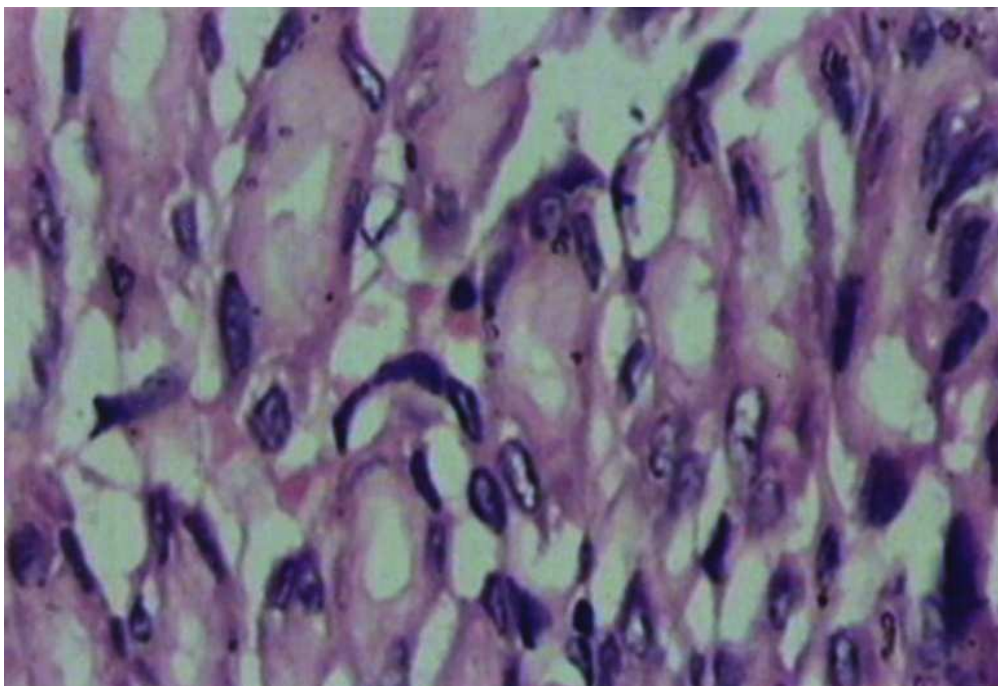


Figure-IV : Chloroform Extract Of *Albizzia Chinensis* Bark 400mg/Kg

CONCLUSION

The present study concluded the beneficial effect of in the control of paw oedema volume in carrageenan induced paw oedema rats. The chloroform extract of *Albizzia chinensis* bark has been controlled by ulcer in ethanol treated rats. This study confirms the rational basis for its use in traditional medicine for the treatment of inflammation and reduced ulcer effect in patients. Further phytochemical and pharmacological investigations are under way to characterize active phytoconstituents and to establish exact mechanism of inflammation action. This work, we believe, will be useful for further inflammation research works.

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