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Original Article

Anti-allergic Potential of Methanolic Extracts of Leaves and Fruit of *Gmelina arborea* Roxb

Daya L Chothani[1,*], NM Patel[2]
[1]Department of Pharmacy, BK Mody Government Pharmacy College, Rajkot, Gujarat, INDIA.
[2]Shri BM Shah College of Pharmaceutical Education and Research (BMCPER) Modasa, Modasa, Gujarat, INDIA.

ABSTRACT

Objectives: An anti-allergic study was performed on a methanolic extract of leaves (GLA) and fruit (GFA) of *Gmelina arborea* Roxb. (Verbenaceae). **Methods:** Anti-allergic study was evaluated using an isolated guinea pig ileum, isolated rat ileum preparation and passive paw anaphylaxis in rats. The effect of methanolic extracts (100, 200 µg/ml) of the fruit and leaves were studied on contraction induced by histamine and acetylcholine on isolated guinea pig ileum and isolated rat ileum, respectively. The inhibition of paw volume was studied (100, 300mg/kg GLA and 100, 300mg/kgGFA, p.o.) and compared with vehicle treatment. Dexamethasone (0.27mg/kg, p.o.) was included as a positive control. **Results:** GLA and GFA showed significant inhibitory contraction of guinea pig ileum ($p<0.01$, $p<0.005$), rat ileum and ($p< 0.01$, $p<0.005$) rat paw volume inhibition. **Conclusion:** The anti-allergic activity of methanolic extracts of the fruit (GFA) and leaves (GLA) of *G. arborea* may be due to presence of phenolic and flavonoid compounds. The effects are noteworthy and highlight these extracts for further studies.

Key words: Anti-allergic activity, Acute toxicity *Gmelina arborea*, Passive paw anaphylaxis, Phytochemical screenig.

Correspondence:
Daya L Chothani
BK Mody Government Pharmacy College, Rajkot-360003, Gujarat, INDIA.
Phone no: +91 8200210300
E-mail: daya.herb@gmail.com
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INTRODUCTION

The prevalence of allergic diseases worldwide is rising dramatically in both developed and developing countries which include asthma, rhinitis, anaphylaxis, drug, food, insect allergy, eczema and urticaria. Indeed, about 30-40% of world population is affected by allergic conditions.[1] Allergens are environmental antigens which cause allergy via the induction of an immune response. Most allergens reacting with IgE and IgG antibody are proteins, often with carbohydrate side chains, or may be pure carbohydrates or other low molecular weight chemicals.[2]

Gmelina arborea Roxb. is an unarmed, moderately sized to large deciduous tree, which grows to about 30m or more in height and a diameter of up to 4.5m. The name *arborea* is derived from the Latin word 'arbor' and means tree-like. Leaves are opposite and decussate. Fruitsare drupes, with enlarged calyx; glossy and yellow when ripe, the exocarp is succulent and the endocarp is bony and is usually 2-celled. *Charaka* is prescribed a as paste of the leaves and other ingredients mixed into a medicated clarified butter. It is given for stiffness of the back, facial paralysis; prescribed the soup of fruits in diarrhoea. *Chakradatta* uses ripe fruits prepared with honey and is used for alleviating haemorrhages. Ripe fruits dried and cooked with cow's milkare given for urticaria.[3] The leaves are used for treatment of cough, scorpion and insect stings.[4] Furthermore, the leaves have been reported to have antiulcer,[5] anthelmintic,[6] Vasodilator,[7] antihyperglycemic,[8] wound healing,[9] antimicrobial,[10] analgesic,[11] and *in vitro* cytotoxic activities.[12] The fruits also have hepatoprotective,[13] antibacterial, antioxidant and antidiabetic[14] and diuretic activities.[15] Both the fruits and leaves of *G. arborea* are also used in the treatment of various allergic reactions although scientific research is lacking to verify these effects. Therefore, the anti-allergic activity was examined out on fruits and leaves of *G. arborea.*

MATERIALS AND METHODS

Plant material

Fresh leaves and fruits plant of *Gmelina arborea* was collected from Waghodia Road, Baroda in the month of May 2011. Plant was identified and authenticated by Dr. P. S. Nagar at Botany Department of The M. S. University, Vadodara, India. Voucher specimen (DC-GM-1) was stored in herbarium of Pharmacognosy Laboratory, Pioneer Degree Pharmacy College, Vadodara.

Reagent and chemicals

All the chemicals and reagents used were of analytical grade and procured from E. Merck (Darmstadt, Germany), Hi-Media Laboratories Ltd., Mumbai, India and Sigma (Chemical Co, St. Louis, MO, USA). All UV-Vis measurements were recorded on a Shimadzu UV-1800 (Japan).

Preparation of extracts

The collected fruits and leaves were dried under shade and powdered using grinder. Twenty gram of fruits and leaves powder were extracted for 24h with 200ml methanol (AR grade, Sigma-Aldrich) separately. The extracts were filtered through whatman filter paper, concentrated by evaporation on water bath at 60-70°C temperature and dried in shade.

Phytochemical screening

The extracts were tested for the presence of various type of phytoconstituents *i.e.* phenols, flavonoid, saponin and sterols by employing standard chemical tests.[16]

Animals

Healthy albinoWistar rats (male/female) weighing 150-200g and guinea pigs (300-600g) of either sex were procured from Sun Pharmaceutical Advanced Research Centre, Vadodara, India. The animals were housed under standardized conditions (12-h light/dark cycle, 24°C, 35 to 60% humidity) and were allowed free access of standard laboratory feed and purified drinking water *ad libitum*. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee (IAEC), The Pioneer Degree Pharmacy College, Vadodara, Gujarat, India.

Acute Toxicity Study[17]

Acute toxicity studies was performed as per the OECD (423) guidelines. Albino rats were used for the toxicity study. The animals were fasted overnight and provided only with water. They were divided in groups, each containing three animals. The methanol fruit and leaf extracts was selected for this study. The 300mg/kg, 2000mg/kg single dose of extracts (suspended in 2.5%w/v Tween 80) were given orally with gavage. The animals were continuously observed for 30 min after dosing, then observed periodically for during the first 24 hr. Thereafter, the animals were observed daily for 14 days. The animals were observed for physiological effects including convulsion, salivation, sleep, movement, body weight, death etc..

In vitro studies on isolated guinea pig ileum

Overnight fasted guinea pigs (300-600g) were sacrificed, their abdomen was opened and the ileum was dissected. A segment of the ileum (2cm long) was suspended in a 30ml organ bath containing Tyrode's solution (NaCl 136.9 mM, Glucose 5.6 mM, $NaHCO_3$ 11.9 mM, KCl 2.68 mM, $MgSO_4$ 1.05 mM, $CaCl_2$ 1.8 mM, NaH_2PO_4 0.37 mM), continuously gassed with air and maintained at 37°C. The tissue was allowed to stabilize for 35 min and the Tyrode's solution was replaced at10 min intervals. After an equilibration period of 10min, histamine (sigma) (10µg/ml) was added to induce contraction and the effect of the extracts (100, 200µg/ml) in presence of same dose of histamine was recorded. A drug tissue contact time of 1min and 5min time cycle was followed for recording the response of histamine by using frontal writing liver. The percentage response of each group was calculated from the height of peaks obtained.[18-,20]

In vitro studies on isolated Rat ileum preparation

Albino rats were fasted overnight. The following day, the animals were sacrificed and a small piece of ileum was isolated and mounted in an organ bath containing Tyrode solution maintained at 37°C. A basal tension of 500mg was applied and the tissue was stabilized for 30min. The tissue was then exposed to graded doses of acetylcholine and contractions were recorded. The effect of selected extracts (100, 200µg/ml) in the presence of same dose of acetylcholine was recorded.[21,22]

Passive paw anaphylaxis in rats

Albino Wistar rats (male/female) weighing 150-200mg were randomly selected and divided into 6 (n = 5) groups. The three doses (subcutaneously) of 100µg of egg albumin(sigma) adsorbed on 12mg of aluminium hydroxide gel (Tarus chemical, IP grade) prepared in 0.5ml of saline on 1st, 3rd and 5th day. A blood sample was collected from the retro orbital plexus on 10th day of sensitization and allowed to clot. The blood was ringed and centrifuged at 1500 rpm to separate the serum. The standard drugs and test extracts were suspended in 2.5%w/v Tween 80 given orally with gavage. The animals belonging to group-I serves as control and was administered vehicle (2.5%w/v Tween80 10ml/kg P.Os) only. Animals belonging to group-II received standard drug (Dexamethasone (Sun pharma, IP grade) 0.27 mg/kg, P.O.). Group III, IV, V, VI animals were received the plant extracts orally at dose of 100mg/kg GLA, 300 mg/kg GLA, 100mg/kgG, 300mg/kg GFAp.o., respectively. The animals were passively sensitized with 0.1ml (undiluted) serum into the left hind paw of animals. An equal volume of saline was administered to contra lateral paw. Controls and plant extracts were given 24h after sensitization. After 1h of drug treatment, the animals were again challenged with 10µg of egg albumin in 0.1ml of saline in the left hind paw and the paw volume was measured using a Plethysmometer[23,25] The difference in the reading before and after antigen challenge indicated the edema volume and the percent inhibition of volume were calculated by using the following formula:

$$\text{Percent Inhibition} = \left[1-\left(\frac{V_t}{V_c}\right)\right] \times 100$$

Where V_t indicated mean relative change in paw volume in the test group and V_c indicated mean relative change in paw volume in control group.

Statistical analysis

The experimental parameters have been reported as mean ±SD for three determination (n=3). The variation in a set of data has been estimated by one way analysis of variance (ANOVA) using Graph Pad Prism version 6.00 and MS excel 2007. Value of p<0.05 was considered as significant difference.

RESULTS

Phytochemical screening

The phytochemical present in leaves and fruit of *G. arborea* are reported in Table 1.

Acute toxicity study

The albino rats were fasted overnight and only providing only water. They were divided in four groups, each containing three animals. The methanol extracts (300mg/kg, 2000mg/kg doses) of the fruit and leaf of *G. arborea* were given orally with gavage. The animals were observed continuously for 30min after dosing then periodically during the first 24hr and thereafter daily for 14 days. There was no change observed in behaviour and no mortality was observed.

In vitro studies on isolated guinea pig ileum preparation

Methanolic extract of fruit and leaves of *Gmelina arborea* showed dose dependant significant (p< 0.05) inhibition of contraction of ileum smooth muscle induced by histamine (Table 2). The methanolic extract of leaves (GLA) showed a higher % inhibition of contraction than the

Table 1: Phytochemical screening of GLA and GFA of *G. arborea*.

Phytoconstituents	GLA	GFA
Carbohydrates	–	+
Proteins	–	–
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannin and phenolics	+	+
Steroids and triterpens	+	–
Where , (- = absent, + = positive		

Table 2: Effect of GLA and GFA of *G. arborea* on isolated guinea pig ileum preparation.

Treatment	Peak height	% inhibition
Histamine (10 µg/ml)	2.47±0.25	-
Histamine + GLA (100 µg/ml)	0.70±0.15*	71.63
Histamine + GLA (200 µg/ml)	0.44±0.06*	82.03
Histamine + GFA (100 µg/ml)	1.38±0.11*	43.94
Histamine + GFA (200 µg/ml)	0.88±0.03**	64.20

Values (n=3) are mean ± SEM; *p<0.01, ** p<0.005 when compared with control (histamine induced) group

corresponding fruit extract.

In vitro studies on isolated Rat ileum preparation

Acetylcholine produces dose dependent contraction of rat ileum. Pre-treatment with methanol fruit extract (GFA) and methanol leaf extract (GLA) induced significant (p<0.01, p<0.005) dose dependent inhibition of contraction of rat ileum induced by acetyl choline (Table 3). The result indicates that the methanolic extract of leaves (GLA) showed higher inhibition of contraction of rat ileum than fruit extract of *Gmelina arborea*.

Passive paw anaphylaxis in rats

Methanolic extract of fruit and leaves of *G. arborea* showed the dose dependant significant (P< 0.05) reduction of paw volume as compared to control (Table 4). The methanolic extract of leaves (GLA) showed higher reduction of paw volume than fruit extract of (GFA).

In the passive paw anaphylaxis model, egg albumin was injected after 1 hr of the administration of dexamethasone, GLA and GFA. Egg albumin increased the paw volume in the sensitized animals. Previously treated animals with GLA (100, 300 mg/kg, P.O.) and GFA (100, 300 mg/kg P.O.) had significantly reduced paw volumes at 1, 2, 3 and 4 hr time interval. GFA (300 mg/kg p.o.) showed 35.4%, 38.8%, 33.7% and 24.5% inhibition at interval of 1 hr, 2 hr, 3 hr and 4 hr respectively. GLA (300 mg/kg p.o.) showed 46.4%, 46.9%, 44.03% and 42.9% inhibition at 1hr, 2 hr, 3 hr and 4 hr respectively as shown in Figure 1.

DISCUSSION

The present investigation screened the anti-allergic activity of GLA and GFA extracts of *G. arborea* using *in vitro* models (isolated guinea pig ileum preparation and isolated rat ileum preparation) and *in vivo* mode using passive paw anaphylaxis. The avoidance of allergen is first step to controlling allergies. Skin allergies can be reduced by using soothing creams and wet wrapping.[26]In *Ayurveda* the main causative factor of allergy is from improperly digested food called *Ama.* Allergic reaction illnesses (ARIs), drug allergies, or hypersensitivities are considered *Kapha* dominated diseases in Ayurveda, e.g. asthma and eczema. In allopathic medicine, the treatment of these diseases clusters around the use of steroids, antihistamines and bronchodilators. The treatment of allergic reactions in Ayurveda essentially consists of identifying the allergens, avoiding the exposure to them, using drugs to relieve acute symptoms, improving digestion and cleaning the intestine of toxic materials in the gut (*ama*). Emesis is recommended to treat allergy and asthma because ARI are considered *kaphaja* disease.[27] Toxicity studies in Wistar albino rats revealed that no lethality or toxic reactions were found at the dose of 2000mg/kg body weight indicating the non-toxic nature of the methanol extract of fruit and leaf of *G. arborea.*

Methanolic extract of leaves and fruits of *G. arborea* showed dose depedent inhibition of contraction on isolated guinea pig ileum preparation induced by histamine and isolated Rat ileum preparation induced by acetyl choline. Methanolic extract of fruit and leaves of *G. arborea* also showed the dose dependent significant (P< 0.05) reduction of paw volume as compared to control. Experimental data in all three model indicate that methanolic extract of leaves and fruits of *Gmelina arborea* showed significant (p<0.05) anti-allergic activity. Further study is required to isolate specific chemical constitutes responsible for anti-allergic activity.

Table 3: Effect of GLA and GFA of *G. arborea* on isolated rat ileum preparation.

Treatment	Peak height	% inhibition
ACH (10 µg/ml)	2.10 ± 0.115	0
ACH + GLA (100 µg/ml)	1.28 ± 0.044 **	38.90
ACH + GLA (200 µg/ml)	0.65 ± 0.061*	68.89
ACH + GFA (100 µg/ml)	1.38 ± 0.060**	34.14
ACH + GFA (200 µg/ml)	0.83 ± 0.035**	60.63

Values (n=3) are mean ± SEM; *p<0.01, ** p<0.005 when compared with control (Acetyl choline induced) group

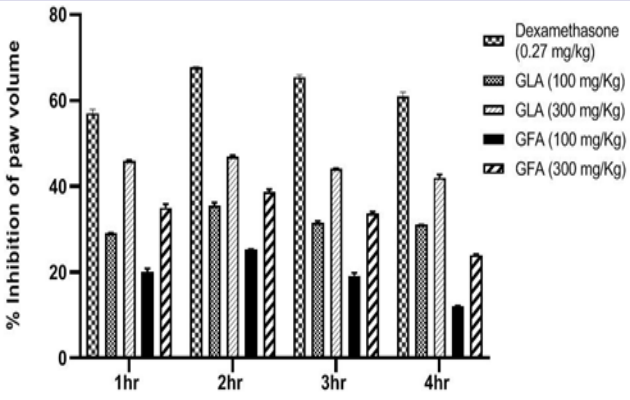

Figure 1: Effect of extracts *G. arborea* on % inhibition of paw volume at various time intervals.

Table 4: Effect of GLA and GFA of *G. arborea* on passive paw anaphylaxis in rats.

Treatment	Paw volume (1h)	Paw volume (2h)	Paw volume (3h)	Paw volume (4h)
Control (10mg/ kg P.O))	0.81 ±0.051	0.74±0.050	0.64±0.077	0.55 ±0.062
Dexamethasone (0.27 mg//kg P.O)	0.34 ±0.042*	0.24±0.027**	0.22±0.030	0.21±0.017**
GLA (100mg//kg P.O)	0.57 ±0.086*	0.48±0.062	0.44±0.067*	0.37±0.046*
GLA (300 mg//kg P.O)	0.44 ±0.045**	0.39±0.029**	0.36±0.041**	0.31±0.028**
GFA (100 mg//kg P.O)	0.64 ±0.055**	0.55±0.075*	0.51±0.056**	0.48±0.046**
GFA (300 mg/kg P.O)	0.53 ±0.0522**	0.45±0.057*	0.42±0.068*	0.41±0.062*

Values (n=5) are mean ± SEM; *p<0.01,** p<0.001 when compared with control (histamine induced) group

CONCLUSION

The methanolic extract of fruits (GFA) and methanolic extract leaf (GLA) of *Gmelina arborea* showed significant ($p<0.05$) anti-allergic activity. The methanolic extract of leaves (GLA) showed better activity than methanolic extract of fruits (GFA) of *G. arborea*. These findings may be useful in treatment of allergic condition, although further study is required to isolate specific chemical constitutes, mechanism of action, safety through toxicity screening, bioactivity determination etc. which contribute anti-allergic activity.

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CONFLICT OF INTEREST

The authors declare that they have no Conflict of interest.

ABBREVIATIONS

GLA: Methanolic extract of leaves of *Gmelina arborea*; **GFA:** Methanolic extract of fruits of *Gmelina arborea*.

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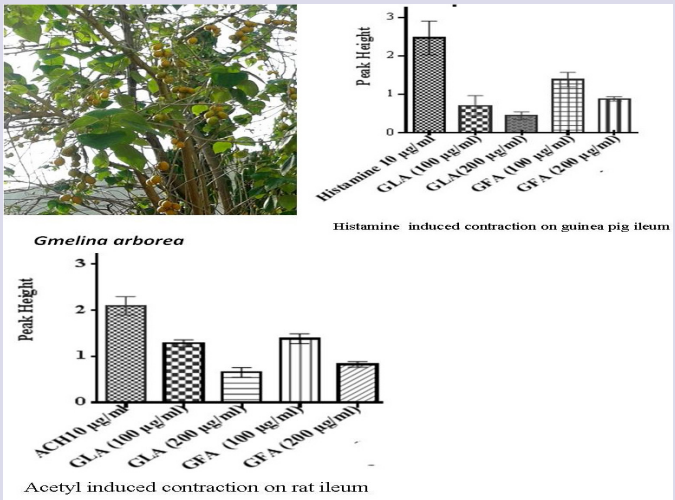
PICTORIAL ABSTRACT



SUMMARY

- *Gmelina arborea* fruits and leaves extracts were screened for anti-allergic activity using isolated guinea pig ileum, isolated rat ileum preparation and passive paw anaphylaxis in rats models. Leaves and fruit extract showed antiallegic activity.
- Acute toxicity study was performed on albino rats. All extracts were found non-toxic.

ABOUT AUTHORS

Dr. Daya L. Chothani: Currently working as an Assistant Professor at B K Mody Government Pharmacy College, Rajkot, (Gujarat, India). She has 11 years academic experience. She has published over 15 research paper in various national and international journals. She received Gold medal from M S University of Baroda, Vadodara during her graduation in 2007.

Dr. N. M. Patel: He guided more than 30 Ph.D. scholars in standardization, method development for estimation of phytochemical, pharmacological evaluation of herbs and development and evaluation of formulation of drugs, resulted inpublication of more than 200 research papers in peer reviewed journals.He hold more than 35 years academic and 20 years of research experience and worked as Principal of Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa, Gujarat, India.