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Research Article

Anti-allergic Potential of Methanolic Extract of Leaves and Fruits of *Careya arborea*

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Abstract

In present investigation, anti-allergic activity was carried out on methanolic extract of leaves (CLA) and methanolic extract of fruits (CFA) of *Careya arborea* (Lecythidaceae). Anti-allergic activity was evaluated using isolated guinea pig ileum, isolated rat ileum preparation and passive paw anaphylaxis in rats. The effect of methanolic extracts (100, 200 μg/ml) of fruits and leaves were recorded 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 CLA, and 100, 300mg/kg CFA, p.o.) against comparing with vehicle. Dexamethasone (0.27mg/kg, p.o.) was used as a positive control.In present investigation, anti-allergic activity was carried out on methanolic extract of leaves (CLA) and methanolic extract of fruits (CFA) of *Careya arborea* (Lecythidaceae). Anti-allergic activity was evaluated using isolated guinea pig ileum, isolated rat ileum preparation and passive paw anaphylaxis in rats. The effect of methanolic extracts (100, 200 μg/ml) of fruits and leaves were recorded 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 CLA, and 100, 300mg/kg CFA, p.o.) against comparing with vehicle. Dexamethasone (0.27mg/kg, p.o.) was used as a positive control. The CLA and CFA showed significant inhibition of contraction of guinea pig ileum ($p<0.01$, $p<0.005$) and ($p< 0.01$, $p<0.005$) rat paw volume inhibition. The anti-allergic activity of methanolic extract of the fruits (CFA) and leaves (CLA) of *C. arborea* may be due to presence of phenolic and flavonoid compounds.

Keywords: Anti-allergic activity, *Careya arborea*, passive paw anaphylaxis, methanolic extract

1.Introduction

The term allergy was originally introduced by von Pirquet in 1906, meaning "changed reactivity [1]. Four different types of hypersensitivity were described by Coomb and Gell [2]: Type-I or anaphylaxis, Type-II or Cytotoxic, Type III or Toxic complex reaction and Type IV or cell-mediated type. *Careya arborea Roxb* belongs to family Lecythidaceae, commonly known as wild Guava and Kumbhi (because the fruits resemble earthen pots) [3]. Leaves are alternate crowned at the end of branches, flower yellowish white and fruits are large, globose, fleshy, indehiscent, crowned with the calyx limb. Numerous seeds are embedded in fleshy pulp. It is habitat from sub-Himalayan tract, from Jammu eastwards to West Bengal, Madhya Pradesh and Tamil Nadu [4-7]. *Careya arborea* bark has been reported to have central nervous system depressant [8], antimicrobial and antioxidant [9], antidiarrhoeal [10], hepatoprotective [11], antitumor [12], analgesic [13], and anticonvulsant activity [14]. Fruits are reported to possess antioxidant activity [15]. The reports on anticancer [16], antileishmanial activity [17], and wound healing activity [18] of the leaves are also available. The anti-allergic potential of fruits and leaf of *C. arborea* remains unexplored. Therefore, the anti-allergic activity was carried out on fruits and leaves of *C. arborea.*

2. Materials and Methods

2.1. Plant material

Fruit and leaves of *C. arborea* were collected from Vadodara, Gujarat, India in June 2012. Plant was identified and authenticated by Dr. P. S. Nagar at Botany Department of The M. S. University, Vadodara, India. Voucher specimen (DC-CA-2) was stored in herbarium of our laboratory.

2.2. 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).

2.3. Preparation of extracts

About 20g of fruit and leaf powder were extracted for 24h with methanol, separately. The extracts were filtered, concentrated by evaporation on water bath and dried.

2.4. Phytochemical screening

The extracts were tested for the presence of various type of phytoconstituents i.e. alkaloid, phenols, flavonoid, saponin and sterols, by employing chemical tests [19].

2.5. Animals

Healthy Albino Wistar rats (male / female) weighing 150-200g and Guinea pigs (300-600g) of either sex were procured from Sun pharmaceutical advanced research centre, Vadodara, India. Animals were housed under standardized condition (12-h light/dark cycle, 24°C, 35 to 60% humidity). The animals 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.

2.6. In vitro studies on isolated guinea pig ileum preparation

Overnight fasted Guinea pigs (300-600g) were sacrificed, abdomen was opened and ileum was dissected out. A segment of the ileum (2cm long) was suspended in a 30ml organ bath containing Tyrode's solution (mM concentration of NaCl 136.9, Glucose 5.6, $NaHCO_3$ 11.9, KCl 2.68, $MgSO_4$ 1.05, $CaCl_2$ 1.8, NaH_2PO_4 0.37), 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 every 10 min intervals. After equilibration period, histamine (10μg/ml) induced contraction and effect of selected 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 [20-22].

2.7. *In vitro* studies on isolated Rat ileum preparation

Albino rats were fasted overnight. On next 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 presence of same dose of acetylcholine was recorded [23-24].

2.8. Passive paw anaphylaxis in rats

Albino Wistar rats (male / female) weighing 150-200g were divided into 10 *(n = 5)* groups. The three doses (s. c) of 100µg of egg albumin adsorbed on 12mg of aluminium hydroxide gel 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. Blood was centrifuged at 1500 rpm to separate serum. The standard drugs and test extracts was given orally. The animals belonging to group-I serves as control and was administered vehicle only. Animals belonging to group-II received standard drug (Dexamethasone 0.27 mg/kg, p.o.). Group III, IV, V, VI animals were received the plant extract orally at dose of 100mg/kg CLA, 300 mg/kg CLA, 100mg/kg CFA, 300mg/kg CFA p.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. Standard 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 [25-26]. 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:

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

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

2.9. 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.

3. Results and Discussion

It was found that leaves contain alkaloid, saponin, steroid, terpenoid, phenolic and flavonoid. A fruit contains carbohydrates, Saponins, flavonoid, phenolics compound and sterols. Methanolic extract of fruit and leaves of *C. arborea* showed dose dependant significant ($p< 0.05$) inhibition of contraction of ileum smooth muscle induced by histamine. There was an increase in % inhibition of contraction of ileum in the presence of CFA and CLA at dose 100, 200μg/ml when compared against histamine (10μg/ml). Results were reported in Table 1.

Table 1. Effect of CLA and CFA of *C. arborea* on isolated guinea pig ileum preparation

Treatment	Peak height	% inhibition
Histamine (10 μg/ml)	2.47±0.25	-
Histamine + CLA (100 μg/ml)	1.60±0.12*	35.14
Histamine + CLA (200 μg/ml)	0.91±0.02**	62.98
Histamine + CFA (100 μg/ml)	1.40± 0.12*	43.25
Histamine + CFA (200 μg/ml)	0.77±0.04**	68.79

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

Acetylcholine produces dose dependent contraction of rat ileum. Pre-treatment with methanol extract of fruits showed significant ($p<0.01$, $p<0.005$) dose dependent inhibition of contraction of rat ileum induced by acetylcholine. While methanolic extract of leaf increased the contraction of rat ileum. The observations were reported in Table 2.

Table 2 . Effect of CLA and CFA of *C. arborea* on isolated rat ileum preparation

Treatment	Peak height	% inhibition
ACH (10 μg/ml)	2.10 ± 0.115	0
ACH + CLA (100 μg/ml)	2.20 ± 0.058**	- 4.76
ACH + CLA (200 μg/ml)	2.78 ± 0.290**	-31.74
ACH+ CFA (100 μg/ml)	1.21 ± 0.058**	42.06
ACH+ CFA (200 μg/ml)	0.78 ± 0.078 *	62.70

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

The methanolic extract of fruit and leaves of *C. arborea* showed the dose dependant significant ($p<0.05$) reduction of paw volume as compared to control. The fruit extract showed higher reduction in paw volume than methanolic extract of leaves of *C. arborea.* Results were reported in Table 3.

Table 3. Effect of CLA and CFA of *C. arborea* on passive paw anaphylaxis in rats

Treatment	Paw volume (1h)	Paw volume (2h)	Paw volume (3h)	Paw volume (4h)
Control(10 mg/ml)	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**
CLA (100mg//kg P.O)	0.71 ±0.061**	0.61±0.072*	0.54±0.085*	0.51±0.060*
CLA (300 mg//kg P.O)	0.61 ±0.083*	0.58±0.082*	0.52±0.063*	0.48±0.040**
CFA (100 mg//kg P.O)	0.65 ±0.079*	0.52±0.069*	0.47±0.046**	0.43±0.065*
CFA (300 mg/kg P.O)	0.55 ±0.064*	0.49±0.058*	0.41±0.051*	0.39±0.039**

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

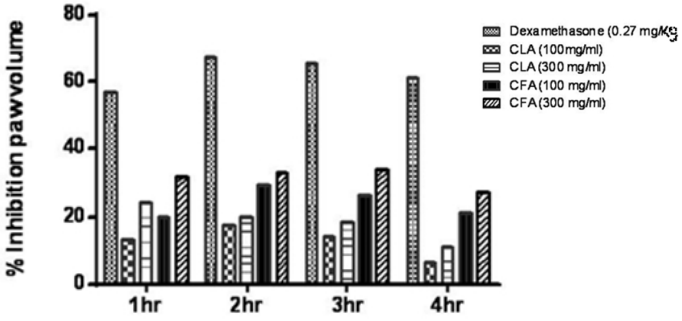

Fig. 1: Effect of extracts *C. arborea* on % inhibition of paw volume at various time intervals.

Allergy is a condition to increase sensitivity to certain substances. The reaction between antigen and antibody cause release of endogenous substance like histamine and produce allergic symptoms in various parts of body viz. skin, nose, eye etc. Allergies can be caused due to food, medicines, pollen, insect stings, dust mites, mold spores. It causes problems like sneezing, runny nose, itching, rashes, urticaria, swelling or even asthma and hay fever [27-28].

The present investigation concerns with screening of anti-allergic activity of CLA and CFA of *C. arborea* using in vitro model (isolated guinea pig ileum preparation and isolated rat ileum preparation) and in vivo mode using passive paw anaphylaxis.

In isolated guinea pig ileum preparation methanolic extract of fruits (CFA) showed higher inhibition of contraction of muscle induced by histamine than methanolic extract of leaves (CLA) of *C. arborea*. CLA and CFA were observed that decrease the % contraction of ileum at doses of 100 and 200μg/ml.

In isolated rat ileum preparation the contraction was induced by acetylcholine. The study was performed with CLA and CFA in presence of same dose of acetylcholine. CLA increased the % contraction of ileum at doses of 100 and 200μg/ml. However, CFA decreased the contraction of ileum at doses of 100 and 200μg/ml. The methanolic extract of fruits showed acetylcholine antagonistic activity.

In passive paw anaphylaxis model, egg albumin was injected after the administration of Dexamethasone, CLA and CFA. Egg albumin increased the paw volume in the sensitized animals. The animals treated previously with CLA (100, 300 mg/kg, p.o.) and CFA (100, 300 mg/kg p.o.) exhibited a significan reduction in the paw volume at 1, 2, 3 and 4h time interval. The CFA (300 mg/kg p.o.) showed a percentage inhibition of 32.92%, 33.88%, 34.91% and 27.84% at intervals of 1h, 2h, 3h and 4h, respectively. The CLA (300 mg/kg p.o) showed a percentage inhibition of 24.82%, 20.84%, 18.55% and 11.72 % (Fig. 1) at intervals of 1h, 2h, 3h and 4h, respectively.

4. Conclusion

The methanolic extract of fruits and leaf of *Careya arborea* showed significant ($p<0.05$) anti-allergic activity. The methanolic extract of fruits (CFA) showed better activity than methanolic extract of leaves (CLA) of *C. arborea*. The fruit extract inhibited acetylcholine as well as histamine while leaves antagonised the action of histamine and increased the activity of acetylcholine in isolated rat ileum preparation. The further study is required to isolate specific chemical constitutes responsible for anti-allergic activity.

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Conflict of Interest

We declare that we have no conflict of interest.

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