EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF *ANACARDIUM OCCIDENTALE* L. LEAVES

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Summary

The ethanol extract of *Anacardium occidentale* L. *(*Anacardiaceae*),* was evaluated for antiasthmatic activity by using various animal models. The *in vitro* animal models like isolated goat tracheal chain preparation and isolated guinea pig ileum preparation were used to study basic mechanism by which extract shows relaxant activity. The ethyl acetate soluble part of ethanol extract showed significant relaxant effects ($p<0.01$) compared to histamine in both Goat tracheal and Guinea pig ileum preparation. Hence ethanolic extract was used for further *in vivo* models instead of ethyl acetate soluble part. The *in vivo* models used were Haloperidol induced catalepsy and milk induced leukocytosis in mice. The ethanol extract showed maximum protection ($p<0.01$) against catalepsy at dose of 375 mg/kg p.o. body weight. Biochemical estimations in Milk induced total leukocytosis count showed maximum decrease in total leukocyte count at dose of 250 mg/kg p.o. body weight. The activity was found to be decreased as the dose was increased. The results of these studies inspire usefulness of Ethanol extract of *Anacardium occidentale* L. in antiasthmatic treatment.

Keywords: *Anacardium occidentale* L.*,* antiasthmatics, catalepsy, histamine, leukocytes.

Introduction

Asthma is a chronic disease with spastic contraction of smooth muscle in the bronchioles characterized by difficult breathing with wheezing. Asthma, a common, chronic inflammatory disorder of the airways, associated with pronounced health and economic consequences, has been identified as one of the five pressing global lung problems [1]. It has many causes but more specifically due to inflammation of air passage, hypersensitivity of afferent glossopharyngeal and vagal ending in larynx and afferent trigeminal endings in the nose, pulmonary edema and congestion of lungs caused by left ventricular failure (cardiac asthma). The prevalence of childhood asthma with wheeze and/or wheezy bronchitis ranges from 9.9 to 33% [2]. There is considerable mortality and morbidity due to asthma [3] the majority of which is avoidable.

There are two types of asthma, Atopic and non-atopic. Atopic occurs in children and young adults who have atopic (type-I) hypersensitivity to foreign proteins. Again when the same antigen comes into contact the antibody/antigen reoccurs resulting in release of histamine and other factors increase mucous secretion and muscular contraction that narrows the airways. Attacks become less frequent and less severe with age. Non-atopic occurs in adult life with no childhood history and is associated with chronic inflammation of upper respiratory tract. Eventually impaired lung ventilation leads to hypoxia, pulmonary hypertension and right sided heart failure. Anti asthmatic drugs like corticosteroids, theophylline, salbutamol are widely used in the treatment of asthma but these drugs produce some adverse effects like immune suppression, cardiac problems [4]. Now a day the approach on herbal medicine to reduce the adverse effects has been increased and *Anacardium occidentale* L. is one of the traditional herbal medicine which claims to have many therapeutically beneficial effects. *Anacardium occidentale* L. kernel contains proteins, fats, fibres, carbohydrates, minerals etc. Leaves comprise tannins (23%), nontannins (18%), β-sitosterol, ethyl gallate, hyperoside, methyl gallate, leucocyanidin and leucodelphidins [5]. Alcoholic extract of leaves show hypoglycemic activity in albino rats. Tar from bark is used as counterirritant; while the kernel is nutritive, demulcent and emollient. In Peruvian herbal medicine, cashew leaf tea is employed as common diarrheal remedy. Bark tea as common antiseptic, vaginal douche and seeds are used for skin infections. Cashew fruit is used in leprosy, psoriasis and as a blister in warts, corns and ulcers [6]. The bark and leaves are used medicinally in curing toothache, sore gum and as a remedy for scurvy. So, the aim of the study was to give a scientific evaluation of anti-asthmatic property of *Anacardium occidentale* L. [7].

Materials and Method

Plant Material: The leaves of *Anacardium occidentale* L. were collected from Y.C.M.O. University campus, Nasik, Maharashtra, India. It was authentified by Botanical Survey of India, Pune, Maharashtra, India and voucher number: SKMAOPL1/2008.

Preparation of extract**:** Leaves were dried under normal environmental conditions, milled to coarse fine powder (60-80 #). Powdered leaves were charged in Soxhlet extractor for continuous hot extraction using petroleum ether (60-80^{o}C), chloroform and ethanol in successive manner. Ethanolic extract was separated into two fractions namely ethyl acetate soluble part and methanol soluble part.

Animals: Mice (20-30 gm) and Dunken Hartley Guinea pig (350-400 gm) of either sex supplied by the Bharat Serum and Vaccines Limited, Mumbai were used for the study. All the animals were housed in the cages with natural light-dark cycle and fed with standard pellet food and had free access to water. The study was approved by the Institutional Animal Ethics Committee of MGV's Pharmacy College, Nasik. The ethical norms as described by CPCSEA were strictly followed during all experimental procedure.

The different models used for the study were**:** a) Goat tracheal chain preparation b) Guinea pig ileum preparation c) Haloperidol induced catalepsy in mice d) Milk induced leukocytosis.

a) **Isolated Goat tracheal chain preparation** [8]:
Isolated adult goat tracheal tissue was obtained from slaughter house. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution and was continuously aerated at 37 ± 0.5^{o}C. Dose response curves of histamine was obtained in Kreb's solution and in Kreb's solution containing 100 µg/ml *Anacardium occidentale* L. extract. Percent of maximum contractile response were plotted to record dose response curves of histamine in the absence and presence of plant extract.

b) **Isolated Guinea pig ileum preparation [**9]
The guinea pigs (overnight fasted) were sacrificed and the ileum was mounted in an organ bath containing Tyrode solution, which was continuously aerated at 37 ± 0.5^{o} C. Dose response curve of histamine in plain Tyrode solution and solution containing 25 µg/ml *Anacardium occidentale* L. extract was performed. Percent of maximum contractile response were plotted to record dose response curves of histamine in the absence and presence of plant extract.

c) **Haloperidol induced catalepsy in mice** [10]
Swiss mice were divided into 5 groups (n=6), control group received saline and other groups received single dose of extracts (250, 375, 500 mg/kg, p.o.) respectively. Chlorpheniramine maleate (10 mg/kg) was used as a positive control. All groups received haloperidol (1 mg/kg, i.p.) one hour after the drug administration and the duration of catalepsy was measured at 0, 30, 60, 90, 120 and 150 minutes.

d) Milk induced leucocytosis in mice [11**]**
Swiss mice were divided into 5 groups (n=6). Control group received saline, other groups received single dose of extracts (250, 375, 500 mg/kg, p.o.). Only milk received group served as an intoxicant. After 1 h of drug treatment except control all groups received boiled and cooled milk injection in dose of (4 ml/kg, s.c.). Total leucocyte count was done in each group before drug administration and 24 after milk injection.

Results

Isolated Goat tracheal chain preparation

In isolated goat tracheal preparation, a right side shift of dose response curve of histamine was observed in the presence of the extract indicating antihistaminic action.

Dose of Histamine (2.5 µg/ml)	Isolated Goat tracheal chain preparation	
	Control Group (% maximum contraction)	Test group (% maximum contraction)
0.1	18.73 ± 1.39	10.35 ± 1.35*
0.2	37.43 ± 5.77	15.08 ± 2.30*
0.3	50.15 ± 3.91	20.69 ± 2.38*
0.4	71.97 ± 2.11	27.16 ± 1.16*
0.5	100 ± 0	33.14 ± 1.16*

TABLE 1 Effect of ethyl acetate soluble part of ethanol extract of *Anacardium occidentale* on Histamine induced contractions in Isolated Goat tracheal chain preparation

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was $p<0.05$ as compared with histamine alone.

Effects of methanol soluble part of ethanol extract of *Anacardium occidentale* on Histamine induced contractions

In isolated goat tracheal preparation, a right side shift of dose response curve of histamine was observed in the presence of the extract indicating antihistaminic action

Dose of Histamine (2.5 µg/ml)	Isolated Goat tracheal chain preparation	
	Control Group (% maximum contraction)	Test group (% maximum contraction)
0.1	20.24±2.09	0.5±2.45*
0.2	37.2±2.37	21.93±5.59*
0.3	56.15±2.98	36.09±5.38*
0.4	76.79±2.48	42.16±8.16*
0.5	100±0	65.8±11.57*

TABLE 2 Effects of methanol soluble part of ethanol extract of *Anacardium occidentale* on Histamine induced contractions.

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was $p<0.05$ as compared with histamine alone.

Isolated Guinea pig ileum preparation

In isolated guinea pig ileum preparation, a right side shift of dose response curve of histamine was observed in the presence of the extract indicating antihistaminic action.

Dose of Histamine (2.5 µg/ml)	Isolated Guinea pig ileum preparation	
	Control Group (% maximum contraction)	Test group (% maximum contraction)
0.1	74.5 ± 13.84	40 ± 4.74*
0.2	87.27 ± 5.64	62.72 ± 10.6
0.3	80 ± 10.58	53.68 ± 5.86
0.4	79.98±11.41	65.32 ± 7.64
0.5	80.83 ± 8.49	55.83 ± 7.75

TABLE 3 Effects of ethyl acetate soluble part of ethanol extract of *Anacardium occidentale* on Histamine induced contractions on isolated Guinea pig ileum

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was $p<0.05$ as compared with histamine alone.

Haloperidol-induced catalepsy:

In this study maximum protection against haloperidol induced catalepsy was observed at dose of 375 mg/kg.

Dose (mg/kg)	Duration of Catalepsy (sec)					
	0 min	30min	60min	90min	120min	150min
Control	3.592 ±0.44	15.47 ±1.39	29.37 ±1.39	24.32 ±1.44	18.78 ±1.44	9.798 ±0.52
AO 250	4.48 ±12.24	12.44 ±0.93	25.5 ±1.34	21.42 ±1.25	16.91 ±1.76	7.77 ±0.22*
AO 375	4.76 ±0.42	12.07 ±1.13	22.52 ±1.21*	18.86 ±1.38*	15.29 ±1.67	7.46 ±0.27*
AO 500	4.85 ±0.38	11.22 ±0.85*	18.37 ±1.19*	16.21 ±1.47*	13.56 ±1.20*	7.16 ±0.56
CPM	4.52 ±0.53	8.53 ±0.15*	11.37 ±1.74*	9.65 ±0.92*	8.35 ±0.49*	7.65 ±0.75*

TABLE 4 Effect of ethanol extract of *Anacardium occidentale* (AO) on Haloperidol-induced catalepsy

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was $p<0.05$ as compared with plain histamine. CPM is Chlorpheniramine maleate (10 mg/kg).

Total Leukocyte Count.

Total leukocyte count was carried out for doses 250, 375 and 500 mg/kg p.o. body weight. The result show maximum decrease in total leukocyte count.

Treatment	Number of leukocytes (mm^3)		
	Before treatment	After treatment	Difference
Control (10ml/kg)	4080±1039	8080±766.4	4000±500
AO 250 + milk (4ml/kg sc)	6680±713	5060±872.2	1900±736.2*
AO 375 + milk (4 ml/kg sc)	5760±1724	4100±255	1720±690.2*
AO 500 + milk (4 ml/kg sc)	4400±641.1	4360±1001	1240±402*

TABLE 5 Effects of Ethanol extract of *Anacardium occidentale* (AO) on Total Leukocyte Count

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was $p<0.05$. All treated groups were compared with control group.

Discussion

Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli, in which many cells play a role. Different agonists like acetylcholine, histamine, 5-HT and bradykinin are responsible for contractile responses. The present study dealt with screening of antiasthmatic activity of ethanol extract of leaves of *Anacardium occidentale* L.

In isolated goat tracheal and guinea pig ileum preparation, a right side shift of dose response curve of histamine was observed in the presence of the extract indicating antihistaminic action (Table 1, Table 2 & Table 3).It was observed that antihistaminic activity in methanol soluble part was comparatively (Table 1 and 2) less. Histamine is the major inflammatory mediator in asthma, causing hyper responsiveness and bronchial airway inflammation [12].

Haloperidol induces catalepsy by inhibiting dopamine D_2 receptors and inhibits dopamine secretion. Dopamine is agonist for adrenaline, while adrenaline is physiological antagonist of histamine. Hence decrease in dopamine levels increases histamine physiologically. In this study significant protection against haloperidol induced catalepsy was observed at dose of 375 mg/kg. (Table 4).

Most allergic and non-allergic asthmatics, including those with mild asthma, having bronchial eosinophilia and there is significant association between eosinophil activation and asthma severity as well as bronchial hyper-responsiveness. Therefore Total leukocyte count was carried out for doses 250, 375 and 500 mg/kg p.o. body weight. The result show significant decrease in total leukocyte count, indicating possible usefulness of *Anacardium occidentale* extract in antiasthmatic treatment. (Table 5)

Thus the possible mechanism of its antiasthmatic activity may be due to inhibition of various inflammatory mediators of asthma, but still exact mechanism is yet to be carved out and separation of active chemical constituents is yet to be done to determine exactly which constituent is responsible for activity.

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