EVALUATION OF ANTHISTAMINIC ACTIVITY OF *CASUARINA EQUISETIFOLIA* FROST (CASUARINACEAE)

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Summary

The isolated goat tracheal chain was used to assess the antihistaminic activity of Methanolic extracts of wood, bark, fruit and leaf of Casuarina equisetifolia. The extracts of wood and bark inhibited the histamine induced contraction of trachea (10-80 mcg/ml) in dose dependent pattern ($P<0.05$) while leaf and fruit extracts were without any effects. The successive chloroform extract (63.30 ± 10.33) demonstrated more activity as compare to petroleum ether (87.5 ± 13.24) and methanolic extract (166.66 ± 23.32) of wood ($P<0.05$).

The chronic treatment of methanolic wood extract (100 mg/kg, i. p.) significantly reduced the clonidine induced catalepsy at 60 and 120 minutes ($P<0.05$) and mast cell degranulation (72.50±8.37) against standard, Disodium cromoglycate, (85.19 ± 4.30) ($P<0.001$).

Concisely, the present study evidenced the traditional claim in the management of asthma claimed by medical practioners of Samoa.

Key words: Casuarina equisetifolia; antihistaminic activity; Histamine; Clonidine; Toluidine blue; Catalepsy.

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Introduction

The plant is evergreen tree; generally attain height up to 50 m, introduced into India. It is cultivated on Coastal regions from Gujarat to Orissa, some parts of West Bengal and in Andamans.[1]

The chemical constituents isolated from different plant parts shows the presence of flavonoid glycosides of kaempferol and quercetin in leaves; [2] alicyclic acids (shikimic and quinic acid), polyols (dextrose, fructose and sucrose) and amino acids in fruit, bark and wood, [3] acetates of β–amyrin and taraxerol, lupenone, glutinol, lupeol,kaempferol,3α-L-arabinoside, afzelin, gallic acid and β– sitosterol in leaves and fruits, [4]6-18% of tannins containing D-gallocatechin in bark, [5,6] gallic acid, protocatechuic acid, hydroquinone, juglanin, abzelin, catechin, gallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate in fruits, gallic acid ,methyl gallate, catechin, epicatechin, gallocatechin, epigallocatechin in wood. [7]

The needles in decoction form used as lotion for swelling. The fruits mixed with powdered nutmeg to treat toothache. [1] The tannin content in different plant parts enable to use as astringent and in the treatment of diarrhea and dysentery. [8] The present work evaluates the antihistaminic activity of various parts of the plant.

Materials and methods

Authentification of plant material: - The plant specimen was collected from Gangapur dam locality, Nashik (M.S.) identified as ***Casuarina equisetifolia*** Linn Family Casuarinaceae, Voucher no. ANA1, Ref. No. BSI /WC/ Tech./2005/867 dated 22.12.2005 by P. S. N. Rao, Joint Director, Botanical Survey of India, Pune (M.S.).

Preparation of Plant extracts: - Coarsely powdered (10#) materials of leaf, bark, wood and fruit (100 g each) were subjected to Soxhlet extraction in two sets comprising of extraction with methanol and later wood powder successively with petroleum ether 60-80^{0}C, chloroform and methanol followed by subsequent filtration and evaporation to yield extracts. The extracts were dried in vacuum oven, labeled as, Set I = MEL, MEB, MEW and MEF, Set II= PEW, CHW and MEWS. The activity was studied by preparing the solutions of the extracts (2.5%w/v) in PEG-400.

Animals: - Swiss Albino mice of either sex weighing around 18-25 gms were procured from National institute of toxicology, Pune, India. They were acclimatized to the standard laboratory conditions at the temperature of 25 ± 1 ^{0}C for 5 days. The animals had free access to food and water, maintained under light and dark cycles of 12 hrs each. All experiments were carried out during day time from 09.00-14.00 hrs. The Institutional animal ethics Committee (IAEC) approved the experimental protocol and cares of animals were taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Isolated Goat tracheal chain preparation: - The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in ice-cold oxygenated Kreb's solution. The spirally cut trachea was suspended in 10 ml of Kreb's solution. Goat trachea was cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Krebs-Henseleit (composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2$, 1.2; glucose, 11.5) and maintained at 37 ± 1ºC, a stream of 5 % CO_2 in oxygen was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S- shaped aerator and the other attached to isotonic frontal writing lever to a smoked drum. The tissue was allowed to equilibrate for 45 min. under a load of 400 g.

The effect of histamine (10, 20, 40, 80 mcg/ml) on isolated Goat tracheal chain was observed in presence of extracts (0.5 ml of 25 mg/ml each).The extracts and Histamine were dissolved in PEG-400 (inert in response to Contraction to trachea).[11]

Graph of maximum percentage of contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of extracts of plants (Table1).

Table 1:- Effect of Histamine on Isolated Goat tracheal chain

Sr.No.	Extracts of Casuarina equisetifolia				
	Percentage contraction (Mean ± SEM)				
	Dose mcg/ml of Histamine	10+ x	20+ x	40+ x	80+ x
1	MEW	*12.44 ± 2.35	*42.64 ± 7.85	*48.78 ± 6.64	*60.62 ± 9.87
2	MEB	24.86 ± 3.27	41.78 ± 6.89	58.43 ± 8.67	72.59 ± 5.97
3	MEF	--	--	--	--
4	MEL	--	--	--	--
5	PEW	5.65 ± 1.39	35.44 ± 8.31	60.87 ± 11.13	87.5 ± 13.24
6	CHW	26.15 ± 5.21	50.76 ± 5.63	46.15 ± 7.38	*63.30 ± 0.33
7	MEWS	38.89 ± 8.83	66.67 ± 12.21	77.77 ± 15.71	*166.66 ± 23.32

n=5,*p<0.05, students t-test (unpaired),x= 0.5 ml of 25 mg/ml of respective extract.
-- = no activity

Clonidine-induced Catalepsy

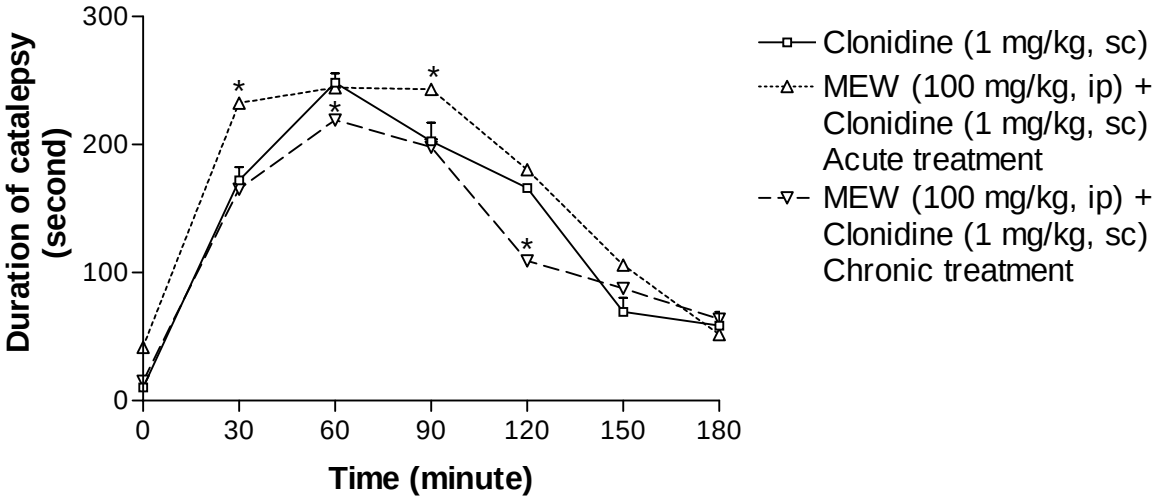
Bar test was used to study the effects of extracts on Clonidine-induced Catalepsy. Clonidine (1mg/kg, s.c.) was injected to Mice (n = 5) pretreated with Vehicle (Water 0.1 ml, i.p.) and MEW (100 mg/kg, i.p.).The duration of Catalepsy was measured at 0, 30, 60, 90, 120, 150, 180 min after Clonidine administration. Both the forepaws of mice were placed on a horizontal bar raised 3-cm above the table and the time required to remove the forepaws from the bar was recorded as the duration of Catalepsy. In another set of experiment, Mice were given MEW (100 mg/kg, i.p. each) once daily for 4 days and received Clonidine 30 min after last dose, and the duration of Catalepsy was measured. Animals were pretreated with vehicle (water 0.1 ml, i.p.), MEW (100 mg/kg, i.p.) 30 min before Clonidine (1 mg/kg, s.c.) administration. The duration of Catalepsy was measured for 3hrs after every 30 min. (Fig. 1). [9,12]

Clonidine-induced Mast cell degranulation

Normal saline (5.0 ml) was injected into the peritoneal cavity of mice. After a gentle massage, the peritoneal fluid was collected and transferred into the test tubes containing 3-4 ml of RPMI-1640 buffer medium (pH 7.2-7.4). Mast cells were then washed by centrifugation at a low speed (400-500 rpm) followed by discarding the supernatant and taking the pellets of Mast cells into the medium. The Mast cells were treated with Clonidine (80 mcg/ml) and incubated at 37ºC in a water bath for 10 min., spread on the microscopic slide; stained with Toluidine blue containing 1% acetic acid and percentage protection against degranulation (indicated by the presence of vacuoles within the mast cells) was calculated. [10]. In the standard drug treated group, Disodium chromoglycate (200 mcg/ml) was added prior to the addition of clonidine. The mean percentages of mast cells were determined by counting 500 cells from each subcutaneous spread.

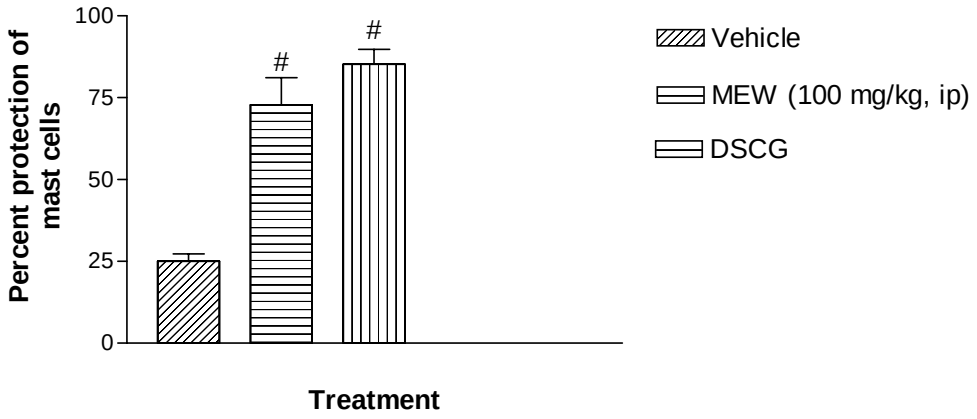
The percentage protection against degranulation was calculated. MEW (100 mg/kg, i.p.) was administered orally for 4 days, prior to collection of Mast cells (Fig.2).

Fig. 1:- Effect of MEW on Clonidine-induced Catalepsy in Mice



n =5, * P < 0.05, One-way ANOVA at different time intervals, followed by Dunnett's test

Fig. 2:- Effect of MEW on Clonidine induced Mast cell degranulation in Mice.



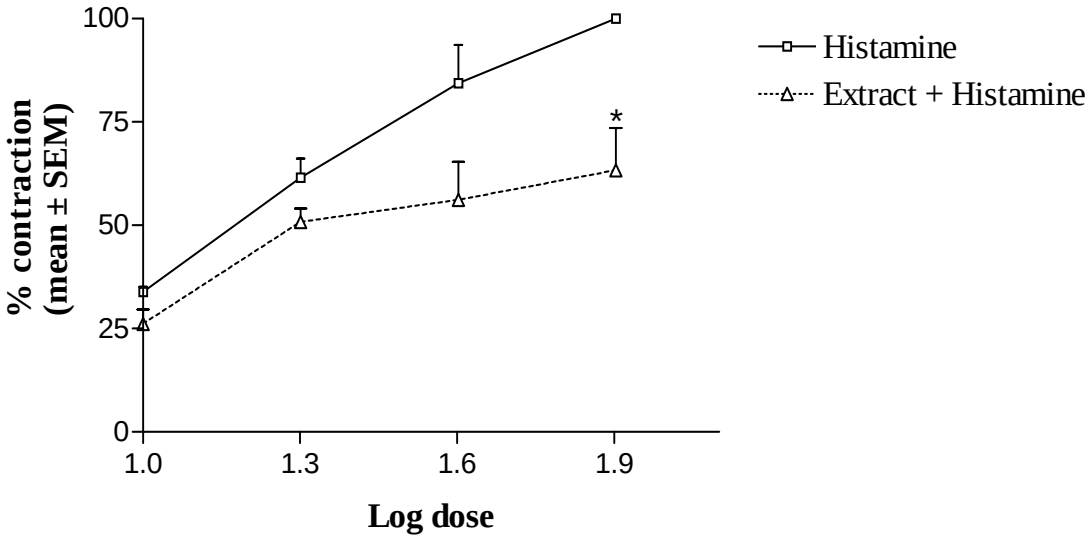
n = 5, # P < 0.001, One-way ANOVA followed by Dunnett's test
Values are expressed as Mean ± SEM

Statistical analysis: - The data is represented as Mean ± SEM. The data was analyzed by Student's –T test and One way Annova followed by Dunnett's test. Prism graph pad 3 was used for statistical analysis. P<0.05 was considered as significant.

Result and Discussion

Isolated goat tracheal preparation: - In a graph of maximum percentage of contractile response Vs negative logarithm of molar concentration of histamine, indicated a dose dependent contraction of goat tracheal chain (Fig.3). Methanolic extracts of wood of C. equisetifolia (60.62 ± 9.8) produced more significant antihistaminic activity (Table1) as compared to (72.59 ± 5.97) bark extract (P<0.05) while Methanolic extracts of leaf and fruit were devoid of effects.

Fig. 3:- Effect of Chloroform fraction on Histamine-induced contraction on Goat Tracheal Chain.



n =5, * P < 0.05, Student's t-test (unpaired)

The chloroform extract of wood (63.30 ± 10.33) showed more significant antihistaminic activity (Fig.1) as compared to petroleum ether (87.5 ± 13.24) and (166.66 ± 23.32) successive methanolic extract of wood (P<0.05).

In the present study, both methanolic extracts of wood and bark showed significant antihistaminic effect. As the chloroform extract has showed a prominent antihistaminic activity in trachea model, reveals that the preparation containing wood extract of C. equisetifolia can be used in reliving the symptoms of asthma.

Clonidine induced catalepsy: - Clonidine produced catalepsy in mice, which was remained for 3 hrs. Maximum catalepsy was recorded 60 min after clonidine (1 mg/kg, s.c.). Single dose of wood extract (100 mg/kg, i.p.) significantly poentiated catalepsy at 30 min. (235.2 ± 2.5) and 90 min. (244.5 ± 2.5) (p<0.05), but on repeated doses once in a day for 4 days reduced catalepsy (Fig. 1) at 60 min. (220.6 ± 3.5) and 120 min.(117.7 ± 4.4) (P<0.05).

The cataleptic effect of clonidine in the mouse is mediated by histamine release from the mast cells and was inhibited by wood extract. The effect of extract on clonidine induced catalepsy is probably due to their mast cell stabilizing or antihistaminic properties.

Clonidine-induced Mast cell degranulation: - The microscopic studies of the subcutaneous tissue spreads from the animal treated with Clonidine and extract revealed the degranulation. The density and homogeneity of the granules were reduced. The clonidine induced mast cell degranulation was inhibited by standard mast cell stabilizer Disodium cromoglycate in vitro by offering the percentage protection 85.19±4.30 (Fig. 3). Pretreatment of wood extract (4 days) produced comparable mast cell protection (72.50±8.37) against clonidine induced mast cell degranulation ($P<0.001$) (Fig. 2).

The Methanolic wood extract offered significant protection against clonidine induced mast cell degranulation, which is evident from its protective effect on mast cell degranulation in mice and decrease in capillary permeability in mice. The antihistaminic activity of wood may be due to stabilization of mast cell membrane or inhibition of antigen induced histamine release by phenolic constituents.[7] The study revealed that wood extract examined have different mode of actions and useful in the management of asthma as claimed by Samoan traditional medicinal practioner.[13]

Further study shall be aim at isolating, characterizing and purifying compounds from chloroform extract of Wood with potential bioactive properties.

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References

1. Anonymous, Wealth of India. Raw Materials Vol.3, Publications& Information Directorate, C.S.I.R., New Delhi 1992: 380-85.
2. Ansary EL, Mohamad A, Ishak Moheb S, Ahmed Ahmed A, Saleh Nabiel AM. Flavonol glycosides of Casuarina equisetifolia. Bioscience 1977; 32: 444 -445.
3. Madhusudanamma W, Sastry KNS, Sundara Rao VS, Reddy KK, Santappa M. Isolation and Characterization of Alicyclic acids, Polyols &Amino acids from Casuarina equisetifolia. Leather Science (Madras) 1978; 25: 369-371.
4. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants Vol.5,Central drug Research Institute, Lucknow and National Institute of Science Communications, New Delhi 1998: 183.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants1 st edition, National Institute of Science Communications (C.S.I.R.), New Delhi 1956: 55.
6. Roux DG. d-Gallocatechin from the bark of Casuarina equisetifolia Linn. Nature 1957;179: 158-159.
7. Madhulata W, Sundara Rao VS, Reddy KK, Sastry KNS. Phenolic constituents present in Casuarina fruits and wood. Leather Science (Madras) 1985; 32: 38-39.
8. Mhaskar KS, Blatter E, Caius JF(Edt). Kirtikar and Basu's Illustrated Indian Medicinal Plants Vol.10, Sri Satguru Publications, Delhi 2000: 3248-50.
9. Dhanalakshmi S, Khaserao SS, Kasture SB. Effect of ethanolic extract of some anti-asthmatic herbs on clonoidine and haloperidol- induced catalepsy in mice. Oriental Pharm. and Expt. Med. 2004; 4:1-5.
10. Lakdawala AD, Dadkar NK, Dohadwala AN. Action of clonidine on mast cells of rats. J. Pharm. Pharmacol. 1980; 32: 790-791.
11. Chaudhari N, Lahiri SC. Use of Goat trachea for an isolated tracheal chain preparation. Ind. J. Pharmacol. 1974; 6:149-151.
12. S.Ferre, T.Guix, G.Prat, F.Jane, M.Cosa. Is experimental catalepsy properly measured?. Pharmacol Biochem. and Behav. 1990; 35: 753-757.
13. Han ST. Medicinal Plants in South Pacific Western Pacific Series No.19, Geneva, Switzerland 1998: 41.