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Research Article

PHARMACOLOGICAL SCREENING OF ANTI-ASTHMATIC ACTIVITY OF *ANISOMELES MALABARICA* (L).R.BR.EXSIMS

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ABSTRACT

Objective: In the study the chloroform and methanolic leaves extract of *Anisomeles malabarica* Linn (Lamiaceae) was investigated for screening of anti histaminic effect by using guinea pig model. Methods: Identified the protective effect against asthmatic activity using different method like both *invivo (*Histamine induced bronchospasm in conscious guinea pigs) and *invitro(*Histamine induced contraction on isolated guinea pig ileum preparation) models. Resuts:. Percentage inhibition of histamine in isolated guinea pig ileum preparation show significant inhibition in standard is 52.26% as like that the extract of MEAM also shows significant inhibition of histamine of about 35.20 %. Conculation:The conculed of the present investigation was find out the anti-asthmatic activity of chloroform and methanolic extract of AM by using histamine induced bronchospsam in conscious guinea pig. The preliminary phytochemical study prove the presence of alkaloids, flavonoids, tri-terpenoids,and saponin. The both extract of CEAM and MEAM was possessing good protection against respiratory disease but methanolic extract 200mg/kg have shows highly protective action than the CEAM.

Keywords: Anti asthmatic, *Anisomeles malabarica,* Histamine, chloroform, Methanol, Guinea pig

INTRODUCTION

It was an integral part of the development of modern civilization[1]. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs[2]. Plant is the rich source of safe and effective medicine.[3] Plant drug is the Source of primary healthcare in world.[4].

It is also estimated that about 25% of the drugs prescribed worldwide are derived from plants, and 121 such active compounds are in use and 13 drugs derived from natural products were approved in the United States[5].

Today, there is an urgent need to develop safer drugs for the treatment of inflammatory disorders, diabetes, liver diseases, and gastrointestinal disorder. Hence, there is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.

Asthma comes from the Greek word for "panting" and has been described as a pathological condition for centuries.[6] Asthma is one of the most common chronic diseases in the world. It is estimated that around 334 million people in the world currently have asthma. Considerably higher estimates can be obtained with less conservative criteria for the diagnosis of clinical asthma.[7]

Asthma is one of the major disease in adults and children's the working definition proposed to be Asthma is a chronic inflammatory disorder of the airways causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and in the early morning [8]. The selection of plant was made based on its ease of availability, therapeutic value. The present study was undertaken to evaluate the asthmatic effect of leaves of *Anisomeles malabarica* (L).

MATERIALS AND METHODS

Taxonomical Identification

The species for the proposed study was identified and authenticated by Taxonomist Dr. V. Ganesan. Professor and Head, Dept. of Botany, Ayyanadar Janakiammal College of Arts and Science, Sivakasi, Virudhunagar Dist, Tamil Nadu. The plant specimen was certified as *Anisomeles malabarica* (L) R.BR.exsims, belonging to family; Lamiaceae. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) Reference Number:SBCP/2013-14/CPCSEA/IAEC-I/3(j),

Extraction method

The leaves parts were collected; shadow dried and then size reduced to small particles manually. The dried material was coarsely powdered before extraction. The extracts were prepared by Soxhlet Apparatus continuous extraction process by using hexane, chloroform, ethyl acetate, methanol and aqueous as the solvents. For each solvent, extraction was continued for 48 hours. The colour and percentage yield of each extracts were calculated[9].

IN VIVO MODEL

Histamine induced bronchospasm in conscious guinea pigs

Symptoms like asphyxia convulsions resembling bronchial asthma can be induced by inhalation of histamine or other bronchospasmogen in guinea pig. The occurrence of these symptoms can be delayed by bronchodilator drugs[10].

Twelve guinea pigs 250-350g were randomly divided into Six groups (n=2/group). I-VI bronchoconstriction induced by histamine dihydrochloride mixing with normal saline (2%) aerosol type throughout the study, and following treatment was given Bronchospasm was induced in guinea pigs by exposing them to histamine aerosol 2% produced by an ultra-sound nebulizer in an aerosol chamber (24x14x24cm) made of perspex glass [11,12]. The time required for appearance of pre- convulsive dyspnea caused by the histamine was recorded for each animals. Prior to drug treatment, each animals was placed in the histamine chamber and exposed to 2% histamine aerosol, the pre-convulsive time (PCT), i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the pre convulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for pre convulsive dyspnoea was recorded to basal value. Guinea pigs were than allowed to recover from dyspnoea for 24 hrs. After 24hrs the animals of CEAM(200mg/kg) and (400mg/kg) received chloroform extract of *Anisomeles malabarica* (L)&MEAM(200mg/kg) and (400mg/kg) received methanolic extract of *Anisomeles malabarica* (L) and also std group received chlorpheniramine maleate.(2mg/kg,p.w,i.p) These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hrs and 24 hrs to determine pre convulsive time (PCT). The protection offered by the treatment was calculated by using the following formula.

Table 1. Treatment protocol for Histamine induced bronchospasm in conscious guinea pig

Group	Drug Treatment	Animals
Control	2%Histamine+ Normal saline	2
Standard	2%Histamine+Chlorpheniramine maleate(2mg/kg i.p.)	2
CEAM 200	2%Histamine+CEAM (200mg/kg p.o.)	2
CEAM 400	2%Histamine+CEAM (400mg/kg p.o.)	2
MEAM 200	2%Histamine+MEAM (200mg/kg p.o.)	2
MEAM 400	2%Histamine+MEAM (400mg/kg p.o.)	2

Bronchospasm was induced in guinea pigs by exposing them to histamine aerosol 2% produced by an ultra-sound nebulizer in an aerosol chamber (24x14x24cm) made of perspex glass [12]. The time required for appearance of pre- convulsive dyspnea caused by the histamine was recorded for each animal. Prior to drug treatment, each animals was placed in the histamine chamber and exposed to 2% histamine aerosol, the pre-convulsive time (PCT), i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the pre convulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for pre convulsive dyspnoea was recorded to basal value. Guinea pigs were then allowed to recover from dyspnoea for 24 hrs. After 24hrs the animals of CEAM(200mg/kg) and (400mg/kg) received chloroform extract of *Anisomeles malabarica* (L)&MEAM(200mg/kg) and (400mg/kg) received methanolic extract of *Anisomeles malabarica* (L) and also std group received chlorpheniramine maleate.(2mg/kg,p.w,i.p) These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hrs and 24 hrs to determine pre convulsive time (PCT). The protection was calculated using formula.

Parameter

Symptoms like increased breathing frequency, and forced inspiration and asphyxia convulsion was absorbed .the time taken to observe PCT (pre convulsion time – time of aerosol exposure to the onset of dysponea).was noted from 1hr, 4hrs and 24hrs. Percentage protection = $(1-T_1/T_2) \times 100$

Where, T_1 = the mean of PCT before administration of test drugs, T_2 = the mean of PCT after administration of test drugs at 1hr, 4hrs and 24hrs.[13]

IN VITRO MODEL

Histamine induced contraction on isolated guinea pig ileum preparation.

To detect antispasmodic and bronchodilator activity, the test compound was first added alone and washed out. The response of apasmogens like histamine was taken and at the height of contraction and the treatment drug was added. It causes relaxation in dose dependent manner.

Overnight fasted guinea pig was sacrificed using cervical dislocation and carotid bleeding. The ileum was rapidly dissected out and cut into pieces. ileum piece was suspended in organ bath containing 20 ml of tyrode's solution and was maintained at 37 ± 1^0C under basal tension of 500mg . The bathing solution was bubbled aerated with carbogen (95% O_2 and 5% CO_2). The tissue was equilibrated for a period of 30 minutes.The PSS in organ bath was changed every 10 minutes. The response of histamine was recorded by 5 min cycle. Using student physiography isotonic transducer. After obtaining a dose response curve of histamine (10µg/ml) on ileum, methanolic extract of areal part of *Anisomeles malabarica* (100µg/ml) was added to the reservoir and same doses of histamine was repeated in presence of plant extract. Same procedure was repeated for standard drug (CPM 10µg/ml) as methanolic extract. The effect of extract of treatment drug and its interaction with contractile response was recorded. The inhibition of contraction produced by spasmogens i.e., Histamine was inhibited by test drug MEAM [14, 15].

Parameter

% inhibition of histamine induced contraction was measured

STATISTICAL ANALYSIS

Values were expressed as mean± SEM. The mean difference in body weight changes, paw volume difference, biochemical parameters and haematological parameters were analysed using One way ANOVA followed by Dunnett's test. The values were considered significant at $P<0.01$ and $P<0.05$. Analysis was performed using GraphPad prism statistical software (Version 5.03).

RESULTS AND DISCUSSION

Percentage yield obtained for each extract were Hexane extract 4.32%. Chloroform, ethyl acetate, methanol and aqueous extracts yield were 7.73, 11.31, 14.33 and 9.62% respectively. The results were showed in (Table 7).

Preliminary phytochemical analysis

The presences of various phytoconstituents were identified by using different chemical test. The Hexane extract possess the presence of steroids, sterols and triterpernoids. The chloroform extract possess the presence of carbohydrates, steroids, sterols, tannins, flavonoids, saponins and triterpernoids. Ethyl acetate extract possess the presence of alkaloids, carbohydrates, glycosides, steroids, sterols, tannins, phenols and tri-terpinoids. Methanol extract possess the presence of alkaloids, glycosides, steroids, sterols, tannins, phenols, flavonoids, saponins and triterpernoids. Aqueous extract possess the presence of alkaloids, carbohydrates, glycosides, tannins and tri-terpinoids. The extracts which contain saponins, flavonoids and steroids are the

phytochemical constituents that are responsible for the anti-asthmatic activity so that chloroform and methanol extracts were selected for further studies. The results were showed in the (Table 1)

IN VIVO MODELS

Histamine induced bronchospsam in conscious guinea pig

The anti-asthmatic screening was carried out in chloroform & methanolic extract of *A.malabarica* in different concentration (200 and 400 mg/kg) by Histamine induced bronchospsam in conscious guinea pig model using chlorpheniramine maleate as a Standard Anti-histaminic drug (2mg/kg i.p). while spraying histamine aerosol (2%) the control animal showed PCT in first 45.75 sec of the experiment. The standard group which shows increase in PCT time of about 154.75±5.31 & 196±4.67 in one and fourth hour as compared to control group. The chloroform and methanolic extract of *A.malabarica* (L) in 200mg/kg p.o shows 106.5±2.32, 134.75±3.63 and 126.25±1.10, 145.75±2.92 less increased PCT int erval in first and fourth hours as compare to control. Chloroform and methanolic extract of *A.malabarica* (400 mg/kg p.o) also posses' increased PCT interval in first and fourth hour but it is very less as compared to control group, and the results were shown in (Table 2, 3)

IN VITRO MODEL

Histamine induced contraction on isolated guinea pig ileum preparation

The anti-asthmatic screening was carried out in methanolic extract of *A.malabarica* in the concentration of (100µg/ml) by Histamine induced contraction isolated of Guinea pig ileum preparation. Contraction response was recorded in the presence of standard drug chlorpheniramine maleate (10µg/ml) and methanolic extract of *A.malabarica* in the concentration of (100µg/ml) .The contraction response of guinea pig ileum was recorded in kymograph during the treatment. In the present study, histamine (10µg/ml) produced dose dependent contraction of guinea pig ileum preparation maximum percentage of contractile response versus negative log molar concentration of histamine. The modified the salt solution contain chlorpheniramine maleate (2mg/kg i.p.) and methanolic extract of AM 100µg/ml were significantly inhibited $p<0.01$ respectively. (Table 4)

Percentage inhibition of histamine in isolated guinea pig ileum preparation show significant inhibition in standard is 52.26% as like that the extract of MEAM also shows significant inhibition of histamine of about 35.20 %.(Table 5) Figure 1

Table 1 Phytochemical screening of leaf extracts of *Anisomeles Malabarica*

Test	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	-	+	+	+	+
Carbohydrates	-	+	+	-	+
Glycosides	-	+	+	+	+
Steroids	+	+	+	+	-
Sterols	+	+	+	+	-
Tannins	-	+	+	+	+
Phenols	-	-	+	+	-
Proteins	-	-	-	-	-
Amino acids	-	-	-	-	-
Flavonoids	-	+	-	+	-
Saponins	-	+	-	+	-
Tri-terpenoids	+	+	+	+	+

+ Presence -absence

Table 2 Effect Chloroform& Methanolic Extract of *Anisomeles Malabarica* on Histamine induced bronchospasm in conscious guinea pigs

Group	Pre convulsive Dyspnea (in Sec) (Mean ± SEM)			
	Before	1hr	4hrs	24hrs
Control	45.75±2.05	48±1.77	51±2.04	51.75±2.05
Standard(CPM 2mg/kg;i.p)	44.25±2.25	154.75±5.31**	196±4.67**	101.75±4.80**
CEAM 200mg/kg;p.o	42.25±2.05	106.5±2.32*	134.75±3.63*	69.25±2.32
CEAM 400mg/kg;p.o	43.75±1.93	65.75±1.49	121.75±1.79	80.25±3.09*
MEAM 200mg/kg;p.o	44±1.29	126.25±1.10*	145.75±2.92*	75.75±3.25
MEAM 400mg/kg;p.o	43±1.77	76.75±1.10	140.5±4.21	91±1.68*

n=2, values are mean ± SEM. Statistical analysis done by using one way ANOVA followed by Dunnet's test.* $p<0.05$, ** $p< 0.01$ significantly different from control.

Table 3 Percentage Production of Pre Convulsive Dyspepsia in *Anisomeles Malabarica* on histamine induced bronchospasm in conscious Guinea Pigs.

Groups	Percentage protection		
	1hr	4hrs	24hrs
Control	4.68	10.29	11.59
Standard(CPM 2mg/kg;i.p)	71.4	77.41	56.51
CEAM 200mg/kg;p.o	60.32	68.64	38.98
CEAM 400mg/kg;p.o	33.46	64.06	45.48
MEAM 200mg/kg;p.o	65.14	69.81	41.91
MEAM 400mg/kg;p.o	43.97	69.39	52.74

Table 4 Effect methanolic extract of *Anisomeles Malabarica* on Histamine induced contraction on isolated guinea pig ileum preparation

S.NO	Dose Volume	Log molar concentration	Percentage of response		
			Histamine10µg/ml	CPM10µg/ml	MEAM100µg/ml
I	0.1ml	0	36.86±9.14	17.54±8.18**	22.61±5.64*
II	0.2ml	0.3010	40.68±13.17	20.65±8.41*	31.53±7.16
III	0.4ml	0.6020	55.98±5.68	32.54±5.81*	46.33±0.37
IV	0.8ml	0.9030	77.51±1.29	38.82±6.37	54.41±6.23*
V	1.6ml	1.2041	100±0.00	47.74±7.90*	64.8±6.78*

n=2, values are mean ± SEM. Statistical analysis done by using one way ANOVA followed by Dunnet's test.* p<0.05, ** p< 0.01, *** p< 0.001 significantly different from control.

Table 5 Percentage inhibition response of different antagonist in histamine induced contraction on isolated guinea pig ileum preparation.

S.No	Treatment (Concentration)	%Inhibition of Histamine
I	Histamine (10µg/ml)	-----
II	Histamine+Chlorpheniramine Maleate(10µg/ml)	52.26%
III	Histamine+MEAM(100µg/ml)	35.20%

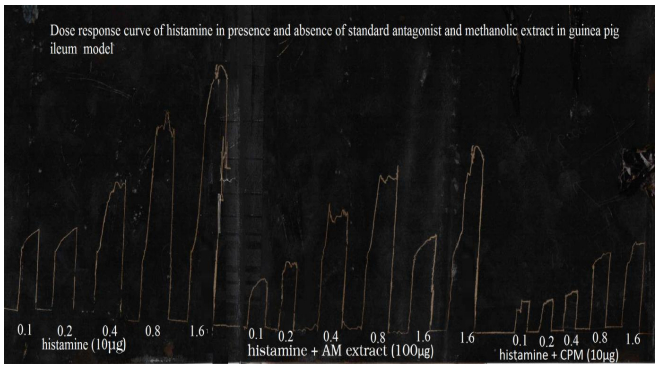

Figure: 1 Dose response curve of histamine in presence and absence of *Anisomeles malabarica* in guinea pig ileum model

CONCLUSION

The present investigation was to find out the anti-asthmatic activity of chloroform and methanolic extract of AM in guinea pig using histamine induced bronchospsam in conscious guinea pig. The preliminary phytochemical study prove the presence of alkaloids, flavonoids, tri-terpenoids,and saponin. The extract of CEAM and MEAM possess good activity but methanolic extract 200mg/kg have shows highly protective action. However the active constituents from the extract should be isolated and evaluation for anti-asthmatic activity of active constituents is needed for characterization of active principle responsible for anti-asthmatic activity. The studies may be extended in future by isolating the phytoconstituents which is responsible for anti-asthmatic activity. The further studies are in progress at department of pharmacology in our college.

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