PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF *CLITORIA TERNATEA* SEEDS EXTRACTS ON CLONIDINE INDUCED CATALEPSY IN MICE

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Summary

Clitoria ternatea L. (Family: Fabaceae) is a perimial twing herb. The roots have a sharp bitter taste and cooling, laxative, diuretic, anthelmintic, anti-inflammatory properties; they are useful in severe bronchitis, asthma and hectic fever Stem and flower are recommended for treatment of snake bite. The seed are Cathartic and the root is diuretic. The powder seed is combination with ginger powder was found to have laxative action. Seed contain fixed oil, a better acid resin, tannic acid, glucose. Polar (ethanol) and non polar (Benzene) extracts of C*litoria ternatea* seed at dose, 75 and 100 mg/kg, i.p. were evaluated on clonidine induced catalepsy on mice. Experimental study observed that ethanol and benzene extract at dose 75 and 100 mg/kg, i.p. showed significantly inhibition of clonodine induced catalepsy as compare to control group. Clonidine induced catalepsy by releasing histamine in brain so present study found that *Clitoria ternatea* seeds are having antihistaminic potential.

Key words: Clitoria ternatea, Clonidine, antihistamine, Chlorpheniramine maleate

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Introduction

Clitoria ternatea L. (Family: Fabaceae) a perennial twing herb, steams are terete, more or less pubescent. There are two varieties of *clitoria ternatea* white-flower and blue flower varieties. Root bark contain starch, tannin's & resins. Seed contain a fixed oil, a bitter acid resin, tannic acid, glucose. The roots have a sharp bitter taste and cooling, laxative, diuretic, anthelmintic, anti-inflammatory properties; they are useful in severe bronchitis, asthma and hectic fever Stem and flower are recommended for treatment of snake bite. The seed are purgative, Cathartic and laxative in combination with ginger powder [1,2]. The fatty acid content of *Clitoria ternatea* seeds includes palmitic, stearic, oleic, linoleic, and linolenic acids[3-5]. The seeds also contain a water-soluble mucilage, delphinidin 3, 3', 5'-triglucoside useful as a food dye[6]; beta-sitosterol[7].

C. ternatea have number of pharmacological activities such as possessing nootropic, anxiolytic, antidepressant, anticonvulsant[8], sedative[9], antipyretic, anti-inflammatory and analgesic activities[10]. Enhance memory, and increase acetylcholine content and acetylcholinesterase activity in rats[11, 13]. Objective of present study was to evaluate Polar (ethanol) and non polar (Benzene) extracts of *clitoria ternatea* seed on milk induced leucocytosis and milk induced eosinophilia.

Material and Methods

Plant material

Seeds of *Clitoria ternatea* were collected from Baramati localities, Pune district (Maharastra), and dried in the shade at room temperature. Dried seeds were coarsely powdered in grinder and powder material was kept in air tight container for further study. The plant was identified and authenticated by Prof. R. B. Deshmukh Head Dept. of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar.

Extraction

Dried and coarsely powder of Clitoria ternatea seeds were macerated for 48 hrs using ethanol (90%) and Benzene, evaporated to dryness in water bath to produce ethanol and benzene extract respectively.

Animals

Swiss albino mice of either sex weighing 25-28 g were housed under standard laboratory conditions, in groups of five. The animals had free access to food and water. The animal ethical committee of the institute approved all the protocols of the study.

Drugs and Chemicals

Clonidine (Unichem, Ltd.); Chlorpheniramine maleate (Alkem, Mumbai)

Statistical Analysis

The results were reported as mean±SEM and analyzed for statistical significance using One way ANOVA followed by student- Newman Keuls test $P < 0.05$ was considered significant

Clonidine-induced catalepsy in mice

Bar test was used to study effect of extracts on clonidine-induced catalepsy, to determine indirect antihistaminic activity. Mice were divided into six groups, five animals in each group. Animals belonging to group I served as control and were administered vehicle the (5 ml/kg, i.p.). Animals belonging to groups II to III received ethanol extract at dose of, 75 and 100 mg/kg i.p. respectively. Whereas animals belonging to Group IV and V received benzene extract at 75 and 100 mg/kg i.p respectively. Standard drug Chlorpheniramine maleate (10 mg/kg, i.p.) was given to group VI. The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar was noted for each animal. All the groups received clonidine (1 mg/kg, s.c.), 30 minute after the drug administration and the duration of catalepsy was measured at 30, 60, 90, 120, 150 and at 180 minute interval[14, 15].

Phytochemical investigation

Extracts were screened for preliminary phytochemicals test using standard procedure [16-18].

Results

Clonidine-induced catalepsy in mice

Clonidine releases histamine from mast cells which is responsible for different asthmatic conditions. Catalepsy produced by clonidine is mediated by histamine via H_1 receptors. The maximum catalepsy is developed after 90 minute of clonidine administration (1 mg/kg, i.p.) in vehicle treated (control) group. Prior treatment with ethanol and benzene extract at dose 75 and 100 mg/kg, i.p. showed significantly inhibition of clonodine induced catalepsy. Among these benzene extract at dose 100 mg/kg showed significantly decrease in ($P<0.001$) duration of catalepsy (as shown in table no.1).

Table no1. Effect of *clitoria ternatea* seed extracts on clonidine induced catalepsy in mice

Sr.No	Treatment	Dose	Time of catalepsy in (sec)					
			30min	**60 min**	**90min**	**120min**	**150min**	**180 min**
1.	Control (saline)	5 ml/kg i.p	16.84 ± 2.422	52.34 ± 3.595	175.5 ± 14.18	88.04 ± 7.91	54.7 ± 11.95	39.00 ± 1.66
2.	Ethanol extract	75 mg/kg	14.34 ± 0.8570	23.3± 1.222***	37.64± 3.808***	21± 4.163***	15.77± 3.50**	11.2± 4.46***
		100 mg/kg	19.14 ± 3.906	15.77± 1.90***	30.37± 3.094***	17.87± 0.829***	16.93± 6.98**	11.7± 4.71***
3.	Benzene extract	75 mg/kg	14.57 ± 2.217	19.06± 2.35***	26.6± 2.78***	17.84± 5.88**	7.87± 0.956**	6.87± 0.834***
		100 mg/kg	14.34 ± 2.325	18.87± 2.603***	14.7± 3.121***	25.64± 4.29	12.94± 4.23**	16.26± 1.48***
4.	Chlorphenir amine maleate	10 mg/kg	9.67± 0.7311	29.9± 7.319**	46.44± 2.107***	45.84 ± 1.811***	29.6± 4.05*	25.53± 1.369**

One way ANOVA followed by student- Newman Keuls test *** P < 0.001, ** P < 0.01, * P < 0.05 as compaired to control group

Phytochemical investigation

Phytochemical study found that presence of carbohydrate, glycosides, alkaloid and tannin in ethanol extract. Benzene extract showed presence of alkaloids.

Table no.2 Preliminary Phytochemicals Screening of Various Extracts of Clitoria ternatea seed extracts

Sr.no.	Chemical Tests	Extracts	
		Ethanol	Benzene
01	**Test for carbohydrate** (Molish test)	+	-
02	**Test for reducing sugar**		
	a)Fehling test	-	-
	b)Benedicts test	-	-
03	**Test for Alkaloids**		
	a)Dragnedroff test	-	-
	b)Mayer test	-	+
	c)Hager test	+	+
	d)Wagner test	-	+
04	**Test for glycosides** (Foam test)	+	-
05	**Test for Flavonoids** (Shinoda test)	-	-
06	**Test for Tannins**		
	a)5%feeric chloride	-	-
	b) Lead acetate sol.	+	-
	c)Bromine water	-	-
	d) Acetic acid sol.	-	-
	e) Dil. Iodine sol.	-	-
	f) Dil. Potassium dichromate sol	-	-
07	**Test for cardiac glycoside**		
	a)Legal test	-	-
	b)Baljetb test	-	-
08	**Test for steroid** (Salkowaski test)	-	-
09	**Test for carbohydrate** (Molish test)	-	-

+ Indicates presence of constituents. - Indicates absence of constituents.

Discussion

Clonidine, a α_2 adrenoreceptor agonist induces dose dependent catalepsy in mice, which was inhibited by histamine H_1 receptor antagonists but not by H_2 receptor antagonist[17]. Clonidine releases histamine from mast cells which is responsible for different asthmatic conditions[18]. Catalepsy produced by clonidine is mediated by histamine via H_1 receptors. Ethanol and benzene extract at dose 75 and 100 mg/kg, i.p. showed significantly inhibition of clonodine induced catalepsy. Clonidine induced catalepsy by releasing histamine in brain so present study found that Clitoria ternatea seeds are having antihistaminic potential.

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