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Research Article

EVALUATION OF THE EFFECT OF *PIPER BETLE* L. LEAVES EXTRACT AGAINST CLONIDINE-INDUCED CATALEPSY AND MILK-INDUCED LEUKOCYTOSIS AND EOSINOPHILIA IN MICE

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ABSTRACT

Objective: The objective of the study was to evaluate the effect of *Piper betle* L. leaves extract against clonidine-induced catalepsy and milk-induced leukocytosis and eosinophilia in mice.

Methods: Methanolic extract of *P. betle* L. leaves was prepared using Soxhlet apparatus. Preliminary phytochemical screening of the prepared extract was carried out using standard chemical tests. Effect of the prepared extract was evaluated against clonidine-induced catalepsy and milk-induced leukocytosis and eosinophilia in mice model.

Results: Maximum duration of catalepsy was observed at 90 min after the clonidine administration. There was a significant inhibition ($p<0.05$) of clonidine-induced catalepsy in the animals pretreated with chlorpheniramine maleate and extract of *P. betle* leaves. Administration of milk (4 mg/kg) subcutaneous route exhibited a significant increase in leucocytes and eosinophil count after 24 h of administration. Methanolic extract of *P. betle* L. leaves showed significant inhibition ($p<0.05$) of milk-induced leukocytosis and eosinophilia.

Conclusion: These results suggest that *P. betle* leaves extract may have the potential therapeutic value in the treatment of allergic diseases.

Keywords: Catalepsy, Leukocytosis, Eosinophilia.

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INTRODUCTION

Piper betle Linn. is a perennial dioecious climber, stems are semi-woody, much thickened at nodes; leaves large, 15–20 cm long, broadly ovate, slightly cordate, shortly acuminate, acute, entire, glabrous, yellowish or bright green, shining on both sides [1]. It is commonly known as the betle vine, it is an important medicinal and recreational plant [2]. *P. betle* is extensively found in damp forests and is propagated in India and other Southeast Asia, such as Vietnam and China [3]. The five main cultivars of *P. betle* Linn. are Bangla, Desawari, Karpoori, and Sanchi [4]. The betle leaves are nutritive and possess antitumor [5], wound healing [6], antimicrobial [7], antibacterial [8], antioxidant [9], gastroprotective [10], neuroprotective [11], antifilarial [12], antimalarial [13], and analgesic activity [14]. The leaves contain a variety of biologically active components like hydroxychavicol, chavicol, piperbetol, chavibetol, piperol A, methylpiperbetol, and piperol. The key component of the leaf is a volatile oil known as betle oil [15]. Karpoori variety possesses highest content of eugenol [16]. The primary goal of this research was to evaluate the effect of *P. betle* L. leaves extract against clonidine-induced catalepsy and milk-induced leukocytosis and eosinophilia in mice.

MATERIALS AND METHODS

Collection of plant material

The leaves of *P. betle* were collected from the rural areas of Baramati Dist., Pune (Maharashtra) and identified in the Department of Botany, Agricultural Development Trust's Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Malegaon (Bk), Tal-, Baramati Dist., Pune, Maharashtra, India (Voucher specimen PASR-142).

Drugs and chemicals

The drugs used were clonidine (Neon Lab. Ltd., India), chlorpheniramine maleate (Pfizer Ltd.), and dexamethasone (Zydus Healthcare Ltd.); all were purchased from a commercial source. Chemical used were methanol (Research Lab. Industries, India).

Extract preparation and phytochemical screening

The leaves of *P. betle* were dried and crushed to a coarse powder. Powdered material was subjected to Soxhlet extraction with methanol as a solvent. The extract so obtained was concentrated to dryness by evaporating the solvent. The percent yield was calculated. Extract was stored at room temperature and protected from direct sunlight. The prepared extract was subjected to preliminary phytochemical screening using standard chemical tests [17].

Animals

Swiss albino mice (20–30 g) of either sex were obtained from National Institute of Biosciences, Pune, India. Animals were maintained in our animal house under standard laboratory conditions. Animals were exposed to day-night light cycle and room temperature (24±2°C). All animals are allowed free access to readymade food pellets and water. Animals were handled according to standard protocols for the use of laboratory animals [18]. The experimental protocol was approved by the Institutional Animal Ethics Committee (1214/ac/08/CPCSEA).

Acute toxicity study

Acute toxicity study was performed by oral route in mice as per OECD guidelines 423.

Clonidine-induced catalepsy in mice

A bar test was performed to study the effect of clonidine-induced catalepsy [19-21]. Clonidine (1 mg/kg, s.c.) was injected to mice (n=6). Before 1 h of clonidine treatment, Group I received dist. water (1 ml/kg i.p.), Group II received chlorpheniramine maleate (10 mg/kg body weight, i.p.), Group III received methanolic extract 250 mg/kg

body weight i.p. (MEPBL250), and Group IV received methanolic extract 500 mg/kg body weight i.p.(MEPBL500). The forepaws of mice were placed on a horizontal bar and the time required to remove the paws for each animal was noted and the duration of catalepsy was measured at 15, 30, 60, 90, and 120 min.

Milk-induced leukocytosis and eosinophilia in mice

Swiss albino mice of either sex weighing between 20 and 30 g were divided into six groups of five animals each. All animals received boiled (boiling temp 70°C and boiling time 20 min) and cooled milk in dose of 4 ml/kg subcutaneously. Animals belong to Group I treated as control and received distilled water 10 ml/kg. p.o., Group II received methanolic extract 250 mg/kg.p.o.(MEPBL250), Group III received methanolic extract 500 mg/kg.p.o. (MEPBL500), whereas Group IV received dexamethasone 50 mg/kg i.p. Extract and standard drug were administered 1 h before milk injection. Blood samples were collected before and 24 h after milk administration from the retro-orbital plexus, under light ether anesthesia. Difference in total leukocyte and eosinophil count before and after 24 h drug administration was calculated [22].

Statistical analysis

The mean ±SEM values were calculated for each group. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test for individual groups compared with control. $p<0.05$ was considered as statistically significant.

RESULTS

Acute toxicity study

Treatment with methanolic extract up to 2000 mg/kg orally to mice did not induce mortality. Hence, LD_{50} was considered to be more than 2000 mg/kg. Based on acute toxicity results, 250 and 500 mg/kg doses were selected.

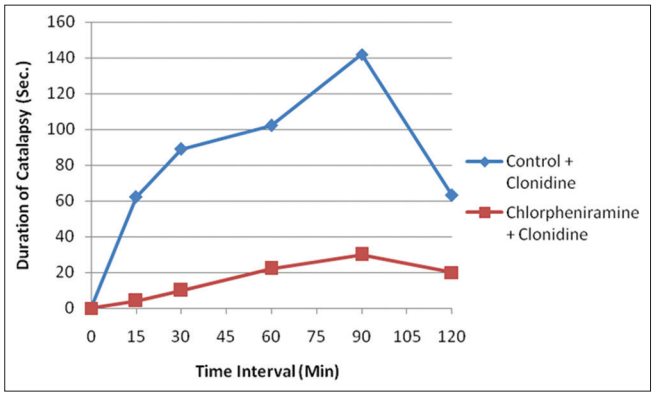

Fig. 1: Effect of chlorpheniramine maleate (10 mg/kg body weight, i.p.) on clonidine-induced catalepsy in mice

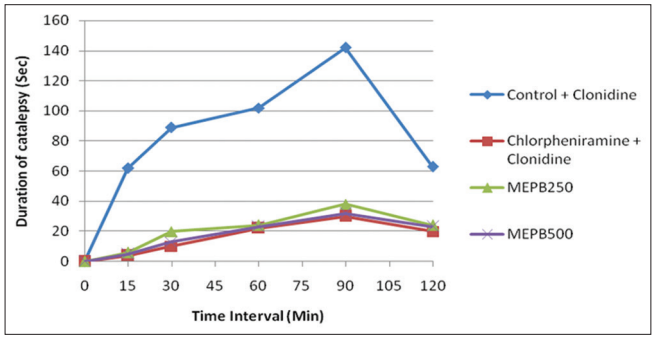

Fig. 2: Effect of *Piper betle* Linn. leaves extracts on clonidine-induced catalepsy in mice

Preliminary phytochemical screening

Preliminary phytochemical screening of the extract showed the presence of alkaloids, carbohydrates, proteins, flavonoids, phenolic compounds, and tannins.

Clonidine-induced catalepsy

All the groups showed a maximum duration of catalepsy at 90 min after the clonidine administration. There was a significant inhibition ($p<0.05$) of clonidine-induced catalepsy in the animals pretreated with chlorpheniramine maleate (Fig. 1), MEPBL250, and MEPBL500 (Fig. 2 and Table 1).

Milk-induced leukocytosis and eosinophilia in mice

Subcutaneous administration of boiled and cooled milk at a dose of 4 ml/kg showed a significant increase in the leukocytes and eosinophils count after 24 h as compared to leukocyte count before milk administration.

DISCUSSION

Catalepsy is a condition in which the animal maintains imposed posture for a long time before regaining normal posture. Catalepsy is the sign of the extrapyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in the brain. Clonidine, a α_2-adrenoreceptor agonist induces dose-dependent catalepsy in mice, which is inhibited by H_1 receptor antagonist but not by H_2 receptor antagonist [23]. It is known that clonidine releases histamine from mast cells [24]. Clonidine-induced release of histamine from mast cells is inhibited by α_2-adrenoceptor blocker. In the present investigation, all groups showed a maximum duration of catalepsy at 90 min after the clonidine administration. There was a significant inhibition ($p<0.05$) of clonidine-induced catalepsy in the animals pretreated with *P. betle* leaves extract. This indicates the antihistaminic activity of *P. betle* leaves.

During asthmatic inflammation, leukocyte release cytokines, histamine, and major basic protein promote ongoing inflammation. An abnormal

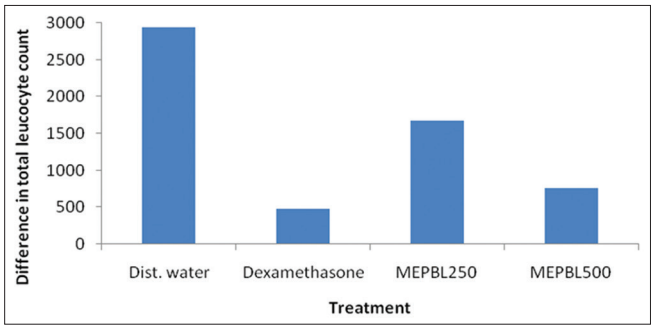

Fig. 3: Effect of *Piper betle* leaves extract on milk-induced leukocytosis in mice

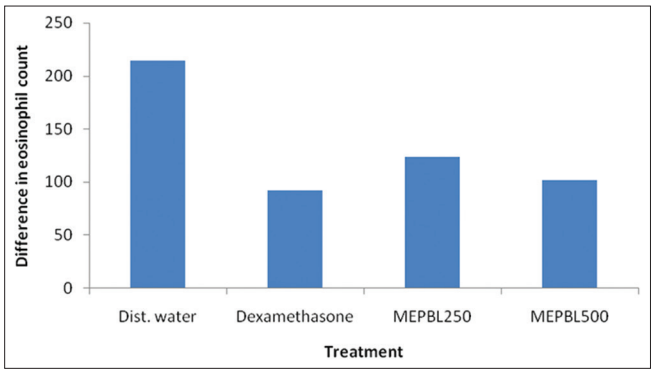

Fig. 4: Effect of *Piper betle* leaves extract on milk-induced eosinophilia in mice

Table 1: Effect of *Piper betle* leaves extracts on clonidine-induced catalepsy in mice

S. No.	Group	Duration of catalepsy (sec) at Mean±SEM				
		15 min	30 min	60 min	90 min	120 min
1	Control	62.00±4.46	89.33±4.82	102.50±3.81	142.33±12.38	63.00±5.19
2	Standard	04.33±0.66*	10.00±1.46*	22.00±3.36*	30.00±2.29*	20.00±2.38*
3	MEPBL 250	06.16±0.94*	19.83±1.88*	24.00±2.74*	38.00±6.19*	23.66±2.10*
4	MEPBL 500	05.00±0.73*	13.00±1.41*	23.00±2.39*	32.00±3.27*	23.50±4.65*

*p<0.05

Table 2: Effect of *Piper betle leaves* extract on milk-induced leukocytosis in mice

Group	Treatment	Difference in total leukocyte count (Per cu mm) (Mean±SEM)
I	Dist. water	2936.00±487.13
II	MEPBL250	1676±177.83*
III	MEPBL500	760.00±131.71**
VI	Dexamethasone (50 mg/kg i.p.)	476.00±110.40***

*p<0.04, **p<0.003, ***p<0.001

Table 3: Effect of *Piper betle* leaves extract on milk-induced eosinophilia in mice

Group	Treatment	Difference total eosinophil count (per cu mm) (Mean±SEM)
I	Dist. water	214.00±38.64
II	MEPBL250	124.00±28.57*
III	MEPBL500	102.00±10.54**
VI	Dexamethasone (50 mg/kg i.p.)	92.00±22.00***

*p<0.098, **p<0.023, ***p<0.025

increase in peripheral eosinophils count more than 4% of total leukocyte is termed as eosinophilia [25]. After the parental administration of milk, there is an increase in total leukocyte and eosinophil count, this stressful condition can be normalized by the administration of an antistress or adaptogenic drug. The involvement of eosinophil in bronchial mucosa, in which allergic inflammation occurs, is a critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion. In the late phase, especially in the development of allergic asthma, eosinophil plays a role as an inflammatory cell. Eosinophil secrets mediators such as eosinophil cationic protein, tumor necrosis factor, eosinophil-derived neurotoxin, and prostaglandin results in epithelial shedding, bronchoconstriction, and promotion of inflammation in the respiratory tract often allergic. In the present study, it has been found that parenteral administration of milk at a dose of 4 ml/kg significantly induced the total leukocyte and eosinophil count (p<0.001) after 24 h. Animals of the group treated with MEPBL250 and MEPBL500 significantly inhibited an increase in the number of total leukocyte (Fig. 3 and Table 2) and eosinophil count. (Fig. 4 and Table 3).

CONCLUSION

The results of the present study revealed that *P. betle* Linn. leaves extract inhibits the clonidine-induced catalepsy and milk-induced leukocytosis and eosinophilia in mice, suggests its potential antihistaminic and antiallergic activities. However, we are screening *P. betle* leaves extract for other animal models of asthma to evaluate its efficacy in the management of asthma. We are also working on a phytochemical investigation of these extracts to pinpoint the chemical constituents responsible for the activity.

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AUTHORS' CONTRIBUTIONS

Dr. Ravindra Y. Patil planned and designed the whole work and Ramdas N. Kale did the whole research work.

CONFLICTS OF INTEREST

The authors confirm that there were no conflicts of interest.

AUTHORS' FUNDING

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