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.

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, or bright green, shining on both sides [1]. It is commonly known as the betel 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 nutritious 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, pipperbetol, chavibetol, piperol A, methylpiperbetol, and piperol. The key component of the leaf is a volatile oil known as betle oil [15]. Karpoori variety possesses 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 methanol extract 250 mg/kg

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 (Neo 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).

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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.

**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 \( \alpha_2 \)-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 \( \alpha_2 \)-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
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          | 62.0±4.46 | 89.3±8.82 | 102.5±3.81 | 142.3±12.38 | 63.0±5.19 |
| 2      | Standard         | 04.3±0.66* | 10.0±1.46* | 22.0±3.36* | 30.0±2.29* | 20.0±2.38* |
| 3      | MEPBL250         | 06.1±0.94* | 19.8±1.88* | 24.0±2.74* | 38.0±6.19* | 23.6±2.10* |
| 4      | MEPBL500         | 05.0±0.73* | 13.0±1.41* | 23.0±2.39* | 32.0±2.37* | 23.5±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 | 293±6.00±87.13                                              |
| II                        | MEPBL250   | 167±17.88*                                                 |
| III                       | MEPBL500   | 760±0.00±13.71**                                            |
| VI                        | Dexamethasone | 476±0.00±11.40***                                           |

*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 | 214.0±3.64                                                |
| II                        | MEPBL250   | 124.0±28.57*                                              |
| III                       | MEPBL500   | 102.0±10.54**                                             |
| VI                        | Dexamethasone | 92.0±22.00**                                              |

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

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.

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References
1. Warrier PK, Namhier VP, Ramanakutty C. Indian Medicinal Plants. Madras, India: Orient Longman Publishers Ltd.; 1996. p. 279.
2. Kumar N, Misra P, Dube A, Bhattacharya S, Dikshit M, Ranade S. Piper betle Linn: a maligned Pan-Asianal plant with an array of pharmacological activities and prospects for drug discovery. Curr Sci 2010;99:922-32.
3. Bhattacharya S, Banerjee D, Bauri AK, Chattopadhyay S, Bandyopadhyay SK. Healing property of the Piper betel phenol, allylylpropocatechol against indomethacin-induced stomach ulceration and mechanism of action. World J Gastroenterol 2007;13:3705-13.
4. Sapna S, Anju D, Sanju N. Pharmacognostic and phytochemical studies of piper betle Linn leaf. Int J Pharm Pharm Sci 2016;8:222-6.
5. Gundala SR, Anuja R. Piper betel leaf: A reservoir of potential xenohormetic nutraceuticals with cancer-fighting properties. Cancer Prev Rev (Philia) 2014;7:477-86.
6. Arif B, Risrs K, Tazyunul QA. Wound-healing test of piper betle leaf extract and Aloe vera in gel preparation. Int J Appl Pharm 2018;10:86-91.
7. Marlia S, Sophi D, Natalia P. Antimicrobial activity of standardized piper betel extract and its mouthwash preparation. Int J Pharm Pharm Sci 2014;6:243-6.
8. Agarwal T, Singh R, Shukla AD, Waris I, Gujirati A. Comparative analysis of antibacterial activity of four Piper betel varieties. Adv Appl Sci Res 2012;3:698-705.
9. Jaiswal SG, Patel M, Saxena DK, Naik SN. Antioxidant properties of Piper betel (L) leaf extract from six different geographical domain of India. J Bioreosour Eng Technol 2014;2:12-20.
10. Majumdar B, Chauhuri SG, Ray A, Bandyopadhyay SK. Effect of ethanol extract of Piper betel Linn leaf on healing of NSAID-induced experimental ulcer—a novel role of free radical scavenging action. Indian J Exp Biol 2003;41:311-5.
11. Chan EW, Wong SK. Phytochemistry and pharmacology of three Piper species: An update. Int J Pharmacogn 2014;1:534-44.
12. Singh M, Shaky S, Soni VK, Dangi A, Kumar N, Bhattacharya SM. The n-hexane and chloroform fractions of Piper betel L. trigger different arms of immune responses in BALB/c mice and exhibit antiinflammatory activity against human lymphatic filarial Brugia malayi. Int Immunopharmacal 2009;9:716-28.
13. Pal M, Chandrashekar K. Mosquito repellent activity of Piper betel Linn. Int J Pharm Life Sci 2010;1:313-5.
14. Alam B, Akter F, Purvin N, Shamim Pia R, Akter S, Chowdhury J, et al. Antioxidant, analgesic and anti-inflammatory activities of...
the methanolic extract of *Piper betle* leaves. Avicenna J Phytoimed 2013;3:112-25.

15. Sarma C, Rasane P, Kaur S, Singh J, Singh J, Gat Y, et al. Antioxidant and antimicrobial potential of selected varieties of *Piper betle* L. (Betel leaf). An Acad Bras Cienc 2018;90:3871-8.

16. Fathima Begam KM, Ravichandran P, Manimekalai V. Phytochemical analysis of some selected varieties of *piper betle* L. Int J Curr Pharm Res 2018;10:89-93.

17. Khandelwal KR. Practical Pharmacognosy Technique and Experiments. 23rd ed. Pune: Nirali Prakashan; 2005. p. 15-29.

18. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC: Institute for Laboratory Animal Research, National Academics Press; 2011. p. 11-40.

19. Ferré S, Guix T, Prat G, Jane F, Casas M. Is experimental catalepsy properly measured? Pharmacol Biochem Behav 1990;35:753-7.

20. Taur DJ, Nirmal SA, Patil RY. Effect of various extracts of *Ficus bengalensis* bark on clonidine and haloperidol-induced catalepsy in mice. Pharmacologyonline 2007;3:470-7.

21. Ghaisas MM, Bulani VD, Suralkar AA, Limaye RP. Effect of *Calotropis gigantea* on clonidine and haloperidol induced catalepsy. Pharmacologyonline 2009;3:484-8.

22. Bhargava KP, Singh N. Anti-stress activity of *Ocimum sanctum* Linn. Indian J Med Res 1981;73:443-51.

23. Jadhav JH, Balsara JJ, Chandorkar AG. Involvement of histaminergic mechanisms in the cataleptogenic effect of clonidine in mice. J Pharm Pharmacol 1983;35:671-3.

24. Lakdawala AD, Dadkar NK, Dohadwalla AN. Action of clonidine on the mast cells of rats. J Pharm Pharmacol 1980;32:790-1.

25. Brekhman LI, Dardymov IV. New substances of plant origin which increase nonspecific resistance. Amn Rev Pharmacol 1969;9:419-28.