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Full Length Research Paper

Evaluation of antihistaminic activity of *Piper betel* leaf in guinea pig

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***Piper betel* Linn. leaves were evaluated for its antihistaminic activity, commonly known as *Pan*, *Vidyache Pan* or *Tamboli*. *P. betel* has a long history of use in India being applied in multiple therapeutic activities like antibacterial, treating eczema, lymphangitis, asthma, treating rheumatism. So selected, the plant *P. betel* is effective in histaminic activity related diseases, but antihistaminic activity of *P. betel* is still not scientifically investigated. In the present study, the pharmacological evaluation of ethanolic extract and essential oil extract of leaves of *P. betel* Linn. has been done for their antihistaminic activity on guinea pig. In isolated guinea pig tracheal chain preparation, there was a right side shift of dose response curve (DRC) of histamine. Chlorpheniramine maleate was used as a standard drug. Moreover extracts of *P. betel* disturbed histamine aerosol induce bronchoconstriction in whole guinea pig, where essential oil was more effective comparatively to ethanolic extract. Thus from the results obtained in the present investigation, it can be concluded that ethanolic extract and essential oil of *P. betel* Linn possess antihistaminic activity.**

Key words: *Piper betel* leaf, H_1-antagonist, guinea pig.

INTRODUCTION

Piper betel Linn. was an edible plant with leaves that have been traditionally used in India, China and Thailand for prevention of oral malodor, since it has anti-bacterial activity against obligate oral anaerobes responsible for halitosis. Aqueous extracts of *P. betle* have also been shown to reduce the adherence of early dental plaque bacteria (Razak et al., 2006) .*P. betle* is a member of the Piperaceae family and its leaves have a strong pungent and aromatic flavour (Evans et al, 1996). As well as use as a mouth freshener, the leaves are used for wound healing (Santhanam et al., 1990) and digestive and pancreatic lipase stimulant activities in traditional medicine (Prabhu et al., 1995). Antioxidant, anti-bacterial and anti-fungal (Tappayuthpijarn et al., 1982), anti-inflammatory, anti-diabetic and radioprotective (Arambewela et al., 2005) activities of *P. betle* have also been reported. In a clinical trial, *P. betle* ointment cured and improved ringworm skin lesions at rates of 40 and 26%, respectively. *P. betle* gel inhibited growth of dermatophytes that cause ringworm and growth of Candida spp. more effectively than tolnaftate and with a similar inhibitory effect to that of clotrimazole (Pongpech et al., 1993; Wongsiri et al., 1997). Histamine was one of the important mediators of bronchoconstriction and inflammation (Goyal et al., 2003). In the living organism, histamine [4-(2-aminoethyl) imidazole] is synthesized from the naturally occurring -amino acid, histidine, by the loss of a carboxyl group through bacterial or enzymatic decarboxylation (Cooper et al., 2005). Histamine was released from degranulated mast cells. Targeting histamine, either prevention of its release from

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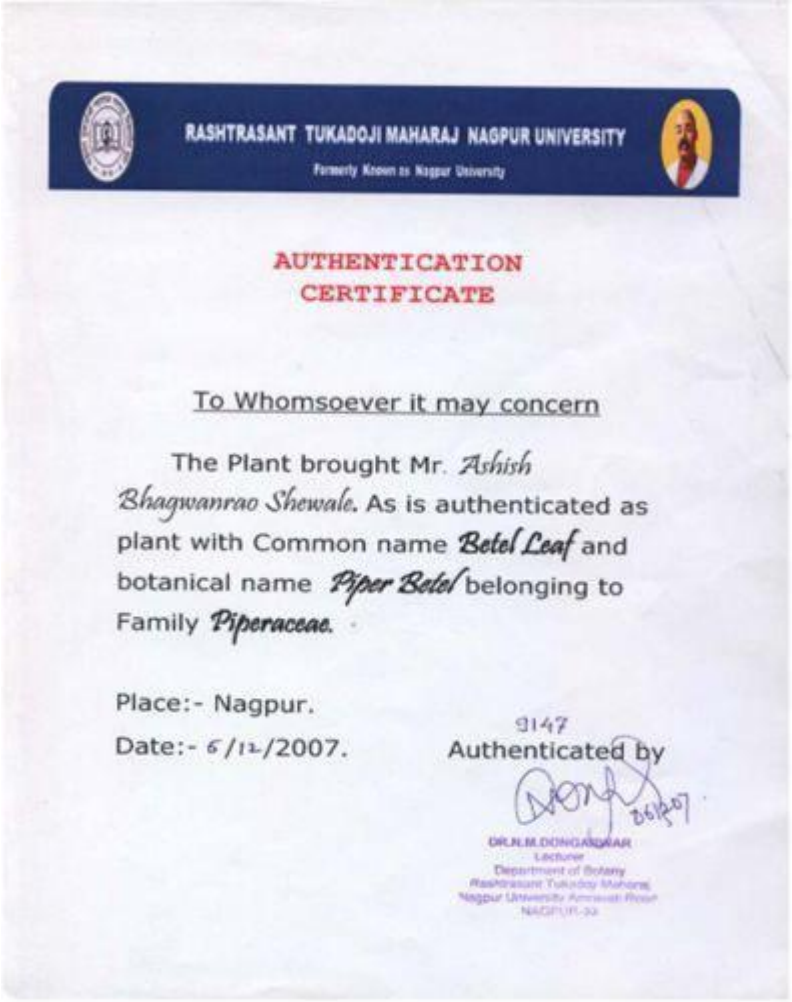
RASHTRASANT TUKADOJI MAHARAJ NAGPUR UNIVERSITY

Formerly Known as Nagpur University

AUTHENTICATION CERTIFICATE

To Whomsoever it may concern

The Plant brought Mr. Ashish Bhagwanrao Shewale. As is authenticated as plant with Common name Betel Leaf and botanical name Piper Betel belonging to Family Piperaceae.

Place:- Nagpur.

Date:- 6/12/2007.

9147

Authenticated by

DR.N.M.DONGARKAR

Lecturer

Department of Botany

Rashtrasant Tukadoji Maharaj

Nagpur University Amravati Road

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Figure 1. Authentication certificate.

mast cells or use of histaminergic receptors antagonists becomes part of antihistaminic therapy (Satoskar et al., 2001). In human body histamine was present in various biological fluids, in platelets, leucocytes, basophiles, and mast cells (Brown et al., 2001). Major portion of histamine was stored in mast cells and circulatory basophiles (Rang et al., 2003) . Histamine also acts as a neurotransmitter participating in many cell physiological processes such as allergic reaction, inflammation, gastric acid secretion, central and peripheral neurotransmission (Barar et al., 2003).

MATERIALS AND METHODS

Procurement of plant materials

The leaves of *P. betel* Linn. were collected from local habitat Faizpur, Dist-Jalgaon, (Maharashtra) India.

Authentication of drug

Plant for the present study was authenticated at Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Amravati Road, Nagpur-33 and authentication no.is 9147. Certificate of authentication was submitted (Figure 1).

Drying and size reduction

After identification and authentication, leaves of *P. betel* Linn. were subjected to drying under shade and then subjected for size reduction to coarse powder by pulverization. The powdered drug was stored in a tightly packed polythene bag (Wallis et al., 1985).

Extraction

Powdered leaves were charged into soxhlet apparatus and exhaustive extraction was carried out using ethanol as solvent. The powdered drug was extracted with solvent until complete extraction was affected. Extract was concentrated by distilling the excess of

Figure 2. *Piper betel* leaf.

solvent to obtain the crude extractive (Singh et al., 2003). *P. betel* leaves were cut into small pieces. The pieces of leaves were placed in a round bottom flask with distilled water and hydrodistillation was carried out for 4 h (Prajapati et al., 2001).

Experimental animals

Dunkin-Hartley Guinea pigs weighing 350 to 400 g were used for the present study. Animals were housed under a standard 12 h: 12 h light/dark cycle and were provided with food and water *ad libitium*. Animals were acclimated to laboratory conditions before testing. Each animal was used once. The animal protocol was approved by the Institutional Animal Ethical Committee and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Acute toxicity studies according to O.E.C.D. guideline

Acute toxicity studies on the leaf extract of *P. betel* was calculated. LD_{50} of test compounds were performed at Tapi Valley Education Society's College of Pharmacy, Faizpur – 425503, Maharastra (India) as per the O.E.C.D guideline 423. Test compounds were suspended in extract solution. The compounds were administered orally to groups of 6 animals. After administration of test compounds the guinea pig were observed for gross behavioral neurological autonomic and toxic effects. The toxicological effects were observed in terms of mortality. No death occurred within 24 h of dose of 100 and 200 mg/kg but at a dose more than 300 mg/kg, 50% mortality was observed. As dose was increased further up to 400 mg/kg, total mortality was found. Hence 200 mg/kg dose was considered as effective dose. From this doses are selected as 100 mg and 200 mg in histamine induced bronchoconstriction. Calcium channel antagonist activity of the constituents of *P. betel* has been reported, from this the dose for isolated guinea pig tracheal chain preparation and isolated guinea pig ileum preparation was selected as 100 mg.

Preparation of drug solution

P. betel ethanolic extract and essential oil extract were dissolved in distilled water. Chlorpheniramine maleate was dissolved in distilled water. Histamine was dissolved in physiological saline. Physiological saline was widely recommended as it is known to be compatible with human tissue, and isotonicity with body fluid (Sibbald et al., 2000; Jacobson, 2004).

Experimental methods

Isolated guinea pig tracheal chain preparation

Procedure: Overnight fasted guinea pig was sacrificed and trachea was mounted in an organ bath containing Krebs solution. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution of the composition: NaCl 5.9, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4$ 0.11, $NaHCO_3$ 2.1, KH_2PO_4 0.16 and glucose 2.0 g/L, which was continuously aerated and maintained at 37 ± 0.5ºC. One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to an isotonic frontal writing lever to smoked drum. Tissue was allowed to equilibrate for 45 min under a load of 400 mg. A dose response curve for histamine was taken in variant molar concentrations, by maintaining 15 min time cycle. After obtaining a dose response curve of histamine on trachea, the *P. betel* ethanolic extract and essential oil extract (100 µg/ml) were added to the reservoir and same doses of histamine were repeated. The extracts were dissolved in PEG 400 and water. (PEG 400 used alone was without any contractile effect.) Graph of percentage of maximum 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 in presence of extracts (Figure 2).

Isolated guinea pig ileum preparation

Procedure: Overnight fasted guinea pig was sacrificed and ileum was mounted in an organ bath containing Tyrode solution. The composition of Tyrode solution (NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, $NaHCO_3$ 1.0, NaH_2PO_4 0.05 and glucose 1.0 g/L) which was continuously aerated and maintained at 37 ± 0.5ºC. One end of ileum was attached to an S-shaped aerator tube and other attached to isotonic frontal writing lever to smoked drum. The tissue was allowed to equilibrate for 30 min. under a load of 500 mg. Contact time of 30 s and 15 min time cycle was followed for recording the response of Histamine. After obtaining a dose response curve of histamine on ileum, the *P. betel* ethanolic extract and essential oil extract (100 µg/ml) were added to the reservoir and same doses of histamine were repeated in presences of extracts. The extracts were dissolved in PEG 400 and water (PEG 400 used alone was without any contractile effect.) Graph of percentage of maximum 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 (Figure 3).

Histamine induced bronchoconstriction in guinea pig

Procedure: Overnight fasted guinea pigs were divided into six groups each containing 6 animals. Group 1 was treated as control, Group 2 received standard drug Chlorpheniramine maleate (2 mg/kg) . Animals belonging to groups 3, 4 received essential oil extract in dose (100,200 mg/kg) and groups 5, 6 were administered ethanolic extract in dose (100,200 mg/kg). All the doses were given orally. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The

Table 1. Results obtained from guinea pig tracheal chain preparation.

S/No.	Dose of Histamine (30 µg/ml)	Log molar concentration of Histamine	Control % maximum response	Standard % maximum response	Essential oil % maximum response	Ethanolic extract % maximum response
1	0.1 ml	6.61	22.430±1.60	9.89± 1.32*	11.20±1.24**	20.02± 2.26**
2	0.2 ml	6.31	44.610±1.56	24.03±1.56*	26.02±1.65**	34.08± 3.72**
3	0.4 ml	6.01	60.450±2.10	29.56±2.02*	32.02±2.78**	39025±3.24**
4	0.8 ml	5.71	75.120±1.56	37.12±1.03*	39.16±2.03**	44.20± 2.12**
5	1.6 ml	5.40	87.320±2.46	46.08±1.89*	50.08±1.03**	58.05± 2.53**
6	3.2 ml	5.10	99.230±1.46	52.06±2.45*	56.06±1.27**	61.02± 1.03**

Values are expressed as mean±SEM (n = 6). *p<0.05 when compared to control group, **p<0.05 when compared to standard group.

preconvulsive time (PCT) was determined from the time of exposure to onset of convulsions (Tripathi, 2001). As soon as the PCT were noted, the animal were removed from the chamber and placed in air. 24 h later the animals of Groups 3 and 4 received essential oil extract and Groups 5 and 6 received ethanolic extract. Group 2 received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol after 1 h of drug administration and PCT was determined. The protection offered by treatment was calculated by using the following formula:

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.

Statistical analysis

Results obtained from each group were expressed as mean ± SEM. The data was analyzed by one way ANOVA followed by Dunnet's multiple comparison test for isolated guinea pig tracheal chain and ileum preparation and Neuman Keul's test for histamine induced bronchoconstriction (Golan et al., 2005). P<0.05 and P<0.001 were considered to be statistically significant respectively.

RESULTS AND DISCUSSION

Histamine was released from mast cells and basophiles by antigenic stimulation causing smooth muscle contraction, increased vascular permeability and mucus formation. Histamine can provoke bronchoconstriction, it may also be responsible for bronchial hypersensitivity which is a common feature of asthma. Mast cells with their mediator can be regarded as centre for initiation and mediation of early phase of allergic reaction and may be responsible for initiation of chronic allergic reaction. The contraction of tracheal chain *in vitro* has often been utilized for the study of contractile responses of agonists as well as antagonist. Guinea pig tracheal chain preparation is suitable for screening of the antihistaminic activity of a drug are demonstrated in Table 1.

Spasmogen such as histamine produces dose dependent contraction of Guinea pig tracheal chain preparation. The Guinea pig tracheal muscle has H_1 receptors. The stimulation of H_1 receptors causes contraction of tracheal chain. In the present study, ethanolic extract and essential oil of *P. betel* Linn. (100 µg/ml) significantly inhibited the histamine-induced contraction of isolated Guinea pig tracheal chain preparation, indicating antihistaminic activity. Guinea pig ileum is used for screening of antihistaminic activity. The stimulation of H_1 receptors produces graded dose related contraction of isolated guinea pig ileum. In the present study, ethanolic extract and essential oil of *P. betel* Linn. (100 µg/ml) significantly inhibited the histamine-induced contraction of isolated guinea-pig ileum preparation indicating H_1 receptor antagonistic activity shown in Table 2.

Conclusion

Histamine when inhaled has been shown to induce bronchoconstriction by direct H_1-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes. In the present study, Table 3 showed the result of ethanolic extract and essential oil of *P. betle* Linn. (100,200 mg/kg) significantly protected the Guinea pigs against histamine-induced bronchospasm. The guinea pigs exposed to histamine aerosol showed signs of progressive dyspnoea leading to convulsions. The ethanolic extract and essential oil of *P. betle* Linn. significantly prolonged the latent period of convulsions (PCT) as compared to control following the exposure of histamine aerosol. The action started after 1 h of drug administration. The antihistaminic drug Chlorpheniramine maleate used in the study produced a significant increase in the latent period of convulsion after 1 h. Therefore, the result of present study indicates the utility of the ethanolic extract and essential oil of *P. betel* Linn. in antihistaminic activity.

Table 2. Results obtained from guinea pig ileum preparation.

S/No.	Dose of Histamine (10 μg/ml)	Log molar concentration of Histamine	Control % maximum response	Standard % maximum response	Essential oil % maximum response	Ethanolic extract % maximum response
1	0.1 ml	7.08	32.56±1.023	12.55±1.560*	17.67±1.93**	24.63±1.05**
2	0.2 ml	6.79	51.91±1.450	23.10±2.065*	26.98±2.01**	33.03±1.33**
3	0.4 ml	6.48	78.26±2.030	35.23±1.020*	39.31±1.31**	46.00±2.53**
4	0.8 ml	6.18	92.90±2.560	42.65±1.670*	47.86±1.20**	53.89±2.48**
5	1.6 ml	5.88	99.58±1.051	51.32±1.250*	55.42±1.43**	61.93±0.56**

Values are expressed as mean±SEM (n=6). *p<0.05 when compared to control group, **p<0.05 when compared to standard group.

Table 3. Results obtained from histamine induced bronchoconstriction in guinea pigs.

S/No.	Group	Onset of convulsion in secondary	% protection
1	Control	91.45 ± 0.093	-
2	Standard	1028.0 ± 4.553*	91.10
3	Essential oil (100 ml/kg)	406.2.± 0.357**	77.48
4	Essential oil (200 ml/kg)	495.1 ± 0.253**	81.5
5	Ethanolic extract (100 mg/kg)	254.0 ±.0.396**	64.0
6	Ethanolic extract (200 mg/kg)	257.6 ± 0.400**	64.5

Values are expressed as mean±SEM (n = 6).*p<0.001 when compared with control group, **p< 0.001 when compared with standard group.

REFERENCES

Arambewela LSR, Arawwawala LDAM, Ratnasooriya WD (2005). Antidiabetic activities of aqueous and ethanolic extracts of *Piper betle* leaves in rats. J. Ethnopharmacol., 102: 239–45.

Barar FSK (2003). In; Essentials of Pharmacotherapeutics, 3rd Edn., S. Chand and Company, p. 517.

Brown NJ, Roberts LJ (2001). In: Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 10th Edn., Limbard Publication, pp. 645-658.

Cooper DJ, Young RC, Durant GJ, Ganellin CR (2005). In: Comprehensive Medicinal Chemistry, Pergamon Press, 3: 335.

Evans WC, Trease GE (1996). In: Textbook of Pharmacognosy. 5th Edn., Saunders Publication, ELBS, p. 471.

Goyal RK (2003). In: Practical in Pharmacology, 3rd Edn., B.S., Shah Prakashan, p. 103.

Golan DE, Tashjian AH, Armstrong EJ, Golanter JM, Rose HS (2005). In: Principles of Pharmacology, Lippincott Wiliams and Wilkins, p. 649.

Jacobson S (2004). The wrong solution. Emerg. Med., 36(8): 13.

Prajapati DS, Purohit SS, Sharma AK, Kumar T (2001). In: Handbook of medicinal plants-Complete source book, Agrobios., p. 478.

Prabhu MS, Patel K, Saraawathi G, Srinivasan K (1995). Effect of orally administered betel leaf (*Piper betle* leaf Linn.) on digestive enzymes of pancreas and intestinal mucosa and on bile production in rats. Indian J. Exp. Biol., 33: 752–756.

Pongpech P, Prasertsilpe V (1993). The study of antimicrobial activity of *Piper betle* cream and gel against some fungi, yeast and bacteria. J. GPO, 19: 8–22.

Razak FA, Othman RY, Haji ZHA (2006). The effect of *Piper betle* and *Psidium guajava* extracts on the cell-surface hydrophobicity of selected early settlers of dental plaque. J. Oral Sci., 48: 71–75.

Rang HP, Dale MM, Ritter JM, Moore PK (2003). In: Pharmacology, 5th Edn., Churchill Livingstone, p. 229.

Santhanam G, Nagarjan S (1990). Wound healing activity of *Curcuma aromatica* and *Piper betle*. Fitoterapia, 61: 458–459.

Singh S, Govil JM, Singh VK (2003). In: Recent progress in medicinal plants. Phytochem. Pharmacol., Stadium Press LIC., 2: 239.

Satoskar RS, Bhandarkar SD, Ainapure SS (2001). In: Pharmacology and Pharmacotherapeutics, 17th Edn., Popular Publication, p. 309.

Sibbald R, Williamson GD, Orsted H, Campbell LK, Keast D, Krasner D, Sibbald D (2000). Preparing the wound bed – debridement, bacterial balance, and moisture balance. *Ostomy/* Wound Manage., 46(11): 14-35.

Tappayuthpijarn P, Dejatiwongse Q, Pongpech P, Leelaporn A (1982). Antibacterial activity of extracts of *Piper betle* leaf. Thai J. Pharmacol., 4: 205–212.

Tripathi KD (2001). In: Essentials of Medical Pharmacology, 4th Edn., Jaypee Brothers, pp. 174-175.

Wongsiri-amnuoy S (1997). Clinical trial of *Piper betle* gel for the treatment of chronic dermatitis. J. GPO, 24: 7–12.

Wallis TE (1985). In: Textbook of Pharmacognosy, 5th Edn., CBS Publishers and Distributors, pp. 22-23.