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Peritoneal mast cell stabilization potential of *Pothos scandens* L

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Abstract

Objective:

To investigate the peritoneal mast cell stabilization activity of *Pothos scandens* extracts

Materials and Methods:

Pothos scandens L. (family- Araceae) aerial part was successively extracted with ethanol and aqueous to prepare extract of the plant. The extracts of *P. scandens* were evaluated for stabilization of mast cell in rat allergic models. The extract of *P. scandens* ethanolic, 50% aqueous ethanolic and aqueous (1, 10 and 100 µg/ml) was studied for peritoneal mast cell stabilization activity in rat mesenteric preparation induced by C 48/80.

Result:

Preliminary phytochemical analysis revealed the presence of carbohydrates, fixed oil, proteins, alkaloids, glycosides, flavonoids and phenolic compounds. The ethanolic, 50% aqueous ethanolic and aqueous extracts of *P. scandens* L. showed dose dependent increase in the number of intact cells when compare with C48/80 at the concentration of 10 and 100 µg/ml. It virtues further work towards the isolation of phytoconstituents from this plant.

Conclusion:

This finding provides evidence that the *P. scandens* L. inhibits mast cell-derived immediate-type allergic reactions and mast cell degranulation. *P. scandens* has a potential as allergic anti- asthmatic agent.

KEY WORDS: Compound 48/80, ethanolic extract, mast cell, *Pothos scanden*

Introduction

Pothos scandens L. (family-Araceae) is a climbing shrub having adventitious acrid roots. The leaves are obovate, lanceolate and coriaceous having a bright green colour.

The leaves are traditionally used to treat skin disorders. An ethnobotanical survey carried out among the ethnic groups (Kani/Kanikaran) in Southern Western Ghats of India revealed the use of *P. scandens* leaf mixed with the fruits of *Capsicum annum* and rhizome of *Allium sativum*.^[1] The mixture is ground into a paste with coconut oil and applied topically on affected places to heal wounds created during delivery.^[2,3] Sri Lankan tribal people use leaves of *P. scandens* to reduce swelling speedily in trauma area.^[4] In China the plants are used as blood coagulant, wounds, tumors and drinking for anti-cough.^[5] In India, the infusion of the leaves of this plant as a bath, is used for curing convulsions and epilepsy.

Apart from that, the stem is also reportedly used to treat asthma, after being cut with camphor and smoked like tobacco.[6]

The previous literature reported that the phytochemical investigation of *P. scandens* leaf extracts showed the presence of chemical compounds such as alkaloid, catechin, coumarin, tannin, saponin, flavonoid, phenol, sugar, glycoside and xanthoprotein.[7] The GC-MS analysis of ethanolic extract of *P. scandens* leaf detected nineteen compounds. The major compounds are dodecanoic acid, tetradecanoic acid, 3,7,11,15-tetramethyl-2-hexadecan-1-ol, n-Hexadecanoic acid, phytol, 9,12-Octadecadienoic acid (Z,Z), 9,12,15-octadecatrienoic acid (Z,Z,Z), 1,2-Benzenedicarboxylic acid, diisooctylester. 9,12-octadecadienoic acid (Z,Z)- and 9,12,15-Octadecatrienoic acid (Z,Z,Z)- have the anti-inflammatory and anti-arthritic property. Among the identified phytochemicals, dodecanoic acid, tetradecanoic acid and n-hexadecanoic acid have the antioxidant property.[8]

Mast cells are found in the skin and in mucosal tissues at homeostasis and their numbers are elevated in asthmatic lungs and inflammatory bowel disease of gastrointestinal tract. Mast cells were first described by Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules, that gave them their name, "Mastzellen" which means well-fed cells, because their cytoplasm was stuffed with granular material. Mast cells are now considered to be part of the immune system.[9] The mast cell was identified as a mesenchymal cell which is stained metachromatically with O-toluidine blue and it was recognized several years later that these cells contained in their granules the majority of the body's histamine. Mast cells play a central role in inflammatory and immediate allergic reactions.[10] The release of potent inflammatory mediators, such as histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid act on the vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells. Histamine is not only released when the body encounters a toxic substance, but also released when mast cells detect injury.[11] The present study was undertaken to evaluate the mast cell stabilization property of the extracts of the aerial part of *P. scandens* on rat mesentery.

Material and Methods

Collection

P. scandens L. aerial parts were collected in the month of August, 2010, from Tirupati district, Andhra Pradesh, India. Dr. K. Madhava Chetty, Botanist, Department of Botany, Sri Venkateswara University, Tirupati authenticated the collected plant. Voucher specimen has been preserved in our laboratory (SVU/SC/09/25/10-11) for future reference.

Chemicals

Ethanol, NaCl, KCl, CaCl₂, NaHCO₃, Dextrose, Xylene, Acetone and Anesthetic ether were purchased from E-Merck (India) Ltd., Mumbai, India. Compound C40/80 and O-toluidine blue were purchased from sigma Pvt. Ltd. etc. All chemicals used were of analytical grade.

Animals

Healthy male albino rats of wistar strain (180–220 g) were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India, and were maintained under standard environmental conditions (22–28 °C, 60–70% relative humidity, 12-h dark:12-h light cycle) and were fed with standard rat feed (M/S Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (Approval no. JSSCP/IAEC/Ph.D/P.Cog/02/2011-12).

Preparation of Extracts

The *P. scandens* aerial part was cleaned thoroughly with water to remove any unwanted matter, dried in shade, ground to a coarse powder with a mechanical grinder and passed through sieve no. 40. Further, it was extracted with cold maceration process using absolute ethanol by intermittent shaking for 10 days, filtered and the dried marc left was macerated with 50% aqueous ethanolic for 10 days, filtered and again

the resultant residue was macerated with aqueous for another 10 days with intermittent shaking. The solvent was removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator (Rota vapor, R-210/215, Buchi, Switzerland). The concentrated semi solid material was kept in a desiccator for drying.

Qualitative Phytochemical Screening

The *P. scandens* L. extracts were subjected for phytochemical tests to find the presence of major phytochemical constituents such as carbohydrates, proteins, amino acids, alkaloids, glycosides, saponins, sterols, flavonoids, phenolic compounds, fixed oil and fat, gum and mucilage, phytosterols and tannins according to standard methods.[12]

Mast Cell Stabilization Activity

The overnight fasted male Wister rats were sacrificed with excess dose of anesthetic ether. The abdomen was cut open to expose the intestine. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out. A small pieces of the mesentery were cut and placed in a beaker containing Ringer Locke (in mM: NaCl 154, KCl 5.6, CaCl₂ 2.2, NaHCO₃ 6.0 and dextrose 5.5) solution for 30 ± 1 min containing different concentration of plant extracts. Later, the tissues were exposed to compound C48/80 (C 48/80 at 0.8 µg/ml to promote mast cell degranulation) and the tissue was incubated for further 30 ± 1 min. The pieces of mesentery were removed and placed in a clean slide. Excess fatty layers and adhering small intestine tissues were removed. The trimmed tissue was dipped in 4% formaldehyde solution containing 0.1 % O-toluidine blue for 20-30 min and then the tissue was transferred through acetone and then xylene (2 changes each) for 5 ± 1 min. Six pieces of mesentery were used for each concentration of the test substance. The stained mesentery pieces were focused through a digital light microscope (M/s. Motic, Japan) at 100x magnification. 100 mast cells were counted, starting from the left hand side of the field and then proceeding clockwise. The number of intact and fragmented or disrupted mast cells was noted. A mast cell was considered disrupted if 4 or 5 granules were found around the mast cells. The percentage of mast cell fragmented or disrupted and of intact mast cells was calculated.[13]

The experimental groups for were as follows:

Group I: Vehicle control (tissues exposed to Ringer Locke solution only)

Group II: Negative control (tissues exposed to 0.8 µg/ml of C 48/80 only)

Group III: Positive control (tissues exposed to disodium cromoglylate-DSCG, 1 mg/ml)

Group IV: Tissues exposed in ethanolic extract of *P. scandens* (PSE) 1 µg/ml

Group V: Tissues exposed in ethanolic extract of *P. scandens* (PSE) 10 µg/ml

Group VI: Tissues exposed in ethanolic extract of *P. scandens* (PSE) 100 µg/ml

Group VII: Tissues exposed in 50% aqueous ethanolic extract of *P. scandens* (50% PSE) 1 µg/ml

Group VIII: Tissues exposed in 50% aqueous ethanolic extract of *P. scandens* (50%PSE) 10 µg/ml

Group VIII: Tissues exposed in 50% aqueous ethanolic extract of *P. scandens* (50%PSE) 100 µg/ml

Group X: Tissues exposed in aqueous extract of *P. scandens* aqueous (PSA) 1 µg/ml

Group XI: Tissues exposed in aqueous extract of *P. scandens* aqueous (PSA) 10 µg/ml

Group XII: Tissues exposed in aqueous extract of *P. scandens* aqueous (PSA) 100 µg/ml

All the groups except group I and II were later exposed to 0.8 µg/ml of C 48/80.

Statistical Analysis

Statistical analysis was done by using one-way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison tests. *P* value < 0.05 was considered to be statistically significant. The analysis was

carried out using Graph Pad Prism software V.5.04.

Results

Extract Recovery Percent

The maximum yield was obtained in 50% aqueous ethanolic compared to other solvents used. The extractive values were found to be: 2.98, 9.73 and 5.39% w/w for ethanolic, 50% aqueous ethanolic and aqueous respectively.

Qualitative Phytochemical Screening

The phytochemical screening on *P. scandens* revealed the presence of primary metabolites such as, carbohydrates, fixed oil and proteins the secondary metabolites such as alkaloids, glycosides, flavonoids and phenolic compounds [Table 1].

Mast Cell Stabilization Activity

Compound 48/80, a known mast cell degranulation agent, produced a significant ($P < 0.001$) reduction in intact mesenteric mast cells, 16.8 ± 1.5 , when compared to the mesentery exposed to the vehicle, Ringer Locke's solution, alone 83.2 ± 2.4 . At the concentration of 10 and 100 $\mu\text{g/ml}$, ethanolic, 50% aqueous ethanolic and aqueous extracts of *P. scandens* produced dose dependent and showed significant ($P < 0.001$) increase in the number of intact cells when compared with C48/80 treated tissues [Figure 1]. Among the different extracts 50% aqueous ethanolic 10 $\mu\text{g/ml}$ (52.2 ± 4.9) and 100 $\mu\text{g/ml}$ (67.8 ± 2.9) showed significant protection of cell as compared other two extracts. The mast cell stabilization activity of different extracts was as follows; 50% aqueous ethanolic > aqueous > ethanolic.

Discussion

It is well known that compound 48/80 (acondensation product of N-methyl-p methoxyphenethylamine with formaldehyde) has the potential to secrete allergy-related factors from mast cells as stimulators.[1] The compound C48/80 has been used to study allergies and anaphylaxis, because it can vigorously activate the release of histamine via the mechanism of cellular exocytosis. Several flavonoids have been shown to possess smooth muscle relaxant and bronchodilator activity.[15] The phytochemical screening on *P. scandens* revealed the presence of such as alkaloids, flavonoids and phenolic compounds. Flavonoids also inhibited the histamine release induced by C48/80.[16] The various phytochemical compounds are present in plant like dodecanoic acid, tetradecanoic acid, 3,7,11,15-tetramethyl-2-hexadecan-1-ol, 1,2-Benzenedicarboxylic acid, diisooctylester. 9,12- octadecadienoic acid (Z,Z)- and 9, 12,15 Octadecatrienoic acid (Z,Z,Z)- have the anti-inflammatory property. Among the identified phytochemicals, dodecanoic acid, tetradecanoic acid and n-hexadecanoic acid have the antioxidant property.[8] Based on present investigation, it can be concluded that *P. scandens* stabilizes mast cells in rat mesenteric tissue. As the mast cell play a major role in Type I hypersensitivity-mediated diseases like allergic asthma and rhinitis.[10] This could be attributed due to the presence of rich chemical constituents in *P. scandens*.

Conclusion

This finding provides evidence that the *P. scandens* L. inhibits mast cell derived immediate type allergic reactions and mast cell degranulation. *P. scandens* will be the potential candidate for allergic anti-asthmatic activity. Studies are underway to evaluate the efficacy of *P. scandens* due to its mast stabilization property in these animal allergic models.

Footnotes

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Conflict of Interest: Authors have no conflict of interest

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Figures and Tables

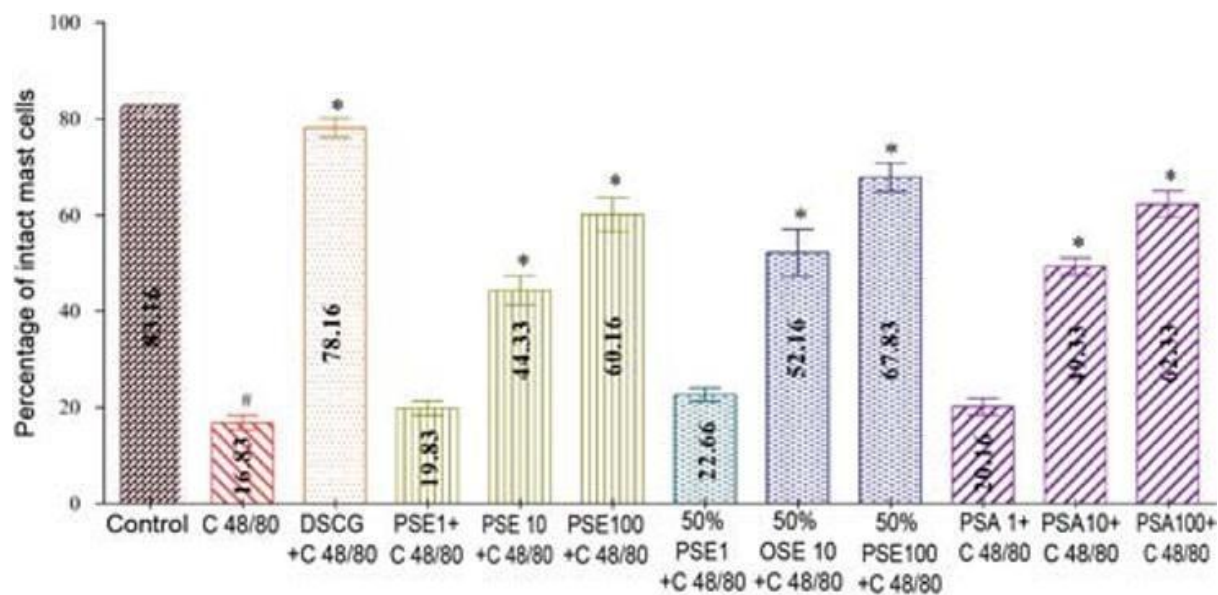
Table 1

Phytochemicals	Ethanollic extract	50% aqueous ethanolic extract	Aqueous extract
Carbohydrates	+++	+++	+++
Proteins	+	++	+
Amino acids	—	—	—
Alkaloids	+	++	+
Saponins	—	—	—
Phenolic compounds	+++	+++	++
Tannins	—	+	+
Flavonoids	++	+++	++
Glycosides	++	+	—
Phytosterols	—	—	—
Fixed oils and fats	+	—	—
Gums and mucilages	—	—	—

(+): Presence of chemical compounds, (-): absence of chemical compounds.
 (+) < (++) < (+++): Based on the intensity of characteristic colour

Phytochemical screening of *Pothos scandens*

Figure 1



Effect of *P. scandens* extracts on mast cell degranulation induced by compound 48/80 in the rat mesentery. # $P < 0.001$ vs. control; * $P < 0.001$ vs. C 48/80; One way ANOVA followed by Tukey's multiple comparison test ($F = 78.88$; $df = 71$; $P < 0.001$). DSSGdisodium cromoglycate 1mg/mL, *P. scandens* extract (ethanolic-PSE, 50% aqueous ethanolic -50% PSE and aqueous-PSA)

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