Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2014; 2(4): 73-76

Received: 20-05-2014
Accepted: 22-06-2014

Khursheed Ahmad Ansari
Assistant Professor, Faculty of Unani Medicine, Jamia Hamdard, New Delhi, India

Correspondence:
Khursheed Ahmad Ansari
Assistant Professor, Faculty of Unani Medicine, Jamia Hamdard, New Delhi, India

The assessment of the effect of extracts of *Bauhinia variegata* against the compound 48/80-induced mast cell degranulation and systemic anaphylaxis

Khursheed Ahmad Ansari

Abstract
Aim: The present investigation was designed to evaluate the possible effect of extracts of *B. variegata* against the compound 48/80-induced mast cell degranulation and systemic anaphylaxis.
Methods: The authenticated plant material was defatted using petroleum ether and extracted with ethanol and distilled water to obtain ethanol (BVE) and aqueous (BVA) extract, respectively. The stabilization potential was studied on mouse mast cells and anti-anaphylactic activity was evaluated by determining the mortality rate of mice treated with toxicant (compound 48/80).
Results: The HPLC analysis recorded 2.09% of quercetin and 0.755% gallic acid in BVE and 0.07% and 8.16% in BVA, respectively. In acute toxicity study, no mortality and behavioral changes were observed up to a dose of 2,000 $mg.kg^{-1}$ p.o. In the compound 48/80- induced mast cell degranulation study, both BVE and BVA showed protection of mast cell degranulation in a dose-dependant manner. The table showed slightly better inhibition revealed by BVE (71.18%) at a dose level of 400 $mg.kg^{-1}$ when compared with standard drug DSCG (70.95%). The lower rate of mortality of mice from compound 48/80- induced anaphylactic shock was 50% at 400 $mg.kg^{-1}$ (BVE) whereas BVA had shown lesser effect on the anaphylactic shock.
Conclusion: It is noteworthy that the extent and potency of inhibition shown by BE against compound 48/80-induced mast cell degranulation and anaphylaxis was almost similar to that of DSCG. The results of the study demonstrated that the ethanol extract of *B. variegata* showed marked mast cell stabilization and anti-anaphylactic activities.

Keywords: *Bauhinia variegata*, compound 48/80, mast cell stabilization, systemic anaphylaxis

Introduction
Mast cells or mastocytes are large connective tissue cells, originated from a distinct precursor in the bone marrow called hematopoietic progenitor cells. These play a pivotal role in defense mechanisms of human body [1]. They are residents of several types of tissues specifically in the vicinity of blood vessels, and are prominent near the boundaries between the outside world and the internal environment, such as the skin, mucosa of the lungs and digestive tract, as well as in the mouth, conjunctiva and nose [2]. They are activated through antigen cross linking of their surface receptors (FcεRI) for IgE antibodies leading to degranulation and the release of vasoactive, and pro-inflammatory mediators that include histamine, cytokines and proteolytic enzymes [3, 4].
Mast cells are involved in variety of ailments such as different types of allergic and inflammatory reactions, atopic dermatitis, asthma, ulcers, psoriasis, obesity, interstitial cystitis, cancer etc [5]. Hence, mast cell stabilizers hold significance in the management of such disorders. They block calcium channels essential for mast cell degranulation, thus stabilizing the cell and thereby preventing release of several mediators. They are classified as chromone-like drugs (disodium cromoglycate, nedocromil sodium) and dual-action antihistaminics (ketotifen, azelastine, second-generation H1-receptor antagonists: cetirizine, loratidine). Several adverse effects associated with these drugs are bronchospasm, wheezing, angiodema, eosinophilic infiltration, anaphylaxis, joint swelling, pain and headache, drowsiness, lethargy, dryness of mouth, etc.
Bauhinia variegata (Caesalpiniaceae), popularly known as Raktakanchan, is a medium-size deciduous tree found throughout India, Burma and China [8].

The bark of the plant is medicinally more important and is used for the cure of a variety of ailments. It is reported that stem bark is highly useful in fever [7], leprosy, skin diseases, wounds [13], goiter [14], and tumor [15]. The bark is reported to be an astringent, tonic to the liver, and beneficial for the cure of dysmenorrhoea, menorrhagia, tuberculosis, asthma, and wounds [10]. The phytochemical studies on the stem bark revealed the presence of flavonone glycoside, β-sitosterol, lupeol, quercetin, saponins, and tannins [11]. Extracts of stem bark of *B. variegata* are reported to possess anthelmintic [12], antidiabetic [9], and Hepatoprotective [6] activities.

The present investigation was designed to evaluate the possible effect of extracts of *B. variegata* against the compound 48/80-induced mast cell degranulation and systemic anaphylaxis.

Methods

Plant Material and Extraction

The plant material (stem bark) was collected from mature trees. The shade-dried coarsely powdered plant material was defatted by Soxhlet extraction using petroleum ether for 12 h. The defatted powder was air-dried to remove solvent and was later subjected to Soxhlet extraction using 95% ethanol for 12 h. The extract was filtered and the solvent evaporated under vacuum to afford a crude ethanol extract (BVE, yield 15.5%). The aqueous extract was obtained by boiling fresh powder in distilled water for 12 h and later by evaporating water from the decanted portion under reduced pressure (BVA, yield 14.75%). The two extracts so prepared were used for investigating mast cell stabilizing effect.

Phytochemical Analysis

Preliminary phytochemical studies of both the extracts were performed for detection of presence of various phytoconstituents, viz., alkaloids, flavonoids, saponins, glycosides, phenols, steroids, tannins, and terpenoids according to published standard procedures [16]. The extracts were subjected for quantitative estimation of known marker compounds present in the plant *B. variegata*, viz., quercetin and gallic acid by high–performance liquid chromatography (HPLC) analysis.

Animals

The original stock of Swiss albino mice (30–40 g) were procured and the animals were kept in the animal house. The animals were housed six per cage at ambient temperature (22±1 °C), relative humidity (55%±5%), and 12 h light-dark cycle. Animals had free access to standard pellet diet and filtered tap water given ad libitum throughout study. The animal experiments were conducted as per protocol approved by the institutional animal ethics committee and as per Indian norms laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi.

Acute Toxicity Study of the Plant Extracts

The acute oral toxicity study was carried out as per the guidelines set by the Organization for Economic Co-operation and Development received from the Committee for the Purpose of Control and Supervision of Experiments on Animals. The BVE and BVA extracts in dose range of 200 to 2,000 $mg.kg^{-1}$ were administered orally to different groups of mice, and mortality was observed up to 72 h.

The mast cell stabilization study was performed based on the method of Venkatesh *et al*. [17] BVE and BVA extracts were administered orally at various doses (200 and 400 $mg.kg^{-1}$, p.o.) for 4 days and the standard drug disodium chromoglycate (DSCG) at a dose 50 $mg.kg^{-1}$ was administered intraperitoneally due to its poor absorption through the oral route. On day 5, 5 mL cold phosphate buffer saline (pH-7.4) was injected intraperitoneally in all groups of mice. The peritoneal fluid–containing mast cells were collected immediately after gentle abdominal massage for 10 sec; the fluid was collected in RPMI- 1640 media (pH 7.2–7.4). The cells were washed several times by centrifugation at low speed (500–600 rpm), discarding the supernatant and re-suspending the mast cell pellets in the medium. Mast cells obtained from the treated and control groups were incubated with compound 48/80 (1 $\mu g.mL^{-1}$), a mast cell degranulator, at 37 °C for 10 min in a water bath. Then the cells were stained with Toludine blue (1%), and protection from degranulation was observed under high–power microscope and calculated accordingly.

Compound 48/80-induced Systemic Anaphylaxis

The compound 48/80-induced systemic anaphylaxis reaction was examined based on earlier reported method by Yi *et al*. [18] Groups of mice (n = 20 per group) were administered with an injection of 0.008 $g.kg^{-1}$, i.p. of the toxicant. BVE and BVA (200 and 400 $mg.kg^{-1}$) were dissolved in carboxymethyl cellulose and administered orally for 4 days prior to injection of compound 48/80. DSCG was used as standard. Mortality was monitored for 1 h after induction of anaphylactic shock. Mortality (%) within 1 h after compound 48/80 injection was represented as the number of dead mice × 100/total number of experimental mice.

Statistical Analysis

Each datum represents the mean and standard error of mean of experiments. The one-way analysis of variance test was used to make a statistical comparison between the groups (p<0.01).

Results

Table 1: Effect of Ethanol (BVE) and Aqueous (BVA) extracts of *B. variegata* on Compound 48/80-induced Mast Cell Degranulation Compared to the Standard Drug Disodium Chromoglycate

Treatment	Concentration ($mg.mL^{-1}$)	% Mast cells degranulation	% Inhibition of degranulation
Positive control (Comp.48/80)	1(µg/mL)	84±1.4	-
Negative control (carboxymethyl cellulose)	2%	2.7±0.3	-
DSCG	50	21.5±1.7	70.95
BVE	200	33.66±1.6	54.51
BVE	400	21.33±1.4	71.18
BVA	200	29±1.4	60.81
BVA	400	24.16±1.4	67.35

The preliminary phytochemical investigation of BVE revealed the presence of an array of active constituents including alkaloids, tannins, flavonoids, steroids, and glycosides whereas BVA showed the presence of alkaloids, tannins, flavonoids, carbohydrates, and proteins. The HPLC analysis recorded 2.09% of quercetin and 0.755% gallic acid in BVE and 0.07% and 8.16% in BVA, respectively. In acute toxicity study, no mortality and behavioral changes were observed up to a dose of 2,000 $mg.kg^{-1}$ p.o. In the compound 48/80- induced mast cell degranulation study, both BVE and BVA showed protection of mast cell degranulation in dose-dependant manner.

Table 2: Effect of Ethanol (BVE) and Aqueous (BVA) Extracts of *B. variegata* on Anaphylactic Shock Compared to the Standard Drug Disodium Chromoglycate

Treatment	Dose ($mg.kg^{-1}$)	Compound 48/80 ($mg.kg^{-1}$)	Mortality (%)
None (saline)	-	-	0
Toxicant	-	0.8	100
DSCG	50	0.8	40
BVE	200	0.8	70
BVE	400	0.8	50
BVA	200	0.8	80
BVA	400	0.8	70

The table showed slightly better inhibition revealed by BVE (71.18%) at a dose level of 400 $mg.kg^{-1}$ when compared with standard drug DSCG (70.95%). The lower rate of mortality of mice from compound 48/80- induced anaphylactic shock was 50% at 400 $mg.kg^{-1}$ (BVE) whereas BVA had shown lesser effect on the anaphylactic shock.

Discussion

Despite the relative success of the most commonly prescribed mast cell stabilizer, disodium cromoglycate, ketotifen, etc. there still remains an urgent need to design new substances that are economical and relatively safer with fewer adverse effects. One of the most established mast cell stabilizer – disodium cromoglycate (DSCG) was first synthesized from a plant chromone – Khellin, present in Ammi visnaga by Roger Altounyan and his colleagues in 1965 [19]. Thus medicinal plants are currently being explored widely for finding new mast cell stabilizers. Plants provide us with several phytoconstituents e.g. simple phenols, coumarins, flavonoids, alkaloids, terpenoids, etc. that have demonstrated potent mast cell stabilizing potential in experimental models [20].

In the past two decades numerous plants have been investigated for management of allergic/ immune disorders involving mast cells degranulation. *In-vitro* and *in-vivo* models have been developed to determine the mast cell functions [21]. *In-vitro* tests involve use of cell lines and isolated mast cells. Mast cells are isolated from bone marrow or from rodent peritoneal cavities; these are called bone marrow mast cells (BMMC) or peritoneal cavity mast cells (PCMC) respectively. PCMC are preferred since these retain their morphology and function and release histamine when exposed to mast cell activators [22]. Mast cells may also be isolated from embryonic stem cells [23]. These isolated cells are exposed to mast cell activators/ degranulators like Compound 48/80, specific allergens, IgE, NSAIDS, iodine containing dyes etc. Compound 48/80 (a polymer formed by condensation of N-methyl-p-methoxyphenethylamine with formaldehyde) is frequently employed in *in-vitro* models as it is causes significant release of histamine from mast cells. The prevention of histamine release from the mast cells is an index of mast cell stabilization [24].

The proposed biological activities of *B. variegata* may be attributed to the presence of alkaloids, glycosides, flavonoids, sterols, and tannins. From the results of the mast cell degranulation study, it can be postulated that protection of mast cells indicates the possibility of inhibition of the phenomenon of sensitization. The drugs used in the treatment of asthma or allergic conditions such as DSCG and dexamethasone inhibit phosphodiesterase and thereby increase the intracellular cyclic adenosine monophosphate (AMP) levels [26]. An increased level in intracellular cyclic AMP is known to have mast cell stabilizing property [27]. Some studies reported that DSCG might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80 and regulate the degranulation of the mast cells by stabilizing membrane fluidity [25].

Quercetin has also been shown to bind to calcium/calmodulin complexes thus preventing the influx of calcium into mast cells and basophils necessary for degranulation of mast cells [26]. It is reported to have an inhibitory effect on histamine release from antigeninduced mast cells and basophils [28] and also found to possess *in vitro* mast cell stabilizing activity greater than sodium cromolyn, a synthetic mast cell stabilizer [29]. The HPLC studies confirm the presence of this constituent in ethanol and aqueous extract of *B. varieata* stem bark.

Conclusion

It is noteworthy that the extent and potency of inhibition shown by BE against compound 48/80-induced mast cell degranulation and anaphylaxis was almost similar to that of DSCG. The results of the study demonstrated that the ethanol extract of *B. variegata* showed marked mast cell stabilization and anti-anaphylactic activities. In conclusion, mast cell stabilization is a complex phenomenon. Thus the need of the hour is methodical evaluation of the plants that have shown mast cell stabilizing potential with a precise goal of developing plant polyphenols as effective and safe mast cell stabilizers.

References

1. Holdsworth SR, Summers SA. Role of mast cells in progressive renal diseases. Journal of the American Society of Nephrology. 2008 Dec 1;19(12):2254-2261.
2. Kobayashi H, Ishizuka T, Okayama Y. Human mast cells and basophils as sources of cytokines. Clinical and experimental allergy (Print). 2000;30(9):1205-1212.
3. Galli SJ, Wedemeyer J, Tsai M. Analyzing the roles of mast cells and basophils in host defense and other biological responses. International journal of hematology. 2002 May;75(4):363-9.
4. Theoharides TC, Enakuaa S, Sismanopoulos N, Asadi S, Papadimas EC, Angelidou A, *et al*. Annals Aller Asthma Immunol. 2012;109:9-14.
5. Bodakhe SH, Ram A. Hepatoprotective properties of *Bauhinia variegata* bark extract. Yakugaku zasshi. 2007 Sep 1;127(9):1503-7.
6. Kapoor SL, Kapoor LD. Medicinal plant wealth of the Karimnagar district of Andhra Pradesh. Bull Med Ethnobot Res, 1980, 1(120).
7. Kirtikar KR, Basu BD. Indian Medicinal Plants. International Book Distributor, Dehradun, India; c1999. p. 892.
8. Koti BC, Biradar SM, Karadi RV, Taranalli AD, Benade VS. Effect of *Bauhinia variegata* bark extract on blood

glucose level in normal and alloxanised diabetic rats. J. Nat. Rem. 2009;9(1):27-34.
9. Nadkarni AK. Indian Materia Medica. Popular Prakashan Pvt. Ltd, Bombay, India; c1954. p. 184.
10. Prakash A, Khosa RL. Chemical studies on *Bauhinia variegata*. Curr. Sci. 1976;45:705-706.
11. Mali RG, Mahajan SG, Mehta AA. Evaluation of *Bauhinia variegata* Linn stem bark for anthelmintic and antimicrobial properties. J Nat. Rem. 2008;8(1):39-43.
12. Sahu TR. An Ethnobotanical study of Madhya Pradesh 1: Plants used against various disorders among tribal women. Ancient science of life. 1982 Jan;1(3):178.
13. Singh PB, Aswal BS. Medicinal plants of Himachal Pradesh used in Indian pharmaceutical industry. Bull Med Ethnobot Res. 1992;13:172-208.
14. Thakur MJ, Misra IN, Chaudhary BK. Ethnobotanical studies of some plants of Madhubani district, Bihar (Part-I). J Econ Tax Bot. 1992;16:383.
15. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media, 1998, Apr 30.
16. Venkatesh P, Mukherjee PK. Mukherjee D, Bandyaopadhyay A, Fukui H, Mizuguchi H. Potential of Baliospermum montanum against compound 48/80-induced systemic anaphylaxis. Pharm. Biol. 2010;48(11):1213-1217.
17. Yi JM, Kim MS, Seo SW, Lee KN, Yook CS, Kim HM. Acanthopanax senticosus root inhibits mast cell-dependent anaphylaxis. Clinica chimica acta. 2001 Oct 1;312(1-2):163-168.
18. Kuzemko JA. Respir Med 1989;83:15-16.
19. Finn DF, Walsh JJ. Twenty-first century mast cell stabilizers. British journal of pharmacology. 2013 Sep;170(1):23-37.
20. Reber LL, Marichal T, Galli SJ. New models for analyzing mast cell functions *in vivo*. Trends in immunology. 2012 Dec 1;33(12):613-25.
21. Malbec O, Roget K, Schiffer C, Iannascoli B, Dumas AR, Arock M, *et al*. Peritoneal cell-derived mast cells: an *in vitro* model of mature serosal-type mouse mast cells. The Journal of Immunology. 2007 May 15;178(10):6465-75.
22. Tsai M, Wedemeyer J, Ganiatsas S, Tam SY, Zon LI, Galli SJ. *In vivo* immunological function of mast cells derived from embryonic stem cells: an approach for the rapid analysis of even embryonic lethal mutations in adult mice *in vivo*. Proceedings of the National Academy of Sciences. 2000 Aug 1;97(16):9186-90.
23. Okayama Y, Benyon RC, Lowman MA, Church MK. *In vitro* effects of H1-antihistamines on histamine and PGD2 release from mast cells of human lung, tonsil, and skin. Allergy. 1994 Apr;49(4):246-53.
24. Kim SH, Choi YK, Jeong SW, Kang HU, Moon G, Shin TY, *et al*. Suppression of immunoglobulin E-mediated anaphylactic reaction by Alpina oxyphylla in rats. Immunoharmacol. Immunotoxicology. 2000;22(2):267-277.
25. Roy AC, Warren BT. Inhibition of cAMP phosphodiesterase by disodium cromoglycate. Biochemical Pharmacology. 1974 Feb 15;23(4):917-20.
26. Spataro AC, Bosmann HB. Mechanism of action of disodium cromoglycate—mast cell calcium ion influx after a histamine-releasing stimulus. Biochemical pharmacology. 1976 Mar 1;25(5):505-510.
27. Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. Effects of luteolin, quercetin and baikalein on immunoglobulin Emediated mediator release form human cultured mast cells. Clin. Exp. Allergy. 2000;30(4):501-508.
28. Otsuka H, Inaba M, Fujikura T, Kunitomo M. Histochemical and functional characteristics of metachromatic cells in the nasal epithelium in allergic rhinitis: studies of nasal scrapings and their dispersed cells. Journal of allergy and clinical immunology. 1995 Oct 1;96(4):528-536. f