Journal of Medical Pharmaceutical And Allied Sciences

www.jmpas.com
ISSN NO. 2320 - 7418

RESEARCH ARTICLE

TRADITIONAL MEDICINE FOR ATOPIC ECZEMA: GILOYA EXTRACT SUPPRESSES INFLAMMATORY MEDIATORS RELEASE FROM MAST CELLS

Tiwari Prabhakar Singh [1*], Sharma Vishnu Kumar [2], Tandan Manisha [3],

1 Department of Pharmacy, AKS University, Satna, India.

2 National Institute of Pharmaceutical Education and Research, Ahmedabad, India.

3 RKDF College of Pharmacy, RGTU University, Bhopal, India

Correspondence
Prabhakar S. Tiwari
Assistant Professor
Faculty of Pharmaceutical Science & Technology
AKS University Satna (m.p.) India E-Mail: pt4477@gmail.com

Keywords
Giloya, Ayurvedic medicine, PGD2, animal sensitization, Mast cells.

Recieved
18/11/2013
Reviewed
25/11/2013
Accepted
30/11/2013

ABSTRACT

Stem paste of Terminalia cardifolia commonly known as Guduchi (‘Giloya' in India) was found to be useful in the management of eczema. In ayurveda, giloy is used to treat acute infections and as a digestive aid, aphrodisiac and diuretic. Since the mechanism of action of is not known, we aimed to investigate the actions of Giloya on mast cell activation. Effects of aqueous extracts of Giloya on mediator release from rat peritoneal mast cells (RPMCs) and cytokine production from HMC-1 were investigated. Aqueous extract of Giloya significantly attenuated histamine release and prostaglandin D 2 synthesis from RPMC activated by anti-IgE and compound 48/80 ($p < 0.05$). However, these herbs failed to affect cytokine production in HMC-1. Inhibition of inflammatory mediator release from mast cells will contribute to the therapeutic efficacy of Giloya.

INTRODUCTION

Atopic eczema (AE) is a common chronic relapsing disease with high prevalence in children . It has been estimated that 15% of school children aged 13–14 years have a history of AE. Inasmuch as the etiology of AE is only partially understood, treatment is empirical and is based on the use of topical or systemic corticosteroids aimed at reducing skin inflammation(1). As there is no definitive cure for the condition among Western medicines, there has been considerable interest in the use of traditional Ayurvedic medicine (TAM) as a potential adjunct therapy for AE(2). This prompted us to investigate the effect of TAM intervention for the treatment of AE. Giloya is an ancient ayurvedic concoction which contains aqueous extract of giloya. In previous randomized study, it was found that Giloya is effective in eczema. These herbs possess anti-allergic, anti inflammatory, anti-pruritic and sedative properties and have been extensively used in rural and tribal region of India for the treatment of allergic diseases including AE, asthma and allergic rrhinitis(3). The giloy plant is a deciduous climbing plant that is native to Southeast Asia and tropical regions of India. The stems are most commonly used for medicinal purposes, although the roots and starch extract can also be used, and it is available in powder or capsule form(4). Giloy is commonly referred to as guduchi, heart-leaved moonseed, amrit and ambrosia. In ayurveda, giloy is used to treat acute infections and as a digestive aid, aphrodisiac and diuretic(5).

It was demonstrated that the Giloya could dose dependently inhibit peripheral blood mononuclear cells proliferation and brain-derived neurotrophic factor secretion upon staphylococcal enterotoxin B stimulation and these in vitro immunomodulatory properties(Vaibhav D. Aher 2010) might account for the clinical benefits in AE patients(6). Mast cells are well known for their critical roles in allergic diseases which are attributable to their potent capability to produce multiple proinflammatory mediators including preformed granular contents such as histamine, newly synthesized lipid mediators such as prostaglandin D2 (PGD2) and cytokines (GM-CSF, IL-6 and TNF) after activation. Subsequent to activation by antigens, cytokines, growth factors or hormones, chemical mediators released from skin mast cells cause immediate skin inflammatory reactions including local vasodilation, T-cell recruitment and

itching(7). In addition to the progression of the immediate inflammatory response, the release of these mediators also initiates the onset of the late-phase inflammatory responses. Inhibition of mast cell activation in skin is thus a potential therapeutic strategy in AE treatment(8). Although in a randomized study showed PHF could reduce the use of topical corticosteroid and improve the quality of life in patients with AE, this treatment did not result in significant decrease in their AE severity(9). We hypothesize that the dosage of giloya in the study might be sub-therapeutic. We hence proposed to further investigate the modulating effects of giloya on inflammatory mediator release and cytokine production from mast cells for the better understanding of the underlying mechanisms of the observed efficacy on AE treatment. The rat peritoneal mast cell (RPMC) is a good model for investigating the contribution of inflammatory mediators released by mast cell degranulation in skin related diseases as it is functionally comparable to human skin mast cells in their responses to substance P and compound 48/80 (Mousli et al., 1994; Emadi-Khiav et al., 1995) while the human mast cell line (HMC-1) is appropriate for investigating the release of cytokines from mast cells(10).

MATERIALS AND METHODS

Sources and authentication of herbal materials

The fresh stem of giloya. The herbs was grown in university campus and then stem part of herb was selected for extract herb was authenticated by a morphological expert. The authenticities of the herb was further verified by thin layer chromatography according to the recommended method described in the Ayurvedic Pharmacopoeia of India(API) 2007 using reference compounds. Herbarium voucher specimens were deposited at the Pharmacognosy Department of the AKS University, Satna; India with voucher specimen numbers assigned: 2806.

Preparation of herbal extracts

Extract was prepared by refluxing in 900 ml of distilled water at 100˚C for 2 h and repeated twice. These three batches of water extracts were mixed together and centrifuged to remove the herbal debris. The combined extract was then vacuum dried and the resulting herbal powder was stored in desiccators until use(11).

Animal sensitization and purification of rat peritoneal mast cells

All animals were handled in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) .Male Sprague–Dawley rats weighing 150–200 g were actively immunized by an intraperitoneal injection of 0.5 ml phosphate buffered saline containing ovalbumin (0.5 mg), Al(OH)3 (120 mg) and Bordetella pertussis (0.8 I.U.). Eight weeks later, the sensitized animals were sacrificed by decapitation after lightly anaesthetized with diethyl ether for the isolation of peritoneal mast cells. Full HEPES-buffered tyrode (FHB) at pH 7.4 (containing: NaCl 137 mM, KCl 2.7 mM, CaCl2 1 mM, NaH2PO4 0.4 mM, N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) 10 mM, glucose 5.6 mM and MgCl2 1.2 mM) was used throughout the study. Mixed peritoneal cells were collected from each rat by peritoneal lavage with 20 ml of FHB supplemented with 1 mg/ml of bovine serum albumin (BSA-FHB). The cells were washed twice in ice-cold BSA-FHB by centrifugation (190 × g, 4˚C, 5 min), resuspended in 1 ml of ice-cold FHB, and then mixed with 4 ml of isotonic Percoll solution (SG = 1.017) in calcium-free FHB. Mast cells were purified by centrifugation (190 × g, 4˚C, 25 min) through the continuous density gradient generated by the Percoll and was pelleted at the bottom of the tube. Residual Percoll was eliminated by two washes in BSA-FHB and a final wash in FHB. The purified mast cells were finally resuspended in the required amount of FHB(12).

Mast cell incubation, histamine and PGD2 assay

Aliquots of purified mast cells (250 µl), which had been equilibrated in FHB at 37˚C for 10 min, were added to 200 µl of FHB with herbal extracts. After incubating the cells with the herbal extracts for 5 min, 50 µl of secretatogues including goat anti-ratIgE (ICN, 1:300 dilution) or compound 48/80 (Sigma, 0.1 µg/ml) were added. The cells were further incubated at 37˚C for 15 min after the addition of secretatogues and the reaction was terminated by the addition of ice-cold FHB(13). The supernatant and cell pellet were then separated by centrifugation (190 × g, 4˚C, 5 min). Histamine contents in mast cell pellets and supernatants were measured spectrofluorometrically using a Bran + Luebbe Auto-Analyzer 2 (Hamburg, Germany). The percentages of total cellular content of histamine (sum of supernatant

content and cell pellet content) released into the supernatant following different treatments were then calculated(14). Prostaglandin D2 was measured using a commercial EIA kit of detection limits of 3.1 pg/ml (Cayman Chemical, USA).

Human mast cell line HMC-1 culture and cytokine release studies

The human mast cell line HMC-1 was a generous gift from Dr. J. H. Butterfield (Mayo Clinic, Rochester, MN, USA). It was maintained in Iscove's modified Dulbecco's media (IMDM), supplemented with 10% fetal bovine serum (FBS), 2 mmol/l l-glutamine, 100 U/ml penicillin G and 10 mg/ml streptomycin (Gibco, Grand Island, NY, USA), and 100 µM α-thioglycerol (Sigma, St. Louis, USA). For cytokine release studies, HMC-1 (106) were pre-incubated for 30 min with 500 g/ml of Giloya extract or dexamethasone (1 M), followed by a 24-h incubation with a mixture of calcium ionophore A23187 (1 µM) and phorbol-12 myristate 13-acetate (50 nM) (A23187 + PMA, Sigma, St. Louis, USA). The cell free supernatants were then assayed for GM-CSF, IL-6, IL-8, IL-10 and TNF-α production by commercially available human cytokine ELISA kits (BD OptEIA, USA) according to the manufacturer instruction with detection limits ranged from 3.1 to 7.8 pg/ml. 2.6. Expression of results and statistical analyses In each experiment, the spontaneous release of histamine or prostaglandin in buffer alone was subtracted from all measurements. Results on rat peritoneal mast cells are expressed as the percentages of control release induced by the secretagogue (anti-IgE or compound 48/80) alone, i.e., histamine release (% of control) or prostaglandin release (% of control), whereas results on HMC-1 are expressed as the actual levels of cytokines released. The levels of mediator/cytokine release induced by the secretagogue in the presence of herbal ingredients were compared with the control levels of mediator/cytokine release induced by the secretagogue alone(15). Statistical analyses were performed using the Student's t-test and differences were considered significant when the probability (p) was <0.05. All data are mean ± standard error of mean (S.E.M.) for n independent observations(16).

RESULTS

Effects of PHF on anti-IgE and compound 48/80 activated RPMC

Rat peritoneal mast cells spontaneously released undetectable level of PGD2 and

less than 15% of total cellular histamine when incubated in buffer alone and none of the tested herbal extracts affected these levels of spontaneous mediator release. Anti-IgE and compound 48/80 alone induced 44.2 ± 4.1% and 58.9 ± 2.3% of histamine release and 2598 ± 280 pg/106 cells and 2642 ± 536 pg/106 cells of PGD2 production, respectively. Extract have significantly inhibited both histamine release and PGD2 production from RPMC stimulated by anti-IgE and compound 48/80 dose-dependently from 500 to 1000 µg/ml ($p < 0.05$). It was more efficacious in inhibiting histamine release and PGD2 production in mast cells that were activated by compound 48/80 than those activated by anti-IgE. At the highest concentration of Giloya Extract tested (1000 µg/ml), anti-IgE induced histamine release and PGD2 production were reduced by 46.9 ± 5.9% and 54.9 ± 11.7%, respectively, while compound 48/80 induced histamine release and PGD2 production were reduced by 71.4 ± 3.5% and 84.9 ± 2.9%, respectively.

DISCUSSION

Mast cells are located in tissues that interface with the external environment including the skin and the number of mast cells is increased during the early phase of AE (Theoharides et al., 2007). The mediators released from activated mast cells, including histamine, prostaglandins and cytokines contribute to prutitus and inflammation in AE (Alenius et al., 2002). Besides immunological trigger, which involves the aggregation of cell surface high-affinity receptors for IgE (FcRI) by their corresponding antigens, activation of mast cells can be achieved by basic secretagogues such as substance P or polycationiccompounds (e.g., compound 48/80 and naturally occurring polyamines) via the peptidergic pathway (Shefler et al., 2008). Serum levels of substance P are associatedwith the disease severity of AE (Hon et al., 2007a), and an elevated level of substance P may contribute to the progression of skin inflammation through activation of mast cells. While the extract of Giloya(*T. cordifolia*) can significantly suppress the release of preformed (histamine) and newly synthesized (PGD2) mediators from both anti-IgE and compound 48/80 activated mast cells, the formulation is more potent against the latter secretagogue[15]. This mast cell stabilizing effect of Giloya may contribute to the clinical effectiveness in AE through suppression of the initial allergen induced immediate acute inflammation and the

subsequent chronic inflammation sustained by elevated level of substance P released from nerve endings.

In addition to the inflammatory mediators, mast cells are also an important source of cytokines, such as IL-6, IL-10, GM-CSF and TNF-, which have been implicated in the pathogenesis of AE. Since clinical use of PHF has been demonstrated to reduce the frequency of steroid use, it was quite unexpected to find that 500 g/ml of Giloya, which significantly suppressed the release of inflammatory mediators from RPMC, failed to demonstrate any modulating effects on cytokine release from the human mast cell line HMC-1 activated by A23187 + PMA. The discrepancy may be due to the fact that HMC-1 is an atypical mast cell like cell line which does not possess intact functional IgE receptors and peptidergic activation mechanism and thus cannot truly represent the responses of skin mast.

REFERENCES

1. Abrams C, Shattil SJ, 1991. Immunological detection of activated platelets in clinical disorders, Thromb. Haemostasis 65, 467-473.
2. Blann A, Kumar P, Krupinski J, McCollum C, Beevers DG, Lip GY. 1999. Soluble intercellular adhesion molecule-1, E-selectin, vascular cell adhesion molecule-1 and von Willebrand factor in stroke, Blood Coagul. Fibrinolysis. 10, 277–284.
3. Bombeli TR, Barbara M, 1998. Adhesion of Activated Platelets to Endothelial Cells: Evidence for a GPIIbIIIa-dependent Bridging Mechanism and Novel Roles for Endothelial Intercellular Adhesion Molecule 1 (ICAM-1), αvβ3 Integrin, and GPIbα,. J. Exp. Med. 187, 329-339.
4. AleniusH, Laouini D, Woodward A, Mizoguchi E, Bhan AK, Castigli E, Oettgen HC, Geha RS, 2002. Mast cells regulate IFN-gamma expression in the skin and circulating IgE levels in allergen-induced skin inflammation. Journal of Allergy and Clinical Immunology 109, 106–113.
5. Aridor M, Rajmilevich G, Beaven MA, Sagi-Eisenberg R, 1993. Activation of exocytosis by the heterotrimeric G protein Gi3. Science 262, 1569–1572.
6. Emadi-Khiav B, Mousli M, Bronner C, Landry Y, 1995. Human and rat cutaneous mast cells: involvement of a G protein in the response to

peptidergic stimuli. European Journal of Pharmacology 272, 97–102.

7. Hon KL, Leung TF, Wong Y, Lam WK, Guan DQ, Ma KC, Sung YT, Fok TF, Leung PC, A pentaherbs capsule as a treatment option for atopic dermatitis in children: an open-labeled case series. American Journal of Chinese Medicine 32, 941–950.

8. Hon KL, Lee VW, Leung TF, Lee KK, Chan AK, Fok TF, Leung PC, 2006. Corticosteroids are not present in a traditional Chinese medicine formulation for atopic dermatitis in children. The Annals, Academy of Medicine, Singapore 35, 759–763.

9. Hon KL, Lam MC, Wong KY, Leung TF, Ng PC, 2007. Pathophysiology of nocturnal scratching in childhood atopic dermatitis: the role of brain-derived neurotrophic factor and substance P. British Journal of Dermatology 157,922–925.

10. Hon KL, Leung TF, Ng PC, Lam MC, Kam WY, Wong KY, Lee KC, Sung YT, Cheng KF, Fok TF, Fung KP, Leung PC, 2007. Efficacy and tolerability of a Chinese herbal medicine concoction for treatment of atopic dermatitis: a randomized, double-blind, placebo-controlled study. British Journal of Dermatology 157, 357–363.

11. Leung DY, 2000. Atopic dermatitis: new insights and opportunities for therapeutic intervention. Journal of Allergy and Clinical Immunology 105, 860–876.

12. Leung R, Wong G, Lau J, Ho A, Chan JK, Choy D, Douglass C, Lai CK, 1997. Prevalence of asthma and allergy in Hong Kong schoolchildren: an ISAAC study. European Respiratory Journal 10, 354–360.

13. Leung AK, Hon KL, Robson WL, 2007. Atopic dermatitis. Advances in Pediatrics 54, 241–273.

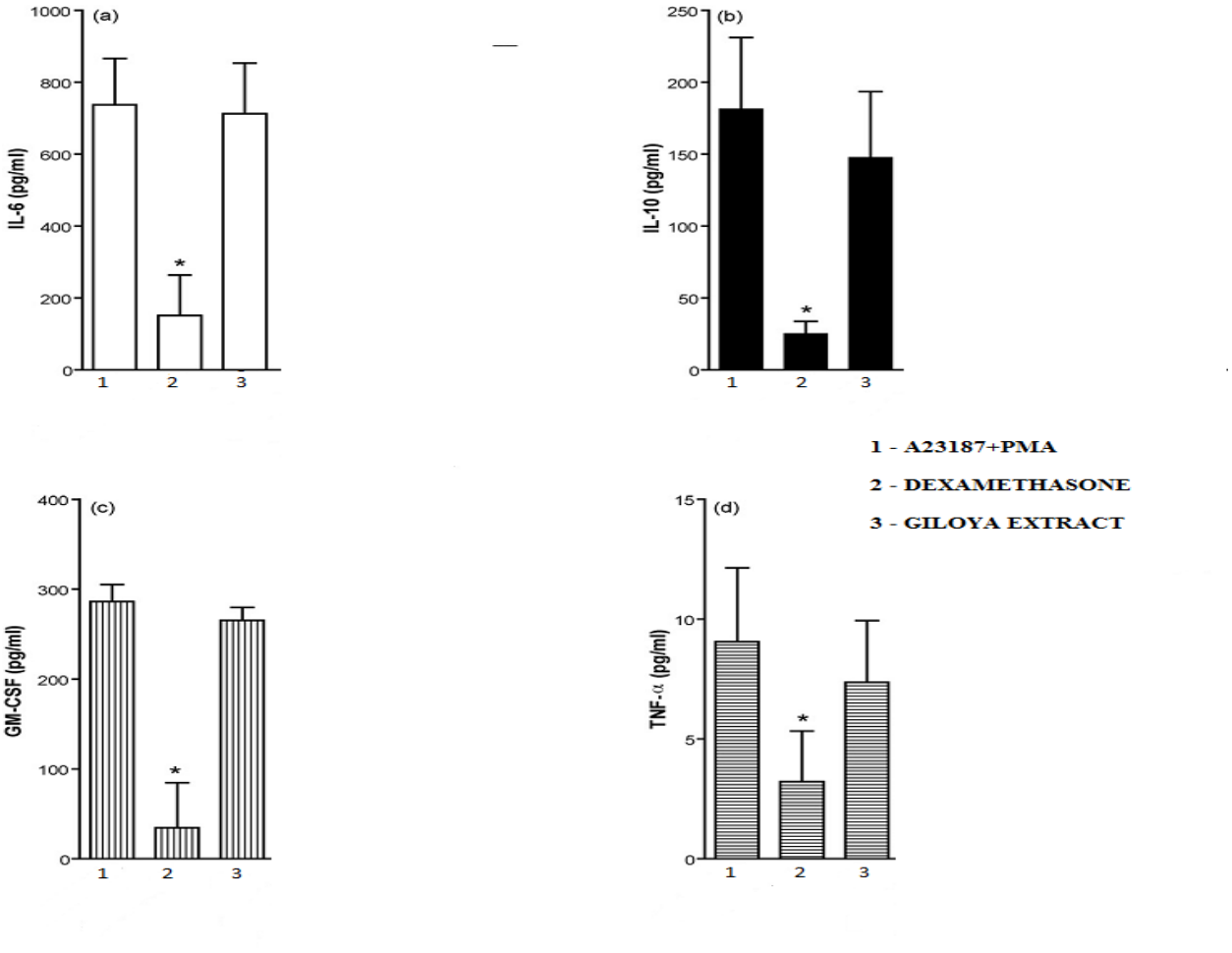
14. Argolo ACC, Sant Ana, Pletsch M, Coelho LCB, 2004. Antioxidant activity of leaf extracts from Bauhinia monandra. Bioresource Technol., 95: 229

15. Atoui AK, Mansouri G, Boskou, Kefalas P, 2005. Tea and herbal

infusion: Their antioxidant activity and phenolic profile. Food Chem. 89: 27-36.

16. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T, Mukherjee B, 2003. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegerative diseases. Journal of Ethnopharmacology 84, 131–138.

FIG.1 EFFECTS OF GILOYA EXTRACT ON THE SYNTHESIS OF CYTOKINES



HMC-1 incubated in amixture of 1µMcalcium ionophore A23187 and 50nM phorbol-12myristate 13-acetate (A23187 + PMA) for 24 h. The control levels of (a) IL-6, (b) IL-10, (c) GM-CSF, and (d) TNF-_ produced by A23187 + PMA activated HMC-1 were 737.6±128.5 pg/ml, 181.5±49.65 pg/ml, 286.3±18.9 pg/ml, and 9.1±3.0 pg/ml, respectively Giloya Extract was tested at 500_g/ml and dexamethasone (1µM) was included as a positive control. Results are given as mean±S.E.M. for $n = 4$. *Indicates $p < 0.05$ when compared with the control level of cytokine release induced by A23187 + PMA.