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Original Article

Anti-anaphylactic effects of *Trichilia monadelpha* (Thonn.) J. J. De Wilde extracts on rodent models of anaphylaxis

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Abstract

Effects of petroleum ether and ethanolic extracts of *Trichilia monadelpha* stem bark (PEE and EAE) on compound 48/80-induced systemic and passive anaphylaxis were determined. Survival rate, extravasation, degranulation of mast cells, and secretion of tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured after pre-treatment with extracts (10-100 mg/kg) and disodium chromoglycate (2.5-250 µg/kg) and induction of anaphylaxis in C57BL/6 mice or Sprague-Dawley rats with compound 48/80. Histopathological assessments were made from skin biopsies of rats. Data was analyzed by Kaplan-Meier Survival Log-Rank Analysis, or One-way ANOVA and Holm-Sidak's post hoc test. PEE and EAE inhibited ($P \leq 0.0001$) tremors in systemic anaphylaxis passive cutaneous anaphylactic reactions and extravasation, stabilized or prevented ($P \leq 0.001$-0.0001) mast cell degranulation, and inhibited ($P \leq 0.001$-0.0001) TNF-α and IL-6 secretion. Per the findings, PEE and EAE of *T. monadelpha* have exhibited substantial anti-anaphylactic and anti-inflammatory property (with PEE performing better) which substantiates its use traditionally in management of allergies and other inflammatory disorders.

Keywords: Mast cells degranulation; *Trichilia monadelpha*; Interleukin-6; TNF-α; Anaphylaxis

INTRODUCTION

Allergies (innate and acquired immune responses) are mostly brought about by mast cells (1) and are associated with immediate-type hypersensitivity and inflammatory responses (2,3). Mast cell-mediated diseases or anaphylactic reactions have increased lately in developed (4) and developing countries such as Ghana. Activation of mast cells initiates inflammation through release and synthesis of vasoactive mediators that increase vascular permeability (e.g. histamine, prostaglandin E_2, leukotrienes), with TNF-α, interleukin-1 (IL-1), and histamine stimulating expression of adhesion molecules (P-selectin, E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) on the endothelial surface (5,6). This brings about activation of resident tissue macrophages, arriving monocytes, and neutrophils by means of interferon-γ, IL-6, and TNF-α which are expected to further amplify leukocyte recruitment and an improved output of pro-inflammatory cytokines (7). IL-6 up-regulates histamine production which induces expression of immunoglobulin E (IgE) and Fc epsilon (FcεI) receptor (8).

In Ghana, herbal practitioners use *Trichilia monadelpha* (Thonn.) J. J. De Wilde (*Meliaceae*) to manage allergic or anaphylactic reactions (9-11). *T. monadelpha* pharmacologically, inhibited carrageenan-induced inflammation in seven–day old chicks and adjuvant-induced arthritis (12), improved haematological parameters, cytokines and oxidative stress (13), inhibited histamine-induced inflammation and reduced neutrophil

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infiltration into synovial membrane of rats induced with arthritis (unpublished work).

The study therefore sought to give scientific evidence of *T. monadelpha* in the traditional management of anaphylactic conditions.

MATERIAL AND METHODS

Experimental animals

Male Sprague-Dawley rats (150–200 g), or C57BL/6 mice (20–25 g) obtained from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, kept in the animal house of the Department of Pharmacology, KNUST, Kumasi, Ghana, were used in this study. Animals were housed in aluminium cages and fed with normal rat diet (GHAFCO, Tema, Ghana) and water, *ad libitum*. Animals were kept according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85-23, revised 1985) and approved by the Departmental Ethics Committee.

Extraction of Trichilia monadelpha

Extraction of the stem bark extracts of *T. Monadelpha* was as described by Ben, *et al.*(11).

Drugs and chemicals used

Compound 48/80 (C48/80), Evans blue dye, toluidine blue dye, disodium chromoglycate (DSCG), formamide, formalin, alcohol, and sodium chloride were purchased from Sigma-Aldrich, Inc. St. Louis MO, USA. Quantikine rat IL-6 and TNF-α immunoassay kit procured from R&D Systems, Inc., Minneapolis, USA.

Compound 48/80-induced systemic anaphylaxis

C48/80-induced systemic reaction was performed as described in literature (14). Briefly, C57BL/6 mice were pre-treated with extracts (10-100 mg/kg *p.o*.) and DSCG (2.5–250 µg/kg *i.p*.) one hour before the induction of anaphylactic reaction. Mice (n = 10) were given intraperitoneal injection of C48/80 (8 mg/kg). The period for observation of mortality was based on the control mice that had died in 15 min. Mortality were monitored for 1 h after induction of anaphylactic shock. The percentage mortality and percentage protection were calculated as follows:

$$\%\ Mortality\ = \frac{Number\ of\ animals\ dead}{Total\ number\ of\ animals} \times 100$$

$$\%\ Protection\ = \%\ Mortality_{control}\ -\ \%\ Mortality_{extract}$$

Compound 48/80-induced passive cutaneous-like anaphylactic reaction

The experiment was done as described by Choi, *et al*. (14). Briefly, extracts (10-100 mg/kg *p.o*.) and DSCG (2.5–250 µg/kg *i.p*.) were administered to each rat 1 h before injection of C48/80, which was injected intradermally (0.25 µg/50 µL) into the dorsal skin. Evans blue solution (1%) was later intravenously injected into the penile vein of each rat and 30 min after injection tissue sections around the intradermal injection site was excised and weighed, followed by extraction of extravasated Evans blue dye by incubation of biopsies in 1 mL formamide at 55 ^{o}C for 24 h (15). Absorbance was measured at 620 nm with UV mini-1240 single beam spectrophotometer (Shimadzu Scientific Instruments (SSI), Kyoto, Japan), and tissue Evans blue concentrations were quantified by interpolation on a standard curve of dye concentrations in the range of 0.01 to 30 µg/mL.

Histological evaluation

PCA was repeated, following protocol described in literature for histological evaluation (16). However lesion portion of the skin was cut out and fixed in 10% formalin. This was cut into sections of 4 µm. A toluidine blue working solution of 5 mL of toluidine stock solution (1 g of toluidine/ 100 mL of 70% alcohol): 45 mL of 1% sodium chloride solution was prepared and used for staining of mast cells. The staining procedure was by first deparaffinising and hydrating tissues in distilled water. This was placed in the toluidine blue working solution for 1-2 min, rinsed in distilled water, 3 times and dehydrated quickly through 95% and absolute alcohol. This was cleared in xylene, covered with coverslip and viewed under a light microscope.

The histopathological features were blindly graded by a board certified pathologist and assigned a score of 0–4, where, 0 = absence of tissue damage, granulation and degranulation, 1= minimal tissue damage, granulation and degranulation, 2 = mild/moderate tissue damage, granulation and degranulation, 3 = intense tissue damage, granulation and degranulation, 4 = extensive tissue damage, granulation and degranulation.

Assay of TNF-α and IL-6 secretion

TNF-α and IL-6, pro-inflammatory cytokine mediators, were analysed using the serum of anaphylactic Sprague-Dawley rats (induced with compound 48/80). Blood was obtained from rat through cardiac puncture, placed in test tubes and allowed to clot for 2 h at room temperature. This was centrifuged at 1000 *g* for 20 min at room temperature. Serum was removed and assayed for the cytokines. The assays were carried out as described in the kit manual of the cytokines. Absorbance was measured at 450 nm using Sunrise microplate reader XREAD PLUS version: V4.30 (Tecan Inc., Switzerland) powered by Smart Magellan data analysis software, version 7.2 Standard (Tecan). The measured absorbance is in proportion to the amount of cytokines in the standard. The sample values were read off the standard curve and computed.

Data analysis

Data obtained was expressed as mean ± SEM using SigmaPlot version 12.3 (Systat Software Inc. Chicago, USA). Mortality data was compared using Kaplan-Meier Survival Log-Rank Analysis. Passive anaphylaxis data was analysed using One-way ANOVA and Holm-Sidak's post hoc test. The $P \leq 0.05$ was considered significant.

RESULTS

Effect of extracts on compound 48/80-induced systemic anaphylaxis

There was dose-dependent increase in probability of survival with time, with PEE considered most significant extract in inhibiting C48/80-induced systemic anaphylaxis. The control group elicited anaphylactic responses such as tremors and eventual death. PEE treatment significantly ($P \leq 0.05$) delayed onset of anaphylactic responses (Fig. 1a) while EAE and DSCG treatments had no significant effect on anaphylactic responses (Figs. 1b and c). However PEE, EAE, and DSCG treatments significantly ($P \leq 0.05 – 0.0001$) improved survival with significant ($P < 0.01– 0.0001$) trends on median survival.

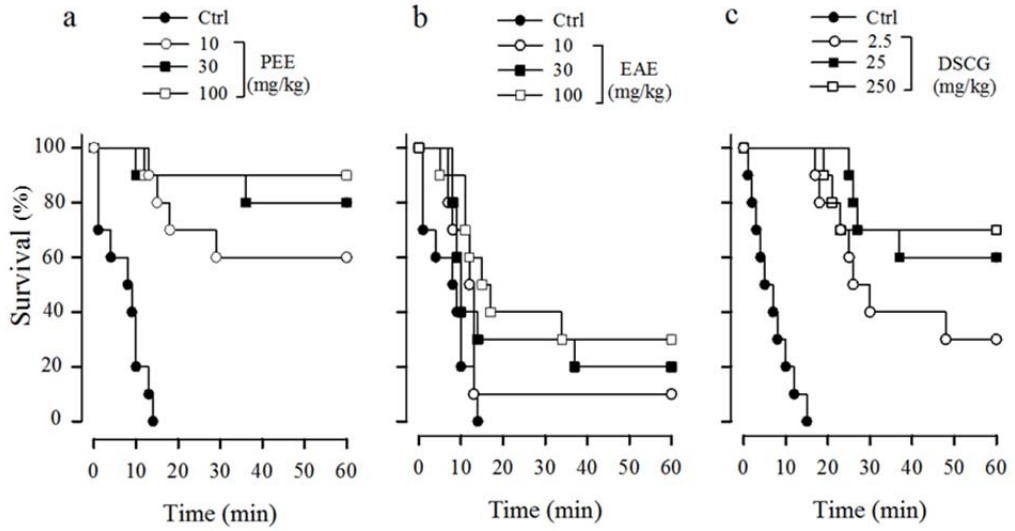

Fig. 1. Percentage survival against time for (a) PEE, (b) EAE, and (c) DSCG in C48/80-induced mortality.

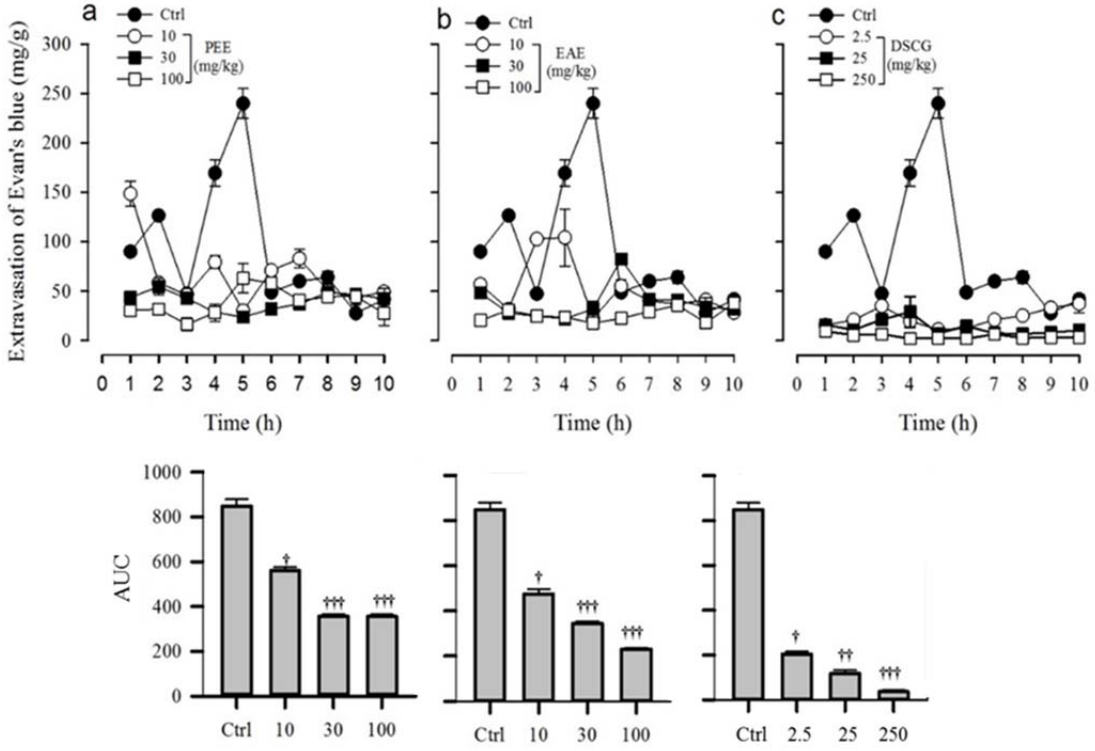

Fig. 2. Time course curve effects of (a) PEE, (b) EAE, and (c) DSCG on extravasations of Evans blue dye in C48/80-induced PCA-like reaction. ††† $P \leq 0.001$, †† $P \leq 0.01$, † $P \leq 0.05$ comparing mean ± SEM using One-way ANOVA (control group *vs* treatment groups). All comparison of variances was done using Holm-Sidak's post hoc test.

Table 1. Effects of extracts on PCA as well as on TNF-α and IL-6 secretion from mast cells

Effects on PCA	**PEE (100 mg/kg)**	**EAE (100 mg/kg)**	**DSCG (250 µg/kg)**
I_{max}	58.2%	72.8%	95.8%
ED_{50}	7.89 ± 2.28	8.06 ± 1.03	0.56 ± 0.14
E_{max}	66.08	77.62	92.36
Effects on TNF-α secretion			
I_{max}	95.4%	92.7%	93.2%
ED_{50}	0.65 ± 0.23	2.75 ± 0.08	0.00031 ± 0.00015
E_{max}	100	100	
Effects on IL-6 secretion			
I_{max}	98.5 %	94.1%	99.5%
ED_{50}	1.71± 0.80	0.54 ± 0.31	9.80e-005 ± 0.0002
E_{max}	100	100	100

ED_{50} = Effective dose at 50%; E_{max} = maximal effect produced; I_{max} = maximum inhibitory effect.

Effects of extracts on passive cutaneous-like anaphylactic reaction

C48/80 induced extravasation of Evans blue in control groups. However, PEE, EAE, and DSCG treatments resulted in significant inhibitions ($P \leq 0.0001$) of PCA reactions in a dose-dependent manner (Fig. 2). PEE and EAE at 100 mg/kg had maximum inhibitory effect of 58.2% and 72.8% respectively (Table 1). Effect of extracts was similar to that of DSCG treatment maximal inhibitory effect of 95.8% at 250 µg/kg (Table 1).

Histopathological assessment

Photomicrographs of rat skin from control group revealed intense degranulation of mast cells and very intensive tissue damage (Fig. 3). PEE and EAE-treated group however showed

significant ($P \leq 0.001 – 0.0001$) and dose-dependent reduction in degranulation of mast cells with little or no tissue damage, confirmed by significant reduction in histopathological score determined (Fig. 4). Maximal effect of PEE and EAE at 30 mg/kg was 61.7% and 61.7%, respectively. Effect of extracts was similar to that of DSCG treatment (maximal effect of 51.1% at 250 µg/kg).

Effects of extracts on TNF-α and IL-6 secretion from mast cells

PEE and EAE significantly ($P \leq 0.01 – 0.001$) reduced TNF-α and IL-6 levels with maximal effects at 100 mg/kg being 95.4 – 98.5% and 92.7 – 94.1% respectively; similar to DSCG (maximal effect of 93.2 – 99.5% at 250 µg/kg) (Fig. 5).

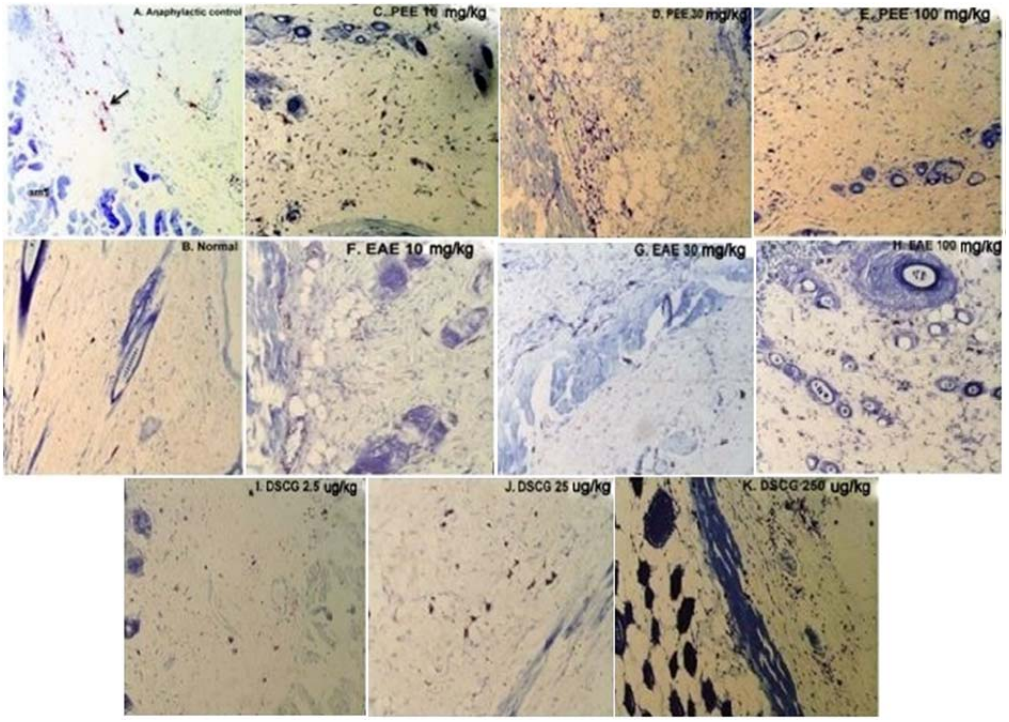

Fig. 3. Photomicrographs of (a) anaphylactic control (b) normal control, (c–e) PEE (10 –100 mg/kg) pretreatment, (f–h) EAE (10 – 100 mg/kg) pretreatment, and (i–k) DSCG (2.5-250 µg/kg) pretreatment; in PCA reaction in rats. Sm-subcutaneous muscular layer. Black arrows show degranulated mast cell. Magnification: 400×.

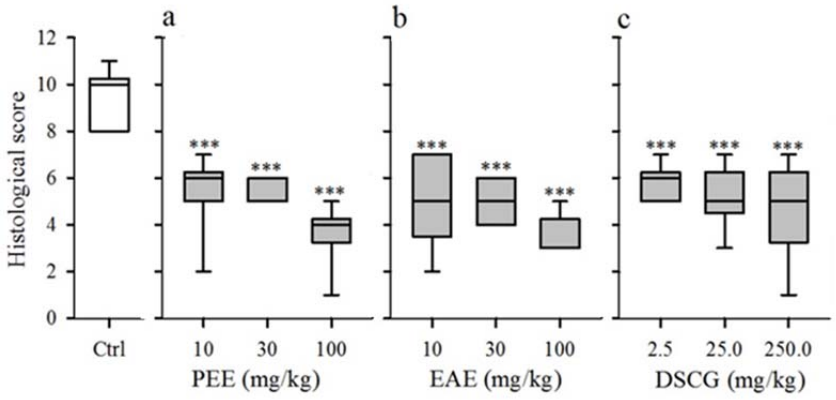

Fig. 4. Histological score of (a) PEE, (b) EAE, and (c) DSCG-treated groups. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ comparing mean ± SEM using One-way ANOVA (control group *vs* treatment groups). All comparison of variances was done using Holm-Sidak's post hoc test.

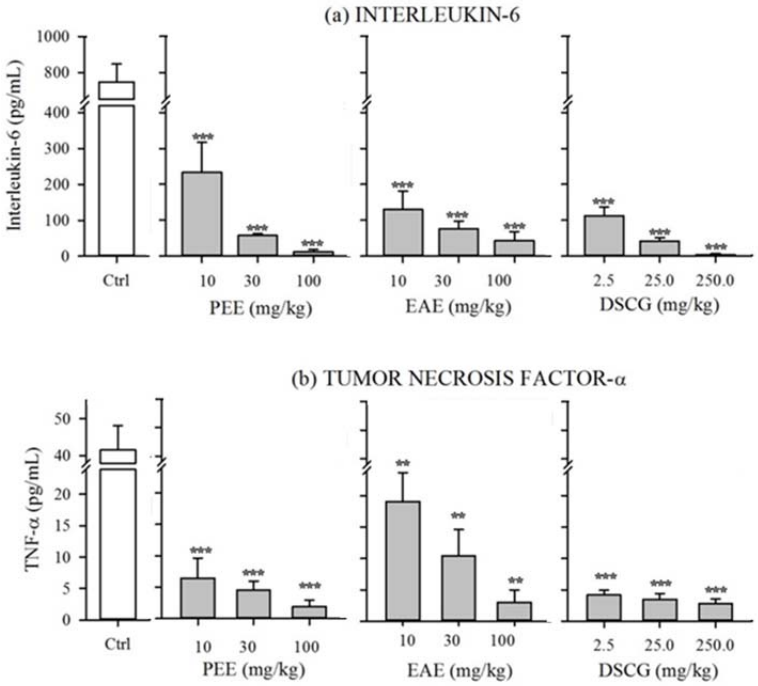

Fig. 5. Effects of PEE, EAE, and DSCG on (a) IL-6 and (b) TNF-α secretion. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ comparing mean ± SEM using One-way ANOVA (control group *vs* treatment groups). All comparison of variances was done using Holm-Sidak's post hoc test.

DISCUSSION

This study seeking to provide pharmacological evidence on use of *T. monadelpha* in management of anaphylactic conditions was initiated by inducing systemic and passive cutaneous-like anaphylaxis, with C48/80, in rodents. C48/80 causes mast cell degranulation resulting in release of histamine (14,17). Histamine mediates anaphylaxis, an acute systemic allergic reaction in response to antigen cross-linking of immunoglobulin E (IgE) bound to FcεI on the mast cells. This leads to activation of phospholipase C (PL_C) (17). PL_C activation results in hydrolysis of phosphatidyl inositol 4, 5-biphosphate (PIP_2) producing IP_3 and DAG. IP_3 binds to receptors on intracellular calcium ions, $[Ca^{++}]_i$ storage site to release Ca^{++}, while DAG activates protein kinase C (PK_C). The increase $[Ca^{++}]_i$ and activation of PK_C leads to degranulation (18). Mast cell degranulation results in release of histamine and a variety of eicosanoid, proteoglycan and protease inflammatory mediators, and chemotactic cytokines (TNF-α, IL-1, IL-4, IL-6, and transforming growth factor-β) (19,20). IL-6 up-regulates histamine production and induces expression of IgE FcεI (21). These mediators start up allergic reactions observed as sneezing, broncho-constriction, tachycardia, tremor, and in severe cases, death (18).

The result showed that both extracts significantly delayed onset of these symptoms, improving and increasing survival time in a manner similar to DSCG, which inhibits C48/80-induced histamine release from isolated mast cells by competitively binding to Ca^{++}-channel opening receptors with IP_3, thus blocking increase in $[Ca^{++}]_I$, inhibiting degranulation (22).

During degranulation, storage granules are transformed into secreting granules; the early histological sign of this change is granule matrix disorganization (17). The disorganization releases, histamine and cytokines that destroy connective tissue of the cell, observed as tissue damage. The histological result revealed that animals pretreated with PEE and EAE had intact tissue structure and intact mast cells granules. It is possible that extracts, especially PEE, are preventing release of mediator from cells via effect involving calcium channel opening or

yet still by other mechanism. In preliminary phytochemical study of *T. monadelpha*, PEE showed presence of alkaloids, sterols, triterpenoids, reducing sugars and coumarins while EAE showed presence of saponins, tannins, alkaloids, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, triterpenoids and steroidal compounds (11). Alkaloids and flavonoids are noted to possess anti-anaphylactic and anti-allergic properties respectively (23-25). The presence of these compounds in the extracts could explain the anti-anaphylactic effect observed. The presence of alkaloids in PEE and not EAE, could possibly explain why PEE had better anti-anaphylactic effect.

PEE and EAE significantly reduced or inhibited secretion of TNF-α and IL-6 (pro-inflammatory cytokines) which lead to inhibition of damage to tissue accompanying anaphylactic reactions. Mediators released from activated and degranulated mast cells increases vascular permeability, with TNF-α, IL-1, and histamine. The pro-inflammatory cytokines trigger and sustain response in mast cells, through 'inside-out' regulation, recruiting circulating leucocytes along gradients of chemotactic mast cell products (leukotriene B4, monocyte chemoattractant protein-1, tryptases and IL-8). These activate resident tissue macrophages, arriving monocytes and neutrophils by interferon-γ, IL-6 and TNF-α, amplifying leucocyte recruitment and an improved output of pro-inflammatory cytokines. IL-6 up-regulates histamine which induces expression of IgE FcεI receptor and is associated with PCA reaction (26).

Both extracts can thus be effective in preventing mast cell degranulation, however PEE had a greater effect in reducing percentage of cytokines released and anaphylactic manifestations, making PEE the preferred extract because it may have effect on mast cell degranulation with major effect observed on manifestations of anaphylaxis.

CONCLUSION

Petroleum ether and ethanolic stem bark extracts of *T. monadelpha* are capable of inhibiting mast cell-mediated systemic and passive cutaneous-like anaphylactic reactions (possibly by stabilizing and strengthening mast cell membrane and pregranular membrane) and inflammatory cytokines secretion. This substantiates its use traditionally in management of allergies and other inflammatory disorders.

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