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Immunomodulatory Effects of *Tinospora cordifolia* Lotion on Interleukin-1, Interleukin-6 and Interleukin-8 Levels In Scabies-Infected Pediatric Patients: A Single Blind, Randomized Trial

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ABSTRACT
Scabies is a contagious, parasitic skin infestation caused by *Sarcoptes scabiei* mite, which has the ability to regulate the host's inflammatory and immune responses. It is a serious community health problem in many less-developed countries. A randomized, controlled, parallel, pilot clinical study was performed to investigate the immunomodulatory effect of the formulated *Tinospora* lotion in clinically diagnosed scabies-infected patients through Enzyme-Linked Immunosorbent Assay (ELISA) for Interleukin-1, Interleukin-6 and Interleukin-8 using blood serum samples. The pediatric patients were treated with *Tinospora* and Permethrin lotions for three consecutive days for two weeks. Blood extraction was performed before and after the second and fourth week of treatment period. *Tinospora* lotion significantly reduced the Interleukin-1 (IL-1) and Interleukin-6 (IL-6) levels from Day 14 to Day 28 (p=0.0002) comparable to Permethrin lotion (p<0.050). Permethrin efficiently decreased Interleukin-8 (IL-8) levels than *Tinospora* at Day 14 (p=0.0155). Down regulation of Interleukin 1, 6, and 8 levels in scabies infestation inhibits hyperkeratosis and infiltration of inflammatory cells into scabietic lesion. The modulation effect of the *Tinospora* lotion on interleukin levels reinforces its anti-scabies activity.

Keywords: Immunomodulatory, interleukin, scabies, *Tinospora*.

INTRODUCTION

Scabies is a highly contagious skin infestation caused by *Sarcoptes scabiei* mite commonly characterized by nocturnal pruritus with mild cutaneous lesions and an increased synthesis of inflammatory mediators observed in dermal cellular infiltrates. [1-2] Around 300 million scabies cases worldwide are reported each year. [3] In the absence of a comprehensive national registry, the exact figures for the incidence of scabies in the Philippines are not known but a sizable number of patients have been reported in several Metro Manila hospitals.

The ectoparasite mite produces antigenic molecules, which modulate the function of the host's immune cells. [4] This adaptation allows mites to avoid the inflammatory and immune responses during early infestation which favor its survival in the host's skin. [2] However, hypersensitivity to dead mites and its products may cause postscabietic itching which may persist for weeks. Therefore, despite the elimination of the mites, there remains the possibility that the host will continue producing inflammatory mediators.

Tinospora cordifolia Boerl is a vine used for the treatment of ulcers, sores, and inflammations. [5] Studies have shown its efficacy as anti-scabies [6-7], antiamoebic [8], antioxidant [9], and immunomodulatory agents [10]. Different active substances like quaternary alkaloids [11], octacosanol [12], G1-4A [13] and α-D-glucan [14] have been isolated and identified in *Tinospora*.

The immunostimulating properties of *Tinospora* extracts such as increased macrophage synthesis, heightened phagocytic activity [10, 15], and rise in white blood cell counts [16] are crucial during the initial phase of scabies infestation. These activities may counteract the immunomodulatory activity of the scabies mites. In this study, we investigated the regulation of interleukins 1, 6, and 8 which are expressed in scabies infestation by the formulated *T. cordifolia* lotion as compared with Permethrin.

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MATERIALS AND METHODS

Preparation of the Plant Material

Mature stems of *Tinospora cordifolia* collected from Bulacan, Philippines were authenticated. After pulverization using Wiley mill, the *T. cordifolia* powder obtained passed the purity and quality standards of the Philippine Pharmacopeia. One kilogram of air-dried powdered *T. cordifolia* stems was percolated with 3 L of 80% ethanol until exhaustion.

Preparation of the *Tinospora cordifolia* Lotion

Tinospora lotion was prepared as previously described. [17] Permethrin lotion was used as a positive control. *T. cordifolia* and Permethrin lotions were transferred and supplied in 60 mL white opaque plastic bottles. All study subjects were given identical dosage instructions.

Experimental Subjects

Subjects, 2-22 years of age, were recruited after clinical diagnosis. Weekly assessment of the symptoms and appearance of local adverse reactions was performed and facilitated by a licensed pediatrician with the assistance of three medical doctors. The clinical testing was approved by the University Santo Tomas College of Rehabilitation Sciences Ethics Committee (no.041/11-12).

Signed, written informed consent was obtained from parents/ guardians and assent was provided before treatment. A signed and dated informed consent was obtained from each patient or legal guardian. All pertinent aspects of the study were explained to each subject or his/her guardian.

Scabies was assessed based on the following clinical features: nocturnal pruritus, occurrence of burrows, vesicles and papules, and the distribution of lesions on the following 15 sites of predilection: face, head, palms, interdigits, sides of fingers, upper and lower extremities, wrists, axilla, nipple, umbilical area and/or lower abdomen, genitalia, inguinal, buttocks and back area. [1] Scabies patients were categorized as either having a mild infestation (<6 sites of predilection); moderate infestation (>6 sites) or severe infestation (>10 sites). [6-7] Test medications were randomly given to each subject. Sixty-six children were assessed including their vital signs were noted (Table 1).

Clinical Trial

A single blind, randomized, controlled, parallel clinical trial comparing the immunomodulation effect of the *T. cordifolia* lotion against 5% Permethrin lotion in treating 66 pediatric patients diagnosed with scabies at the Manila Youth Reception Center, Reception Action Center and Tanglao Detention Center in Malolos, Bulacan, Philippines from July-October 2011 (Table 1). To detect a decrease of δ=2.24 with a standard deviation σ=4.65 in the severity scores of the erythema after one month of *T. cordifolia* lotion treatment, at 80% power of the statistical test, observing 0.05 level of significance, a sample size of at least 30 in each group was required for the study. Sample size was calculated using GPower ver 3.1.2. [17-18] Patients were randomly assigned to two groups using Microsoft Excel in which 34 subjects were blindly given Lotion A (*T. cordifolia)* and 32 subjects were instituted with Lotion B (Permethrin).

Subjects who used topical or oral scabicidal treatment and corticosteroid four weeks before the trial, subjects with concomitant secondary bacterial infections with systemic manifestation like fever, malaise, chills, edema, and subjects who are enrolled in other clinical study were excluded. [19]

The patients were instructed to apply the assigned lotion from neck down the feet especially on the sites of predilection after a night bath using a mild soap. [1, 17, 20] This was done daily for three consecutive days per week for two weeks until clear response is achieved defined as clearing of lesions seen as post-inflammatory hyper/hypopigmentation and disappearance of pruritus, burrow or vesicles during the observation period. The treatment period is two weeks for both *T. cordifolia a*nd Permethrin lotions. [17, 20] Assessments of the patient conditions were made every week using the global evaluation score of signs and symptoms. Treatment was provided to the roommates given that they consented to enter the study and if they fulfilled the criteria for the diagnosis of scabies. Figure 1 presents the flowchart for recruitment, allocation and follow-up of patients.

Treatment was discontinued in patients who presented exacerbation of clinical condition, with significant adverse reactions and those who received concomitant scabicidal drugs. Rescue medication (Permethrin) was provided to patients with lesions that failed to clear after 28 days. [7, 20]

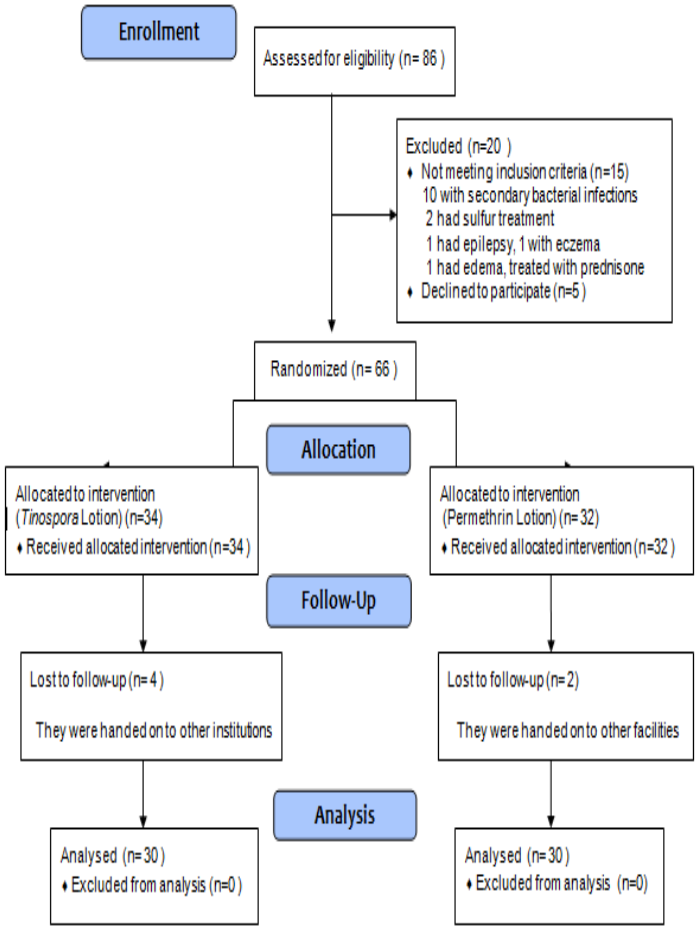

Fig. 1: Flow chart of Recruitment, allocation and follow-up of participants involved in the study [17]

ELISA for Interleukin-1, Interleukin-6 and Interleukin-8 Measurements

Immunomodulatory Activity of *Tinospora* Lotion

Specific Sandwich enzyme-linked immunosorbent assay (ELISA) kits (Biolegend) were used for measuring the serum levels of IL-1, IL-6 and IL-8, according to the manufacturer's protocol. Each sample was tested in duplicate assay. Three trials of each test were performed. [21] The peripheral blood samples from antecubital vein were collected from each patient pre-treatment (Day 0), on Day 14, and one week post-treatment period (Day 28). The peripheral venous blood samples (5 mL) were drawn, allowed to clot at room temperature and serum were separated from by centrifugation

at 9000 rpm for 20 minutes. The serum samples were stored frozen at -20°C until use. [21]

ELISA plates were coated with 100µL diluted capture antibody followed by overnight incubation at 4°C. Two hundred microliters (200µL) assay diluent was added followed by incubation at room temperature for one hour. One hundred microliters (100µL) of diluted standards and samples were added in appropriate wells and was incubated for two hours at room temperature with constant shaking. One hundred microliters (100µL) of diluted detection antibody were added to the wells and incubated for one hour at room temperature. Avidin-HRP (100µL) was incorporated into the wells and was incubated for 30 minutes at room temperature. Plates were washed three times between incubation steps. One hundred microliters (100µL) of TMB substrate solution were added after washing the plate three times. The plate was incubated for 15 minutes in the dark at room temperature. This was followed by addition of 100µL of stop solution. A coloured product is formed in proportion to the amount of human antigen present in the sample or standard. The reaction is terminated by addition of HCL and absorbance was measured at 450 nm using Biotek EL860. A standard curve was prepared.

Statistical Analysis

Means and its 95% confidence interval were used to determine the levels of different cytokines during the treatment. Student's test and Fisher's exact test compared the demographic profile of the two groups of patients. Repeated measures analysis of variance was used to compare the effect of the *Tinospora* lotion with the standard known drug (Permethrin) in terms of regulation of interleukin-1, 6, and 8 levels, with Tukey's HSD for post hoc analysis. Spearman correlation determined if the severity of scabies is correlated with interleukin-1, 6, and 8 levels. P-values less than 0.05 were considered significant.

RESULTS

Among eighty-six (86) patients who were diagnosed with scabies, a total of sixty-six (66) subjects were enrolled in the study. Others were excluded from the study due to secondary bacterial infections, edema, and use of sulphur while some decline to participate in the study. Six patients (9.09%) did not return after the baseline treatment and were considered as drop-outs from the study.

Sixty patients (91.91%) followed up one week after the treatment period (Day 28). All the subjects were diagnosed with scabies based on the criteria of having lesions on the classic sites, nocturnal pruritus and presence of the same lesions within the household members. Table 1 presents the demographic profile of the recruited patients.

Degree of Infestation

The degree of infestation showed that during the pre-treatment period (n=60), majority of the patients 51.67% had mild infestation, 46.66% had moderate infestation, and 1.66% with generalized infestation. At baseline, 60% vs 43.33% had minimal infestation while 40% vs 53.33% of patients had moderate infestation for *T. cordifolia* and permethrin-treated patients. On Day 28, 70% vs 50% exhibited complete cure while 30% vs 50% of the *T. cordifolia* and permethrin-treated patients had mild infestation (Table 2).

Interleukin-1, 6, and 8 were all expressed in the serum samples at Day 0. All patients (n=60) had elevated levels of the three cytokines at baseline. The mean level of the three cytokines (in picogram) is significantly higher (p<0.001) than the normal values at baseline. The normal values of IL-1, IL-6 and IL-8 are 3.72, 4.14 and 6.99 picogram (pg), respectively. [22-23]

Comparison of the Cytokines Levels with the Degree of Infestation

The amount of inflammatory mediators expressed in patients with different degrees of infestation was compared and determined. There was a significant difference in the IL-1 (p<0.001), IL-6 (p<0.001) and IL-8 (p=0.014) levels in patients diagnosed with scabies which were classified as having mild, moderate, or severe infestation. A direct relationship between the levels of IL-6 (r = 0.346, p=0.007) and severity of scabies was observed. IL-1 (r = 0.218, p=0.094) and IL-8 (r=0.103, p=0.432) do not correlate with the severity of scabies.

Some patients exhibited an increased level of inflammatory mediators at Day 14. This increased amount of cytokines may be attributed to the post-scabietic hypersensitivity experienced by the patients after effective treatment. The percentage of patients which had elevated IL-1 (p=0.030), IL=6 (p=0.836) and IL-8 (p=0.067) at Day 14 were statistically the same for both *Tinospora* and permethrin lotions as shown in Figure 2.

Table 1: Demographic profile of the patients included in the study

Demographics	TOTAL (n = 66)	*Tinospora* (n = 34)	Permethrin (n = 32)	*p*-value
Age (yrs)	15.58 (14.54 – 16.62)	15.88 (14.66 – 17.10)	15.25 (13.48 – 17.02)	0.552
Sex: Male (n, %)	61; 92.4% (82.5 – 97.2%)	31; 91.2% (75.2 – 97.7%)	30; 93.8% (77.8 – 98.9%)	0.693
sBP (mm Hg)	103.73 (101.09 – 106.37)	105.29 (101.93 – 108.65)	102.66 (98.42 – 106.90)	0.326
dBP (mm Hg)	67.22 (65.12 – 71.06)	68.09 (65.12 – 71.06)	65.22 (61.67 – 68.77)	0.209
Body temperature (˚C)	36.51 (36.29 – 36.61)	36.45 (36.29 – 36.61)	36.57 (36.33 – 36.81)	0.447
Pulse Rate (bpm)	80.32 (76.32 – 83.24)	79.78 (76.32 – 83.24)	80.88 (77.90 – 83.86)	0.635
Respiration rate (breathes/min)	18.59 (17.73 – 18.91)	18.32 (17.73 – 18.91)	18.88 (18.15 – 19.61)	0.233
Weight (Kg)	45.42 (41.95 – 49.81)	45.88 (41.95 – 49.81)	44.92 (40.29 – 49.55)	0.747

Values expressed as mean (95% confidence interval in parentheses); *p*-values are based on Student's t-test and Fisher's exact test.

Table 2: Number of Patients According to Degree of Infestation on the 28-day Treatment

Degree	Day 0		Day 7		Day 14		Day 21		Day 28	
	T	P	T	P	T	P	T	P	T	P
Clear	0 (0%)	0 (0%)	5 (16.7%)	2 (6.7%)	8 (26.7%)	7 (23.3%)	18 (60%)	16 (53.3%)	21 (70%)	15 (50%)
Mild	18 (60%)	13 (43.3%)	25 (83.3%)	28 (93.3%)	22 (73.3%)	23 (76.7%)	12 (40%)	14 (46.7%)	9 (30%)	15 (50%)
Moderate	12 (40%)	16 (53.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Severe	0 (0%)	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

T - *T. cordifolia* and P - Permethrin, n = 30

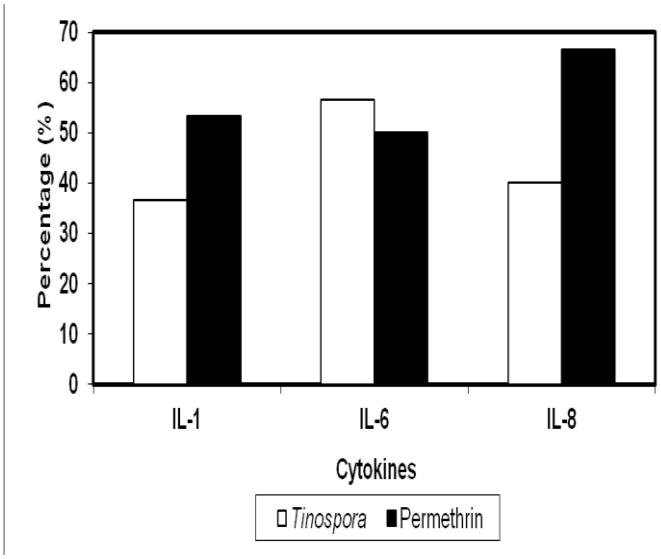

Fig. 2: Percentage Distribution of Patients Exhibiting Increased Cytokine Levels at Day 14

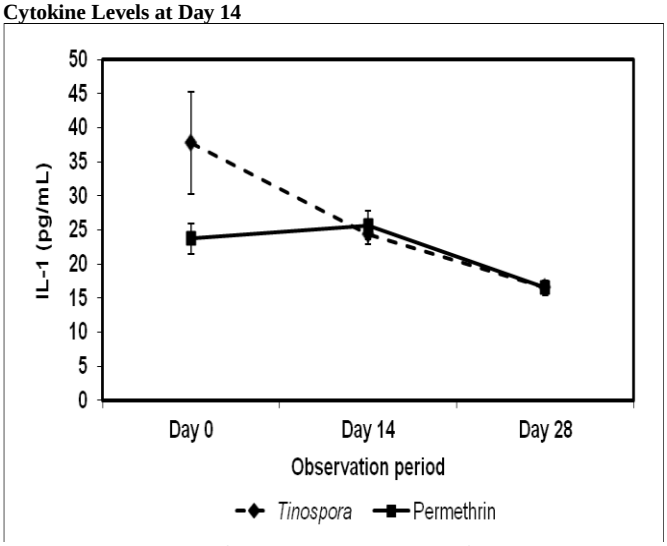

Fig. 3: IL-1 Levels of Patients Over Time after Treatment with *Tinospora* (n=30) and Permethrin (n=30) as Determined by ELISA

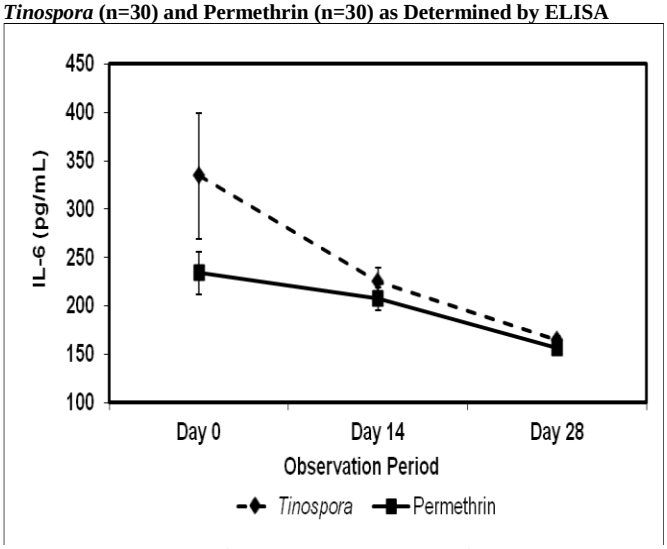

Fig. 4: IL-6 Levels of Patients Over Time after Treatment with *Tinospora* (n=30) and Permethrin (n=30) as Determined by ELISA

With the increased cytokine levels at Day 14 observed among patients treated with *Tinospora* and permethrin lotions, post scabietic hypersensitivity was not significantly inhibited by both lotions. Although, there is a lower percentage of patients treated with *Tinospora* lotion who exhibited an increased in IL-1 and IL-8 levels.

There were 27, 32, 32 treated patients who had elevated levels of IL-1, IL-6 and IL-8 after two weeks of treatment, respectively. Significant increase in IL-1 [baseline (95% CI: 13.90-18.51): mean, 16.20); Day 14 (95% CI: 25.20- 35.00): 30.10; $p<0.001$] and IL-6 [baseline (95% CI: 156.65-209.08): 182.86; Day 14 (95% CI: (194.64-293.08): 243.86 ; $p<0.001$] cytokine levels (in pg/mL) were observed from Day 0 to Day 14 in patients treated with *Tinospora* lotion. Patients treated with Permethrin had significant increase in IL-1 [baseline (95% CI: 14.53-18.91): 16.72; Day 14 (95% CI: 23.84-37.89): 31.00; $p<0.001$], IL-6 [baseline (95% CI: 161.71-176.37): 169.04; Day 14 (95% CI: 187.54-254.26): 220.90; $p=0.005$], and IL-8 [baseline (95% CI: 51.78-70.86): 61.32; Day 14 (95% CI: 115.49-194.60): 155.05; $p<0.001$]. No significant increase [baseline (95% CI: -9.76-111.93): 51.09; Day 14 (95% CI: - 48.10-291.79): 121.84; $p=0.264$] in IL-8 levels was observed for *Tinospora*-treated patients.

There was a significant reduction in the mean IL-1 levels in *Tinospora* and permethrin-treated patients from Day 0 [95% CI: 22.52-53.00 vs. 19.11-28.35], day 14 [95% CI: 21.38-27.44 vs. 21.48-29.92] to Day 28 [95% CI: 14.32-18.75 vs. 15.05-18.13]. Significant decrease ($p<0.001$) in IL-1 levels was noted from Day 14 to Day 28 *($p=0.0005$)*. Both *Tinospora* and permethrin lotions significantly decreased ($p<0.001$) the IL-1 levels over time *($p=0.0002$)*. The two lotions exhibited comparable ($p=0.126$) reduction of IL-1 levels patients as shown in Figure 3.

The mean % reduction in serum IL-1 levels from Day 0 to Day 14 and from Day 0 to Day 28 for *Tinospora* and permethrin-treated patients is 57.85% vs 63.17% and 36.30% vs 26.96%.

A gradual decline in the mean IL-6 levels in Tinospora and permethrin-treated patients from Day 0 [95% CI: 201.18-467.54 vs. 188.79-279.39], Day 14 [95% CI: 195.13-254.91 vs. 183.27-231.85] to Day 28 [95% CI: 154.25-174.13 vs. 148.92-163.88] was observed. Significant decrease ($p<0.001$) in IL-6 levels was noted from day 14 to day 28. Significant decrease ($p<0.001$) in the IL-6 levels over time was noted for among *Tinospora* and permethrin-treated patients. The reduction of IL-6 levels in patients were comparable in both lotions ($p=0.075$) as shown in Figure 4.

The mean % reduction in serum IL-6 levels from Day 0 to Day 14 and from Day 0 to Day 28 for *Tinospora* and permethrin–treated patients is 35.61% vs 29.21% and 24.15 % vs 20.40 %.

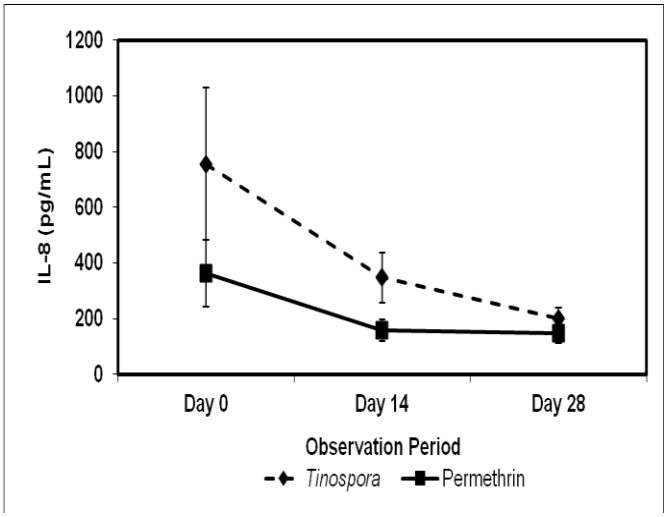

Fig. 5: IL-8 Levels of Patients Over Time after Treatment with *Tinospora* (n=30) and Permethrin (n=30) as Determined by ELISA

A significant reduction in the mean IL-8 levels in *Tinospora* and permethrin-treated patients from Day 0 [95% CI: 195.59-1314.43 vs. 115.67-608.96], Day 14 [95% CI: 164.77-530.90 vs. 82.14-235.49] to Day 28 [95% CI: 124.97-278.78 vs. 74.22 vs. 221.30] was noted. Significant decrease (p=0.007) in IL-8 levels was observed from Day 0 to Day 28. However, a significant difference (p=0.016) between the two lotions in reducing IL-8 levels in patients was noted. Permethrin lotion is more effective in reducing IL-8 levels during the first two weeks (Day 0-14); however, *Tinospora* lotion decreased the cytokine level to the same level as permethrin on Day 28 as shown in Figure 5.

The mean % reduction in serum IL-8 levels from Day 0 to Day 14 and from Day 0 to Day 28 for *Tinospora* and permethrin-treated patients is 95.77% vs 121.85% and 240.56% vs 188.68 %.

DISCUSSION

The scabies-infected patient manifested clinical signs and symptoms of the disease after three to four weeks of infestation as a result of an adaptive immune response of the host. Hence, patients with primary infestation have delayed onset of these symptoms due to long incubation period. [3] As previously established, the mite produces antigenic molecules like salivary secretions, excretory or fecal materials which modify the host's innate and immune cells' functions and responses. Therefore, the mite can avoid the inflammatory and immune responses of the host during an early infestation which allows the parasite to flourish and establish itself in the host skin. [2, 4] In addition, the scabies mite-inactivated protease paralogs (SMIPPs) and serine protease inhibitors (SMSs) like SMSB4 and SMSB3 which are secreted in the mite gut, eggs and excreted in feces have the ability to inhibit the three pathways of the human complement system thereby minimizing the complement-mediated gut damage in mites. [24-25]

The increased inflammatory cytokine levels at baseline during scabies infestation could be attributed to the interaction of Toll-like receptors with certain Pathogen-associated molecular patterns (PAMPs) like lipopolysaccharides and lipoproteins which may be present as antigenic substances in the scabies mite saliva, secretions, and feces. [26-27] These interactions cause a signaling cascade which stimulates production of inflammatory mediators and cytokines responsible for the immune response.

The increased cytokines level at Day 0 specifically IL-6 and IL-8 provide initial protection to the patient, where IL-6 induces the production of acute-phase and complement proteins in the liver which may be responsible for phagocyte accumulation at the scabeitic lesion; [28] IL-6 can stimulate leukocyte recruitment in the scabeitic lesion. [29] On the other hand, IL-8 is responsible for the recruitment and activation of neutrophils to the site of infection, which can generate respiratory burst responses through the production of toxic oxygen, degranulation and release of lysosomal enzymes and antimicrobial peptides. In addition, IL-8 serves as chemotactic agent for NK cells, T cells, basophils, and GM-CSF, thus imparting a role in innate immunity against scabies mite and its toxins. [30]

Some patients exhibited an increased level of inflammatory mediators on Day 14. This increased amount of cytokines may be attributed to the post-scabietic hypersensitivity experienced by the patients after effective treatment. The percentage of patients which had elevated IL-1 (p=0.2995), IL-6 (p=0.8361) and IL-8 (p=0.0665) at day 14 were statistically the same for both *Tinospora* and permethrin lotions. A significant increase in the mean values of IL-1 (p<0.05) and IL-6 (p<0.05) from Day 0 to Day 14 was noted in patients treated with both *Tinospora* and permethrin lotions. Despite the rapid IL-8 expression compared to other cytokines after cell stimulation by scabies mite and toxins along with the presence of IL-1β [30], no significant increase in IL-8 levels was observed for *Tinospora*-treated patients (p=0.13194), where p values <0.05, show significant increase in the cytokine level from Day 0 to Day 14. There was stimulation of cytokine production after two weeks of treatment with both lotions except IL-8 after treatment with *Tinospora.*

Around 40% (14/30) of *Tinospora*-treated patients have elevated levels of IL-8 as compared with 67% (20/30) of Permethrin group which means IL-8 was not significantly induced by *Tinospora* unlike IL-1 and IL-6 after two weeks of application. During this period, there could be less neutrophil migration into the scabietic lesions and less cellular injury caused by lipid peroxidation leading to clearing of lesions particularly in those patients with low cytokines levels specifically IL-8 on Day 14. [31]

Interleukin-1 (IL-1) usually derived from blood monocytes, dendritic cells and macrophages, serves as the pivotal cytokine alarm which induces the synthesis of inflammatory mediators through T cell activation and proliferation. [26] It activates the innate immune response and stimulates the adaptive immune system during an infection. [26, 32] It induces the production prostaglandins, leukotrienes, nitric oxide synthase, and cytokines like TNF and IL-6. It is mainly involved in inflammation enhancement and immune response of the host during scabies infestation because of its potentiation effects on the proliferation, differentiation and function of many innate cells. [26, 30] In addition, IL-1 is also responsible in the infiltration of inflammatory cells like leukocytes into scabietic lesion by its ability to regulate the synthesis of adhesion molecules at site of infection. [2] The decrease in IL-1 levels of patients may indicate a decrease in the synthesis of inflammatory mediators like cyclooxygenase 2, phospholipase A2 and nitric oxide synthase which are the primary cause of inflammation. [26] This effect may be involved in the decrease in inflammation, pustule formation and healing of scabeitic lesions.

Interleukin-6 (IL-6), is both a proinflammatory and anti-inflammatory cytokine which depends on the immune cells secreting it and the nature of stimuli. In case of scabies, it serves as inflammatory agent inducing the production of acute-phase and complement proteins by hepatocytes which are responsible for phagocyte accumulation and activation, pathogen opsonization and phagocytosis, and inflammation at the scabeitic lesion. [28, 30] It can also induce leukocyte recruitment in the scabeitic lesion and stimulates production of MCP-1. [29-30] The significant reduction in IL-6 levels after treatment with *Tinospora* could be responsible for the anti-inflammatory activity of the *Tinospora* lotion through reduced synthesis of acute phase proteins like C-reactive proteins and inhibition of leukocyte recruitement into the scabietic lesion thereby contributing to the improvement and clearance of scabies. [29]

Interleukin-8 (IL-8) is the main chemoattractant for neutrophil. Its synthesis can be induced by IL-1β, TNF-α,

bacterial LPS, retinoic acid, nitric oxide, or irradiation in immune cells like monocytes and macrophages, neutrophils, and lymphocytes. [30] It is a pro-inflammatory cytokine which is locally found in keratinocytes and usually expressed in response to an oxidative stress. [33] The increased IL-8 levels in scabies infected patients may be induced by the oxidative stress during scabies infestation due to significant oxidant/antioxidant imbalance as supported by works of S. K. Singh *et al.* (2011). [31]

The % IL-8 reduction is significantly higher compared to the % IL-1 and % IL-6 reduction after treatment with both lotions. This significant reduction of IL-8 levels which is about 100% to 200% reduction in mean values from baseline could be attributed to the IL-6-induced IL-8 to MCP-1 switch as supported by the works of Marino *et al.* (2008) on human myoblasts and Romano *et al.* (1997) on IL-6-induced endothelial cells. [34-35]

Since IL-8 expression can be influenced by oxidative stress, IL-8 reduction after treatment with *Tinospora* may be also attributed to the inhibition of oxidative stress by *Tinospora cordifolia* which exhibited antioxidative properties like suppression of thiobarbituric acid reactive substances (TBARS). Premanath and Lakshmidevi (2010) [36] reported that the ethanolic extract of *Tinospora* leaves has the highest antioxidant activity as compared with other solvent extracts.

Modulation of cytokine secretion by means of herbal medicines may be used in the management of several diseases. According to the works of Sharma *et al.* (2012), [37] the ethylacetate, water fraction, and hot water extracts of *Tinospora cordifolia* stems stimulate the phagocytic function of human neutrophils and increased NO production of macrophage thereby imparting immunomodulatory activity. The stimulated phagocytic activity, decreased altered macrophages, increased NO synthesis, increased myeloperoxidase (MPO) levels, and restored killing capacity of peritoneal macrophage are also exhibited by aqueous extract of *Tinospora* in CCl_4-treated male albino mice. [38] This increase in leukocyte count might have protected the scabies infected patients from secondary bacterial infections.

In addition, the immunomodulatory effect of *Tinospora* lotion, particularly the significant reduction in Interleukin-1, 6, and 8 levels may be accounted to the (1,4)-α-D-glucan or RR1 as reported by Nair *et al*, (2004); as well as to G1-4A, which binds to B cells and macrophages as shown by Desai *et al*. (2007) and which activates B and T cells and macrophages as reported by Raghu *et al.* (2009); and as well as to the Immunomodulatory protein (ImP), which have the ability to stimulate macrophage activation [39] and cytokines as well as chemokines synthesis. These interactions may be responsible to the increased interleukin levels or immunostimulation from Day 0 to Day 14, after a two-week application of the lotion in majority of the patients.

Moreover, the induction of leukocytosis with neutrophilia, enhancement of phagocytic activity, and stimulation of GM-CSF activity could possibly aid in the healing processes of scabeitic lesions like growth factor activation, angiogenesis and granulation tissue formation. [40-41] The antioxidant property of *Tinospora* as well as the decreased in lipid peroxidation and inhibition of oxidative stress may have contributed to the decrease in IL-8 levels after treatment with *Tinospora* lotion consequently preventing further cellular injury. [31, 33] Flavonoids and phenolic compounds isolated from *Tinospora* may also impart radical scavenging and antioxidants effects similar to other reported plants like *Aloe vera, Bacopa monniera, Moringa oleifera, Zingiber officinale, Zanthoxylum armatum and Aesculus indica*. [9, 10, 42-44] These as whole contributed to the clearance of scabeitic lesions and the general clinical improvement of the patients.

Results presented herein showed the immunomodulatory effect of *Tinospora* through downregulation of IL-1, IL-6, and IL-8 which indicates the anti-inflammatory property of the extract. The observed downregulation of these cytokines may be responsible for the inhibition of inflammatory mediators synthesis and migration into the scabeitic lesion as well as hyperkeratosis. The downregulation of IL-1 would inhibit synthesis of inflammatory mediators and would prevent production of adhesion molecules thereby inhibiting migration of inflammatory cells into scabeitic lesions. The reduction in IL-6 levels could be associated with the reduction of phagocyte accumulation and inhibition of leukocyte recruitement at the scabeitic lesion as a result of inhibition acute-phase, complement proteins and MCP-1 synthesis. Moreover, IL-8 regulation by *Tinospora* could be related to the proven antioxidant property of the extract and its cellular damage inhibition which may lead to the general improvement and clearance of scabeitic lesions. In addition, the modulation effect of the *Tinospora* lotion on interleukin levels potentially reinforces its anti-scabies activity.

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