

Effectiveness of *Tinospora sinensis* (Menispermaceae) extracts in dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis (ACD) mice model

Agnes L. Castillo^{*1-3}, Rachele Ann D. Letran¹, Stella Madj Y. Modina¹, Joselle Anne F. Payuran¹, Clare Louise T. Reyes¹, JessaMae C. Villena¹, Bill A. Que¹, & Rowen T. Yolo^{1,4}

¹Faculty of Pharmacy; ²Research Center for the Natural and Applied Sciences; ³The Graduate School; ⁴Faculty of Medicine and Surgery, University of Santo Tomas, España Boulevard, 1015 Manila, Philippines

Tinospora sinensis, a Philippine medicinal plant with reported anti-inflammatory activity, is traditionally used for wounds and scabies. *Tinospora* extract could exhibit activity against allergen-induced contact dermatitis which is characterized by itching with erythema and vesicles. To explore the effectiveness of the *Tinospora* extracts in contact hypersensitivity, BALB/c mice were sensitized with 0.5% dinitrofluorobenzene (DNFB) and were treated orally with the *Tinospora* extract for seven days. The ear thickness was then measured at 0-h, 24-h, and 48-h after elicitation. On the 24th hour, mean percentage change in ear thickness of mice treated with 1000 mg/kg BW of ethanolic extract did not differ significantly with the change observed in normal mice ($p=0.786$). Those given the 500 mg/kg BW had a mean percentage change significantly less than Prednisolone ($p<0.001$). After 48 h, the mean percentage change of the normal group did not differ with the groups treated with ethanolic extract ($p=0.956$). In terms of reduction in erythema, the mean diameter of the five groups significantly differ [$F_{4,32} = 144.179, p<0.001$], indicating that the mean erythema diameter of the normal group, and those given with ethanolic extract did not differ ($p=0.505$). In addition, *Tinospora* extract reduced inflammatory infiltration and damage to the epidermis based on histopathological examination of the ears. In conclusion, *Tinospora* ethanolic extract significantly reduced the swelling and erythema produced during 24 h and 48 h post-challenge. *Tinospora* ethanolic stem extract is an effective anti-inflammatory agent for DNFB- induced ACD mice model.

Keywords: allergic contact dermatitis, anti-inflammatory, Dinitrofluorobenzene, *Tinospora sinensis*

INTRODUCTION

Contact dermatitis (CD) is a common inflammatory skin condition characterized by an

erythematous and pruritic skin lesion that occurs after skin contact with a foreign substance [1]. It is a type IV delayed hypersensitivity wherein skin changes manifest only after re-exposure of the skin to the foreign substance. Based on manifestations, CD is classified as acute when

*To whom correspondence should be addressed:
alcastillo@ust.edu.ph

there is indication of itching with erythema, vesicles and bullae, or chronic when lichens with cracks and fissures are present [2, 3]. It proceeds through two phases: sensitization and elicitation phases [4]. ACD reaction is mediated by T cells which occur at the challenge site of the hapten on the skin.

ACD has always been in the ten most common skin diseases among Filipinos according to Philippine General Hospital Outpatient Department (PGH-OPD) Section of Dermatology and ranked first among the most common skin diseases in the Philippines according to a study conducted by Jose Reyes Memorial Medical Center (JRMMC) wherein 30–50% of the 10–15% CD patients are those with ACD type. Moreover, in 2015, 492 outpatient department (OPD) patients were diagnosed with ACD out of 10,361 OPD patients in the University of Santo Tomas Hospital (USTH).

Treatment of ACD includes the use of topical or oral glucocorticoids; however, their long-term use is associated with several complications [5, 6]. Several medicinal plants with anti-inflammatory property have been explored as potential inhibitors of ACD. Phytochemicals such as aloperine [6], paeoniflorin [7], veratroylzygadenine [8] and extracts from *Sasa veitchii* [9] and *Rhus verniciflua* [10] were found to suppress 2,4-dinitrofluorobenzene (DNFB)-induced ACD in mice model.

This study focuses on the effect of *T. sinensis*, a member of the Menispermaceae family, on contact dermatitis induced by DNFB. *Tinospora sinensis* is reported to possess anti-inflammatory property [11]. It has been linked with various biological activities including anti-inflammatory, analgesic, hypoglycemic, hepatoprotective, cytotoxic, antiallergy, antioxidant, antimalarial, antiscabies and antibacterial [11–17]. Several *Tinospora* species have also been found to possess anti-inflammatory activity. Decoction from the stem

of *T. crispa* has been used as antipyretic, in the treatment of internal inflammations and for the maintenance of good health [13]. *T. cordifolia* extract has strong analgesic, anti-inflammatory and anti-pyretic effect [11, 13]. Ethanolic and aqueous extracts of *T. cordifolia* and *T. sinensis* stems exhibited immunomodulatory effects in a cyclophosphamide-induced animal model [18].

MATERIAL AND METHODS

Preparation of *T. sinensis* extracts. Mature stems of *T. sinensis* were gathered from Angat, Bulacan, Philippines in August 2016 and authenticated by the University of Santo Tomas Herbarium Section (Manila). The stems were cut into small pieces, air-dried until the stems were brittle and ground to a powder using a Thomas-Wiley mill. One hundred fifty (150) grams of the air-dried powdered stems was percolated with 1 L of 80% ethanol. After the percolation, the ethanol extract was lyophilized. For the decoction, another 50 g of air-dried powdered stems of *T. sinensis* was boiled in 1 L of water and the aqueous extract was lyophilized.

Experimental animals. Six-week old female BALB/c mice were purchased from the Research Institute of Tropical Medicine (Muntinlupa, Metro Manila). The mice were housed in a room under conditions specific for ACD experiments, with food and water available ad libitum. Upon purchase, the mice were acclimatized for 7 days and were randomly divided into seven groups. The experiment was carried out in accordance with the guidelines set by the University of Santo Tomas Institutional Animal Care and Use Committee (UST-IACUC).

Induction and suppression of ACD. Initially, an area (about 1.0×1.0 cm) of abdominal skin of the mice was shaven. After 2 days, the mice were sensitized by applying 100 µL of 0.5% DNFB in acetone-olive oil (4:1) mixture on the surface of the shaven abdomen. After 7 days, the mice were

challenged by painting the right ear with 10 μ L of 0.2% DNFB in acetone-olive oil (4:1) mixture.

To study the suppression effect of the *T. sinensis* extracts, aqueous solutions of each of the following substances were administered separately and orally for 7 days right after the sensitization of the mice: Prednisolone (20 mg/kg BW), ethanolic *T. sinensis* extract (500 mg/kg and 1000 mg/kg BW), and aqueous *T. sinensis* extract (500 mg/kg and 1000 mg/kg BW) [19].

The experiment was conducted on 42 mice which were randomly divided into seven groups, each with six mice. The experiment done on each group of mice after sensitization are as follows:

- Group 1 — Normal mice without any sensitization and treatment
- Group 2 — Sensitization and given water
- Group 3 (Positive control) — Sensitization and treatment with prednisolone solution)
- Group 4 and Group 5 — Sensitization and treatment with two different doses of ethanolic extract
- Group 6 and Group 7 — Sensitization and treatment with two different doses of aqueous extract

Measurement of ear thickness and erythema.

The ear thickness was measured before elicitation for the baseline readings, and after 24 h and 48 h after elicitation, using the dial thickness gauge (G-1A, Ozaki Mfg., Co., Ltd., Tokyo, Japan). The diameter of erythema was measured in mm through a Vernier caliper. The efficacy of the test samples was expressed in terms of % change in ear thickness and erythema scores [19].

The individual score for erythema is based on the sum of the total scores (0–no symptom; 1–slight; 2–mild; 3–moderately severe; and, 4–severe) for each sign and symptom of dermatitis, namely: erythema, edema, excoriation/erosions, and dryness. The maximum value of the individual score is 12. All test

animals were sacrificed through cervical dislocation and the right ear was excised and dissected for future analyses after the 48th hour of measurement [20].

Histopathological studies. Biopsy samples of the ear tissues from all test groups were fixed with 10% buffered neutral formalin, processed and embedded in paraffin, microsectioned, and stained using Hematoxylin and Eosin (H&E). The specimen slide preparations were then microscopically examined at 40 \times , 100 \times , and 400 \times total magnifications. The degree of inflammation was estimated by the degree and extent of leukocyte infiltration, presence of interstitial edema, and local vascular congestion. The infiltrated immune cells were counted using a cell counting grid to evaluate the immune cell index of ear. The immune cell numbers collected were categorized into five reproducible subgroups: 0–no detectable inflammatory cell; 1–if number of inflammatory cells is between 1 and 20; 2–if number of inflammatory cells is between 21 and 40; 3–if the number of inflammatory cells is between 41 and 60; and, 4–if the number of inflammatory cells is greater than 60 [20]. Scale used are: 4–Severe; 3–Moderate; 2–Mild; 1–Slight; and 0–normal.

Phytochemical analysis. The crude alcoholic and aqueous *T. sinensis* stem extracts were subjected to various phytochemical color reactions to determine the presence of secondary metabolites such as cyanogenic, saponin, flavonoid, anthraquinone and cardiac glycosides, tannins and alkaloids [21].

Statistical analysis. Means \pm standard error (SE) was used to summarize the ear thickness, percentage change in the ear thickness, and the erythema diameter of the different groups. Single-factor analysis of variance, with Tukey's honest significant difference (HSD) for post hoc analysis were performed to compare the means of the different groups, while paired *t*-test was used to compare the results after 24 h and 48 h

in each group. Median and interquartile range were used to summarize the results of the erythema scores. Kruskal-Wallis test was performed to compare the groups, with Mann-Whitney test for post hoc analysis. All the statistical tests were performed in SPSS ver. 20.0. p -values less than 0.05 indicate significant differences.

RESULTS

Effect of T. sinensis extracts on ear swelling.

Twenty-four (24) hour after the challenge, swelling and erythema in the ear area where DNFB was applied, were predominantly observed in the negative control group indicating the successful induction of contact sensitivity and elicitation of inflammation. The groups treated with prednisolone (positive control) and the ethanolic and aqueous extracts from *T. sinensis* were found to have less erythema and swelling of the ears compared to negative control group.

The effect of treatment with the ethanol extract on the ear thickness measured 24 h and 48 h after challenge is presented in Fig. 1. The groups treated with 500 mg/kg BW and 1000 mg/kg BW ethanolic *T. sinensis* extract exhibited a 9% and 3% change in ear thickness, respectively, after 24 h. These changes were significantly lower

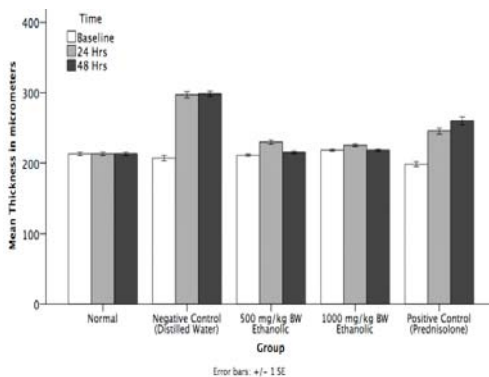


Figure 1. Mean ear thickness of the different mice groups treated with the ethanolic *T. sinensis* extract.

than the 24% change observed in the group treated with the positive control prednisolone (for the two doses: $p < 0.05$). These results show that the ethanolic extract was more effective than the Prednisolone in reducing inflammation, and that the suppression effect was dose-dependent.

After 48 h, both doses of ethanolic extract reduced the ear thickness to greater extent as compared to that seen on the 24th hour. The 1000 mg/kg dosage reduced the ear thickness or swelling of the mice to its baseline (i.e. ~ 0% change). The 500-mg/kg body weight (BW) dose was not as effective, causing a change of about 2%. These observations indicate the efficacy of the *Tinospora* stem ethanolic extract against DNFB-induced ACD as seen in the dose dependent reduction of ear inflammation.

The effect of the treatment of the mice with the aqueous extract from *T. sinensis* is summarized in Fig. 2. Similar to the ethanolic extract, the two doses of the aqueous extract caused significantly less changes (15% and 8% for doses of 500 mg and 1000 mg/kg BW, respectively) in the ear thickness measured 24 h after the challenge compared to the positive control Prednisolone (24%) (for the two doses: $p < 0.05$). These results indicate that aqueous extract was better than the positive control (Prednisolone) in reducing ear inflammation and

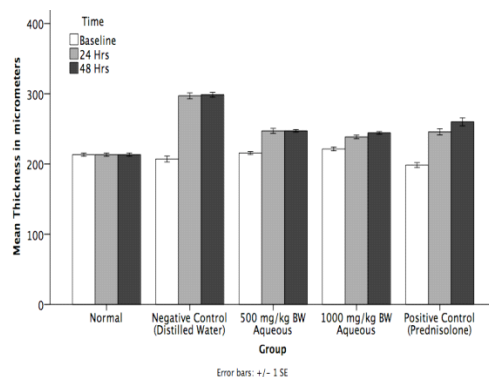


Figure 2. Mean ear thickness of the different mice groups treated with the aqueous *T. sinensis* extract.

Effectiveness of *Tinospora sinensis* (Menispermaceae) extracts

that dosage affected the extent of the effect. The reduction in the ear thickness observed after 24 h and 48 h is the same in the groups treated orally with 500 mg and 1000 mg/kg BW of aqueous extract ($p=0.169$ and $p=0.659$, respectively).

Effect of *T. sinensis* extracts on ear erythema.

Table 1 shows the erythema diameter observed for the different experiment group, as well as the result of an ANOVA test with post hoc HSD analysis. For the mice groups treated with the ethanol extract of *T. sinensis*, the erythema diameter measured at 24 h after challenge was significantly less than the diameter of the treated with the positive control ($p<0.001$). The diameter of the ear erythema in the normal group and the groups given 1000 mg/kg BW of ethanolic extract did not differ significantly ($p=0.947$), while the diameter of the mice which received the 500 mg/kg BW of ethanolic extract were significantly higher ($p<0.001$). After 48 h, the ear

erythema diameter observed in the mice treated with the two doses of the ethanolic extract did not differ significantly from the normal mice ($p=0.505$). These observations indicate that the ethanol extract was more effective in inhibiting the development of redness in the sensitized area better than the positive control, even 48 h after the challenge.

For the mice treated with the aqueous extract, the effect on the erythema diameter was similar to that observed in the mice treated with the ethanol extract, the area of the developed redness being significantly less for the two doses than those which were treated with the positive control ($p<0.001$). However, unlike in the ethanolic extract, the effect observed after 48 h for the two doses of the aqueous extract differed significantly from that observed in the normal mice ($p<0.001$). This indicates that the ethanolic extract is more effective in reducing the erythema diameter which may show that the

Table 1. Mean ear erythema diameter after 24 h and 48 h using *Tinospora* stem extracts

	Normal (A)	Negative Control (B)	500 mg (C)	1000 mg (D)	Prednisolone (E)	p-value	post hoc
<i>Tinospora</i> ethanolic extract							
Baseline	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-
After 24 h	0.00±0.00	12.23±0.22	3.64±1.73	0.86±0.86	10.79±0.15	<0.001	(A=D)<C<(B=E)
After 48 h	0.00±0.00	11.86±0.24	1.14±1.14	0.00±0.00	10.93±0.23	<0.001	(A=D=C) <(B=E)
<i>Tinospora</i> aqueous extract							
Baseline	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-
After 24 h	0.00±0.00	12.23±0.22	6.73±0.3	1.14±1.14	10.79±0.15	<0.001	(A=D)<C<(B=E)
After 48 h	0.00±0.00	11.86±0.24	8.50±0.19	9.29±0.43	10.93±0.23	<0.001	A<(C=D)<(B=E)

Values expressed as mean ± SEM

Table 2. Erythema scores of the five groups after 24 h and 48 h using *Tinospora* stems extract

	Normal (A)	Negative Control (B)	500 mg (C)	1000 mg (D)	Prednisolone (E)	p-value	post hoc
<i>Tinospora</i> ethanolic extract							
Baseline	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	-
After 24 h	0 (0-0)	3 (3-3)	0 (0-0)	0 (0-1)	2 (2-2)	<0.001	(A=C=D)<E<B
After 48 h	0 (0-0)	3 (3-3)	0 (0-0)	0 (0-0)	3 (2-3)	<0.001	(A=C=D)<E<B
<i>Tinospora</i> aqueous extract							
Baseline	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	1.000	-
After 24 h	0 (0-0)	3 (3-3)	1 (1-1)	0 (0-0)	2 (2-2)	<0.001	(A=D)<C<E<B
After 48 h	0 (0-0)	3 (3-3)	2 (2-2)	1 (1-1)	3 (2-3)	<0.001	A<D<C<E<B

Values expressed as median (IQR)

active components are semipolar in nature and are present in the ethanolic extract.

The erythema scores show the intensity of redness seen on the ear after the observation period. Table 2 summarizes the erythema scores for the various treatment. Results after 24 h from elicitation showed that the erythema scores of those given with 1000 mg/kg BW of aqueous extract did not differ significantly with the normal group ($p>0.05$). Mice given a dosage of 500 mg/kg BW of aqueous extract had significantly higher erythema scores ($p<0.05$) compared to those given with 1000 mg/kg BW and the normal group, but significantly less than the positive control group ($p<0.05$). However, 48 hours after challenge, the erythema scores for the mice given 500 mg/kg and 1000 mg/kg BW aqueous extract were significantly higher (for both doses: $p<0.05$) than the normal group, but significantly less than the positive control group ($p<0.05$). These results suggest that the ethanolic extract was more effective in suppressing erythema than the water extract after the observation period. Moreover, the two doses can be considered to be more effective than the positive control (Prednisolone) even after 48 h.

Effects of T. sinensis extracts on histopathological changes of ear tissues.

Histopathological results of the studies were expressed in terms of scores which were based on the number of immune cells index using light microscopic observations in H&E stained slide preparations of the tissues. The scores based on the histopathological results observed for the different experiment group are presented in Table 3. The histopathologic scores for the mice treated with 1000 mg/kg BW ethanolic, 500 mg and 1000 mg/kg BW aqueous extract were significantly greater ($p=0.001$) than those for the mice given the dose of 500 mg/kg BW ethanolic extract, which had the same erythema score as with the positive and negative control groups ($p=0.895$).

Table 3. Histopathological result

Group	Score	p-value
Normal	0 (0–0)	<0.001
Negative control	2.5 (2.5–2.5)	
Prednisolone	2.5 (2.5–2.5)	
Ethanolic extract (500 mg)	2.5 (2.0–3.0)	
Ethanolic extract (1000 mg)	3.0 (2.5–3.0)	
Aqueous extract (500 mg)	3.5 (3.0–3.5)	
Aqueous extract (1000 mg)	3.0 (2.5–3.5)	

Values expressed as median (IQR)

Scale used are: 4 – Severe; 3 – Moderate; 2 – Mild;

1 – Slight; 0 – normal

These observations suggest that suppression of inflammation may be dose-related, and that a greater degree of an inflammatory reaction, may require a higher dose of the extract to suppress the response. Furthermore, greater suppression with the ethanolic extract appears more effective than in the aqueous extract, suggesting that the active phytochemical component responsible for its anti-inflammatory properties may possibly be non-water soluble in its chemical composition.

Additional observation for the group of mice given 500 mg/kg BW ethanolic extract show that the inflammatory cell infiltrates, predominantly neutrophils, plasma cells and eosinophils were seen on the thinner part (tip) of the ears, and were not as dense compared to the thicker portion (basal) of the ears. This may be related to the thickness of the connective tissue layer and perhaps the swelling caused by edema fluids which may play a role as a protective physical barrier, in which DNFB when applied to the thinner layer of the ear, could incite a more pronounce inflammation, compared to the thicker layer at the basal portion. The extracts may probably be more effective in suppressing inflammation in the thicker area where a lesser degree of the inflammatory response is present. We cannot also discount the possibility of an overlying superficial bacterial infection, most notably in the thin layer of the ear brought about by physical injuries due to inevitable biting of the animals within a limited enclosure.

Effectiveness of *Tinospora sinensis* (Menispermaceae) extracts

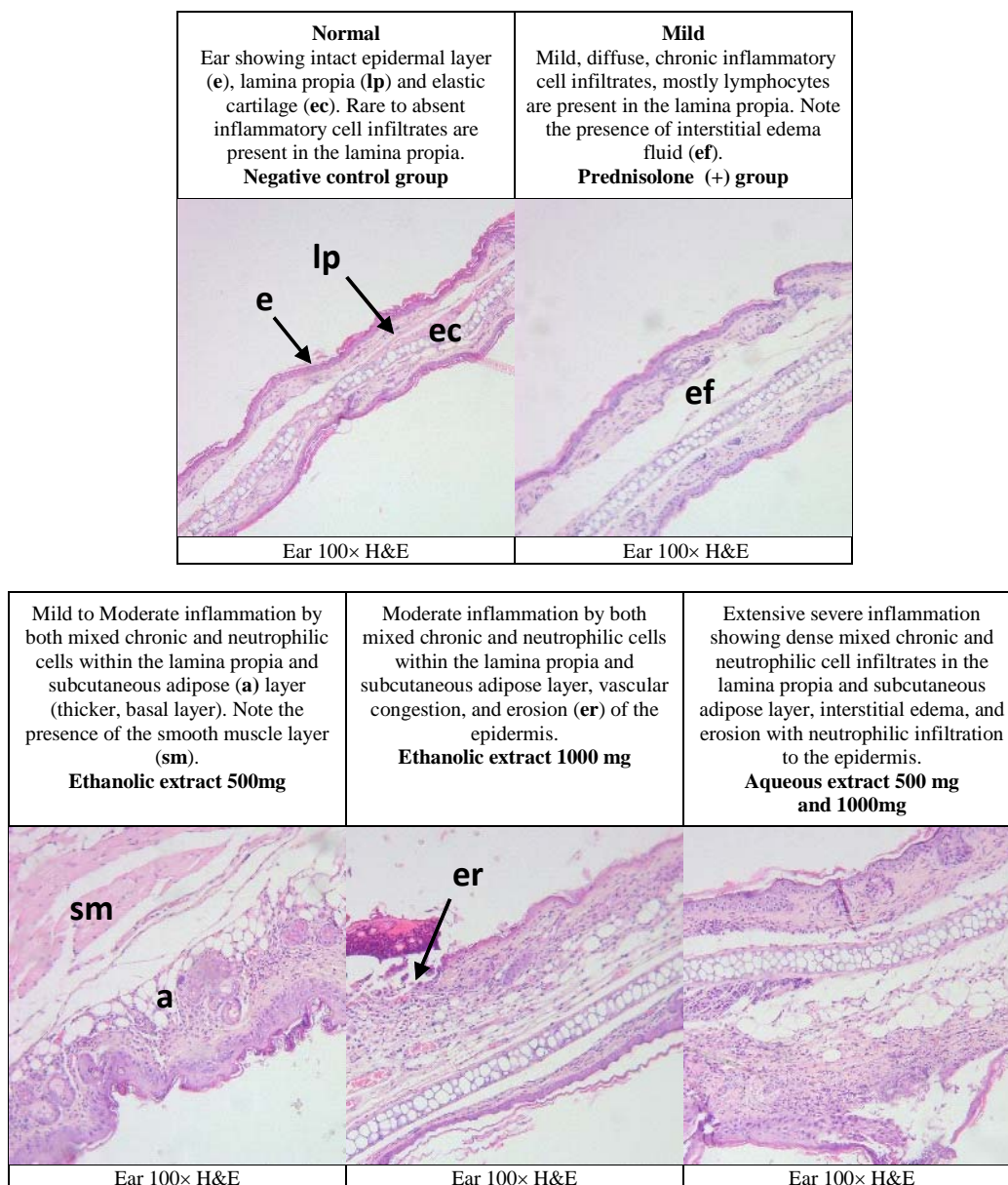


Figure 3. Illustrations of the histopathological studies of the treatment groups

Phytochemical components. The phytochemical tests performed on the ethanolic and aqueous extracts from *T. sinensis* stem revealed the presence of several bioactive compounds (Table 4). The presence of alkaloids and flavonoids in the *T. sinensis* extract may

have contributed in the anti-inflammatory activity which was similarly reported in *T. cordifolia* stem extract [22]. In the same study, saponins present in *T. cordifolia* were responsible for the higher anti-inflammatory activity which, however, was absent in *T.*

Table 4. Phytochemical components of the ethanolic and aqueous extracts of *T. sinensis*

Phytochemicals	Ethanolic extract	Aqueous extract
Alkaloids	+	+
Antraquinone	-	-
Cardiac glycosides	-	-
Cyanophore glycosides	-	-
Flavonoids	+	+
Saponins	-	-
Tannins	+	+
Steroids	+	-
Quaternary amines	+	+

(+) Presence; (-) Absent

sinensis stem extract. In addition, flavonoids are known to inhibit the chemical mediators of inflammation, hence possessing an anti-inflammatory property [11].

In the study of Phienwej *et al.* (2015), the chemical constituents isolated from *T. crispata* were alkaloids, flavones, and phenolics which were also found in the ethanolic and aqueous *T. sinensis* extracts. However, G1-4A, a polysaccharide isolated from *T. cordifolia*, enhanced the release and function of BM-DCs, IL-2, and TNF- α [24] which may be responsible for the presence of erythema and inflammatory factors seen in the ears even after treatment with *Tinospora* extracts.

DISCUSSION

Nowadays, medicinal plants have become an important source of essential immunomodulatory components. Immunomodulation can either strengthen, weaken, or suppress the immune system. Immunosuppression is essential in an anti-inflammatory response. Inflammation is described as “the pathophysiological response of living tissues to injuries that leads to an accumulation of plasma and blood cells at the injured site” [2, 11]. This inflammatory reaction is essential for survival against environmental pathogens and injuries [2, 11].

The principal immunomodulatory components of *T. cordifolia* are phytochemicals such as carbohydrates, glycosides and alkaloidal in nature [25]. *Tinospora cordifolia* extracts were found to have good anti-inflammatory activity which was observed to be dose dependent and may be attributed to the flavonoid content of the plant.

According to Tiwari, Dwivedi, and Kakkar (2014), the 50% ethanolic extract of *T. cordifolia* can downregulate the release of pro-inflammatory genes iNOS, COX-2, and ICAM-1 which results in the decrease of inflammatory response. This was due to the reduction of pro-inflammatory mediators such as TNF- α , IL-4, NO, and IgE in asthma which eventually led to prevention of inflammation. The extract was also able to increase levels of I κ B which can regulate translocation of NF κ B to the nucleus indirectly. This is a possible mechanism of the anti-inflammatory of the *T. sinensis* on the reduced percentage of the ear swelling. There could be a possibility that the aqueous and ethanolic extracts have prevented the activation of pro-inflammatory factors and maturation of the dendritic cells together with its subsequent mediation of the CD4⁺ and CD8⁺ which proliferates and causes inflammation on the ears of the mice.

In the DNFB- induced ACD mice model, sensitization is the initial phase that occurs at first contact of skin with the haptens, DNFB, which leads to the generation of the DNFB-specific T-cells in the lymph node (LN). Haptens are found to possess proinflammatory properties which activate the skin's innate immunity and deliver signals able to induce migration and maturation of cutaneous dendritic cells (DC) and leads to the priming of specific CD8⁺ and CD4⁺ T lymphocytes which then recirculate between the lymphoid organs and the skin. This phase usually lasts for 5–10 days in mice. On the other hand, elicitation phase or challenge phase, occurs when specific T

lymphocytes are activated and trigger inflammatory process responsible for cutaneous lesions. This phase is categorized into two; the early phase which is 2 h after re-exposure and the late phase which is about 24 h after re-exposure. This lasts for several days and progressively decreases upon physiological downregulating mechanisms and was observed after the treatment with *Tinospora* extracts [27].

According to Moody, Robert, Connolly, and Houghton (2006), the methanolic fruit extract of the Menispermaceae plant, *Spenocentrum jollyanum* contains furanoditerpenes namely, columbin, iso-columbin, and fibleucin. Moreover, a flavonoid-rich fraction (FDE) was also isolated from the plant extract. Among the constituents, columbin and FDE had a significant ($p < 0.05$) dose dependent anti-inflammatory activity. It has been suggested that the inhibition of enzymes I and II of cyclooxygenase mediates the anti-inflammatory activity of the furanoterpenoids and columbin which were also isolated from *T. crispa* (29, 11). Hence, the inhibition of cyclooxygenases may be a possible mechanism of anti-inflammation for both the extracts of *T. sinensis*.

The inflammation of the right ear on the 24th hour and 48th hour has been inhibited after the subsequent challenge of 0.2% DNFB. It was also proven that *Tinospora* extracts were effective as an alternative treatment for ACD. The results elucidated that extracts of *T. sinensis* were also comparable to that of the standard drug, Prednisolone, because there were no significant difference on the mean percentage change on the thickness of the right ear after several hours compared to the mean percentage change on the ear thickness of the negative control which did not receive any treatment ($p = 1.000$).

The effects of the alkaloids in inhibiting inflammation, or a possible mechanism involved in the inhibition of ear thickness after the challenge phase could also have contributed to

the extract's anti-inflammatory activity. Inflammation can be triggered by complex signal transduction cascades and thus involves many pro-inflammatory factors. Meanwhile, immunosuppression may result through the inhibition of the production or synthesis of these inflammatory factors, reduced recruitment, and reduced sensitivity of these factors to the antigens presented to them. This study only focused on the elucidation of the anti-inflammatory property of *T. sinensis* in vivo and the comparison of different doses to determine the dose at which the extract exhibits better anti-inflammatory property. Thus, the pathways presented are the possible mechanisms of *T. sinensis* which could explain the anti-inflammatory activity. This study also sought to explore if the both the extracts are comparable to the standard drug, Prednisolone. The standard drug, a corticosteroid, inhibits phospholipase-A2 which prevents the conversion of phospholipids to arachidonic acid. This prevents cyclooxygenase and lipoxygenase enzymes to act on arachidonic acid to synthesize pro-inflammatory factors. It can be inferred that this primary mechanism could be the possible mechanism of the *T. sinensis* extracts that were able to inhibit the inflammation of the ears of the mice challenged by 0.2% DNFB.

CONCLUSION

The ethanolic extract of the *T. sinensis* appears to have a dose-dependent manner in terms of its anti-inflammatory action compared to the aqueous extract that did not show any difference between its two doses. The results suggest that the most effective dose of *T. sinensis* is the 1000 mg/kg BW ethanolic extract. This study demonstrates that it was able to inhibit the ear inflammation significantly reducing the ear thickness back to its baseline measurement in the 48th hour after elicitation.

In conclusion, *Tinospora* ethanolic extract significantly reduced the swelling and erythema produced during 24 h and 48 h post-challenge. *Tinospora* ethanolic stem extract appears to be a potentially effective anti-inflammatory agent for DNFB- induced ACD mice model, parallel in efficacy to that of prednisolone.

ACKNOWLEDGMENT

Grateful acknowledgement is due to Dr. Reginald B. Salonga for the training and equipment he has provided to the researchers. Financial support were provided by the Faculty of Pharmacy and The Research Center for the Natural and Applied Sciences, University of Santo Tomas.

REFERENCES

- [1] Usatine RP & Riojas M. Diagnosis and Management of Contact Dermatitis. *American Family Physician* **2010**, 82(3):249–255.
- [2] Imbesi S, Miniciullo P, Isola S, & Gangemi S. Allergic contact dermatitis: Immune system involvement and distinctive clinical cases. *Allergologia et Immunopathologia* **2011**, 39(6):374–377. DOI:10.1016/j.aller.2011.05.001
- [3] Tonèiæ RJ, Lipozenèiæ J, Martinac I, & Greguriæ S. Immunology of Allergic Contact Dermatitis. *Acta Dermatovenerol Croat* **2011**, 19(1):51–68.
- [4] Saint-Mezard P, Rosieres A, Krasteva M, Berard F, Kaiserlian D, & Nicolas J. Allergic Contact Dermatitis. *European Journal of Dermatology* **2004**, 14:284–295.
- [5] James WD, Elston DM, & Berger TG. Contact Dermatitis and Drug Eruptions. In: *Andrew's Diseases of the Skin: Clinical Dermatology*, pp. 91–99). (China: Elsevier Inc., **2011**).
- [6] Yuan XY, Liu W, Zhang P, Wang RY, & Guo JY. Effects and mechanisms of aloperine on 2, 4-dinitrofluorobenzene-induced allergic contact dermatitis in BALB/c mice. *European Journal of Pharmacology* **2009**, 629:147–152.
- [7] Shi D, Li X, Li D,... & Liu W. Oral Administration of Paeoniflorin Attenuates All Allergic Contact Dermatitis by Inhibiting Dendritic Cell Migration and Th1 and Th17 Differentiation in Moude Model. *Immunopharmacol* **2015**, 432–439. DOI:10.106/j.intimp.2015.02.031
- [8] Cholakova M, Bratanov M, Christov V, Kostova N, Gantcheva M, & Nikolova E. The Veratum Alkaloid, Veraroylzygadenine, Suppresses Contact Dermatitis in Mice. *Journal of Medicinal Plants* **2009**, 3(1):1109–1112.
- [9] Usuda H, Fujii H, & Nonogaki T. Sasa veitchii extracts suppress 2,4-dinitrofluorobenzene-induced contact hypersensitivity in mice. *Food and Agriculture Immunology* **2015**, 27(4):523–534.
- [10] Park DK, Gi Lee Y, & Park HJ. Extract of *Rhus verniciflua* Bark suppresses 2,4-dinitrofluorobenzene-induced allergic contact dermatitis. *Evidence-Based Complementary and Alternative Medicine* **2013**. DOI:10.1155/2013/879696.
- [11] Ahmad W, Jantan I, & Bukhari S. *Tinospora crispa* – A Review of Its Ethnobotanical, Phytochemical, and Pharmacological Aspects. *Frontiers in Pharmacology* **2016**, 7:59. DOI:10.3389/fphar.2016.00059
- [12] Bishayi B, Roychowdhury S, Ghosh S, & Sengupta M. Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl4 intoxicated mature albino rats. *The Journal of Toxicological Sciences* **2002**, 27(3):139–146. DOI:10.2131/jts.27.139
- [13] Hussain L, Akash MH, Ain N, Rehman K, & Ibrahim M. The Analgesic, Anti-Inflammatory and Anti-Pyretic Activities of *Tinospora cordifolia*. *Advances In Clinical And Experimental Medicine* (Official Organ Wroclaw Medical University) **2015**, 24(6):957–964. DOI:10.17219/acem/27909
- [14] Kaur M, Singh A, & Kumar B. Comparative antidiarrheal and antiulcer effect of the aqueous and ethanolic stem bark extracts of *Tinospora cordifolia* in rats. *Journal of Advanced Pharmaceutical Technology & Research* **2014**, 5(3):122–128. DOI:10.4103/2231-4040.137417
- [15] Kumar V, Mahdi F, Chander R, Husain I, Khanna A, Singh R, Saxena J, Mahdi A, & Singh RK. *Tinospora cordifolia* regulates lipid metabolism in allaxon-induced diabetes in rats. *International Journal of Pharmacy and Life Sciences* (IJPLS) **2013**, 4(10):3010–3017.
- [16] Niljan J, Jaihan U, Srichairatanakool S, Uthaipibull C, & Somsak V. Antimalarial activity of stem extract of *Tinospora crispa* against plasmodium berghei infection in mice. *J. Health Res.* **2014**, 28:199–204.

Effectiveness of *Tinospora sinensis* (Menispermaceae) extracts

- [17] Castillo AL, Osi MO, Ramos JA, De Francia JL, Dujunco MU, & Quilala PF. Efficacy and safety of *Tinospora cordifolia* lotion in *Sarcoptes scabiei* var *hominis*-infected pediatric patients: A single blind, randomized controlled trial. *Journal of Pharmacology & Pharmacotherapeutics* **2013**, 4(1):39–46. DOI:10.4103/0976-500X.10766
- [18] Manjrekar P, Jolly C, & Narayanan S. Comparative studies of the immunomodulatory activity of *Tinospora cordifolia* and *Tinospora sinensis*. *Fitoterapia* **2000**, 71(3):254–257. DOI:10.1016/s0367-326x(99)00167-7
- [19] Salonga R, Hisaka S, & Nose M. Effect of the Hot Water Extract of *Artocarpus camansi* Leaves on 2,4,6-Trinitrochlorobenzene (TNCB)-Induced Contact Hypersensitivity in Mice. *Bio. Pharm. Bull.* **2014**, 37(3):493–497.
- [20] Kim EC, Lee HS, Kim SK, Choi MS, Lee S,.. & Min BI. The bark of *Betula platyphylla* var. *japonica* inhibits the development of atopic dermatitis like skin lesions in NC/Nga mice. *J Ethnopharmacol.* **2008**, 116:270–278.
- [21] Guevara B (Ed.). *A guidebook to phytochemical screening: phytochemical and biological.* (Manila: UST Publishing House, **2005**).
- [22] Yadav RNS & Agarwala M. Phytochemical Analysis of Some Medicinal Plants. *Journal of Phytology* **2011**, 3(12):10–14.
- [23] Phienweij H, Swasdichira I, Amnuoyopol S, Pavasant P, & Sumrejkanchanakij P. *Tinospora crispa* extract inhibits MMP-13 and migration of head and neck squamous cell carcinoma cell lines. *Asian Pacific Journal of Tropical Biomedicine* **2015**, 5(9):738–743. DOI:10.1016/j.apjtb.2015.07.001
- [24] Desai V, Ramkrishnan R, Chintalwar G, & Sainis KB. G1-4A, an Immunomodulatory polysaccharide from *Tinospora cordifolia*, modulates macrophage responses and protects mice against lipopolysaccharide induced endotoxic shock. *International Immunopharmacology* **2007**, 7:1375–1386.
- [25] Sharma U, Bala M, Kumar N, Singh B, Munshi RK, & Bhalerao S. Immunomodulatory active compounds from *Tinospora cordifolia*. *Journal of Ethnopharmacology* **2012**, 141(3):918–926. DOI:10.1016/j.jep.2012.03.027
- [26] Tiwari M, Dwivedi UN, & Kakkar P. *Tinospora cordifolia* Extract Modulates COX-2, iNOS, ICAM-1, Pro-inflammatory Cytokines, and Redox Status in Murine Model of Asthma. *Ethnopharmacol* **2014**, 153(2):326–337. DOI:10.1016/j.jep.2014.01.031
- [27] Vocanson M, Hennino A, Saint-Mezard P, et al. CD8 + T Cells Are Effector Cells of Contact Dermatitis to Common Skin Allergens in Mice. *Journal of Investigative Dermatology* **2006**, 126:815–820. DOI:10.1038/sj.id.5700174.
- [28] Moody J, Robert V, Connolly J, & Houghton P. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). *Journal of Ethnopharmacology* **2006**, 104(1-2):87–91. DOI:10.1016/j.jep.2005.08.051
- [29] Choudhary MI, Ismail M, Ali Z, Shaari K, & Lajis NH. Alkaloidal constituents of *Tinospora crispa*. *Nat. Prod. Commun.* **2010**, 5:1747–1750.