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Biphasic inflammatory effects of *Voacanga globosa* ethanolic leaf extract in ICR mice

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Voacanga globosa **is an endemic plant traditionally used as remedy for boils in the Philippines. Four concentrations of the ethanolic leaf extracts (0.5, 5, 50 and 100 mg/kg bw) were administered intraperitoneally to 6 to 8 weeks old female imprinting control region (ICR) mice. Mice treated with 5 mg/kg bw *V. globosa* extract significantly potentiated the inflammatory response, exhibiting the highest edema index and enhanced leukocyte infiltration and hypertrophy. Inflammatory response was reduced at higher concentrations, suggesting that *V. globosa* might be immunosuppressive at high concentrations. These indicate that the ethanolic leaf extract of *V. globosa* exhibit biphasic inflammatory effects since it appears immunostimulatory at low concentration and immunosuppressive at high concentrations.**

Key words: *Voacanga globosa*, delayed-type hypersensitivity test, biphasic response, immunomodulation, histopathology, ICR mice.

INTRODUCTION

Numerous medicinal plants have been studied and developed as modulators of the immune system. These modulators have two major modes of action. One is to potentiate the innate immune response by macrophage activation and enhancement of the functions of T and B lymphocytes and natural killer cells while the second mode of action is the suppression of the immune response mechanisms (that is, inhibition of cytokine production). Some plant-derived immunostimulatory drugs have been utilized for self-medication and also in research work involved in the development of pharmaceuticals (Auttachoat et al., 2004). Examples include *Panax ginseng* (Friedl et al., 2001), *Rehmania glutinosa* (Hong et al., 2002) and *Salicornia herbacia* (Im et al., 2006).

Voacanga globosa (Blanco) Merr. is a tree that is endemic to the Philippines. However, in the village of Kanawan, Morong, Bataan, Philippines, the Aeta communities use the plant species as a remedy for boils and skin inflammation. It has also been reported that its leaf extract is used by the locals to stupefy fish. Biological activities of various *Voacanga* species include potent anti-inflammatory capacity, anti-nociceptive action and anti-ulcer properties (Olaleye et al., 2004; Tan et al., 2000). There is limited information available about the biological activities of *V. globosa.* Ethanolic leaf extracts of *V. globosa* have been shown to be cytotoxic against lung non-small cell adenocarcinoma (A549) and colon carcinoma (HCT 116) cell lines (Canoy et al., 2011) but failed to scavenge the free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Jacinto et al., 2011). The cytotoxicity and lack of anti-oxidant capacity warrants an investigation

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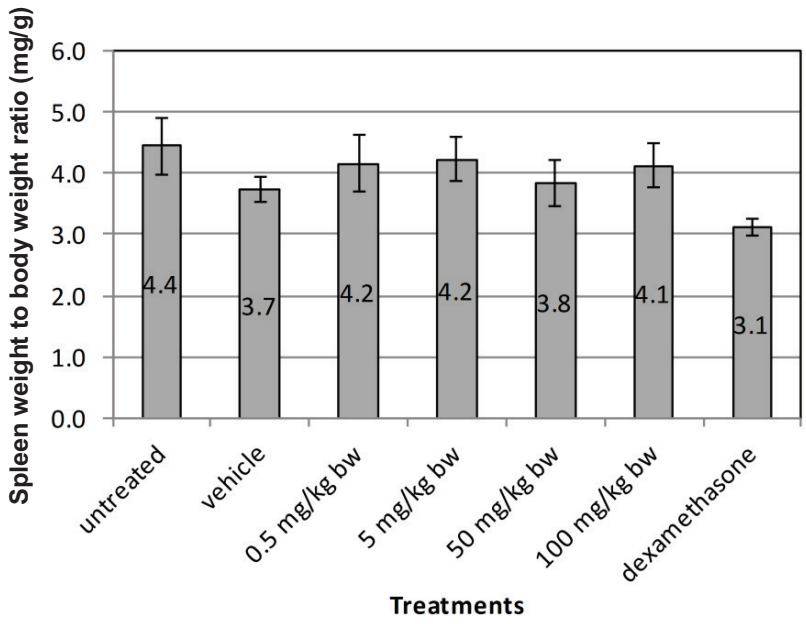

Figure 1. Mean spleen weight to body weight ratio (standard errors indicated) of mice (n=5) treated for seven days with *V. globosa* ethanolic extract (0.5, 5, 50 and 100 mg/kg bw), phosphate buffered saline (vehicle) and 0.2 mg/kg bw dexamethasone (control).

on the effect of this extract to normal cells. In an *in vivo* system, the first types of cells that the extract would interact with are the immune cells under the innate effector arm. Thus, the objective of the study was to assess the effect of *V. globosa* extract to an *in vivo* system with emphasis on the consequent inflammatory response.

In order to examine the effect of *V. globosa* extract on the innate immunity, ICR mice were intraperitoneally injected daily with the selected doses for seven days. The ICR strain of mice has been widely used for therapeutic drug testing and is prescribed for toxicology studies (Lu et al., 2007; Pan et al., 2013). Being a pioneer study, the dosages selected reflects a wide range from 0.5 to 100 mg/kg bw. Data from this study provided preliminary information on the immunomodulatory properties of *V. globosa* in female ICR mice. Evaluation of delayed type hypersensitive reaction and histopathological examinations were employed to determine immunomodulatory properties of the crude plant extract. Three parameters were evaluated: (1) spleen weight to body weight ratio (Figure 1), (2) paw edema index (Figure 2), and (3) visual inspection of leukocyte infiltration (Figure 3).

MATERIALS AND METHODS

This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care of the College of Science Animal Care and Use Committee (CSACUC), University of the Philippines Diliman.

Preparation of crude leaf extract

Leaves of *Voacanga globosa* (Blanco) Merr. were collected from the mountainous forests of Kanawan, Morong, Bataan. The collected leaves were air-dried to crisp and then homogenized using a blender. The homogenized leaves were soaked in 95% ethanol for two days, after which the leaf suspension was filtered. Concentration of the filtrate was done using a rotary evaporator set at the temperature of 40°C in order to separate the solvent and eventually to obtain the crude extract. For the subsequent assays, the obtained crude extract was diluted with phosphate buffered saline (PBS) to the desired concentration.

Treatment of ICR mice with *V. globosa*

Female ICR mice at 6 to 8 weeks old were housed under standard laboratory conditions and were given *ad libitum* access of food and water. Seven groups of mice (five mice per group) were used for the study. Four groups were injected intraperitoneally with *V. globosa* extract (0.5, 5, 50 and 100 mg/kg bw). One group was composed of untreated mice and served as the control group. Another group of mice was injected with PBS intraperitoneally and thus served as the vehicle group. The seventh group was composed of immunosuppressed mice injected with dexamethasone (0.2 mg/kg bw). Dexamethasone is a widely used synthetic analogue of corticosterone which is potently immunosuppressive. The dose volume was 0.2 ml for all the treated groups.

Delayed type hypersensitive reaction and histopathology

To induce delayed type hypersensitivity (DTH), the seven groups of mice were sensitized by subcutaneous injection in the neck at Day 0 with 1×10^8 (0.1 ml) sheep red blood cells (SRBC). From Day 1 to Day 7, the mice were given the appropriate plant extract treatment

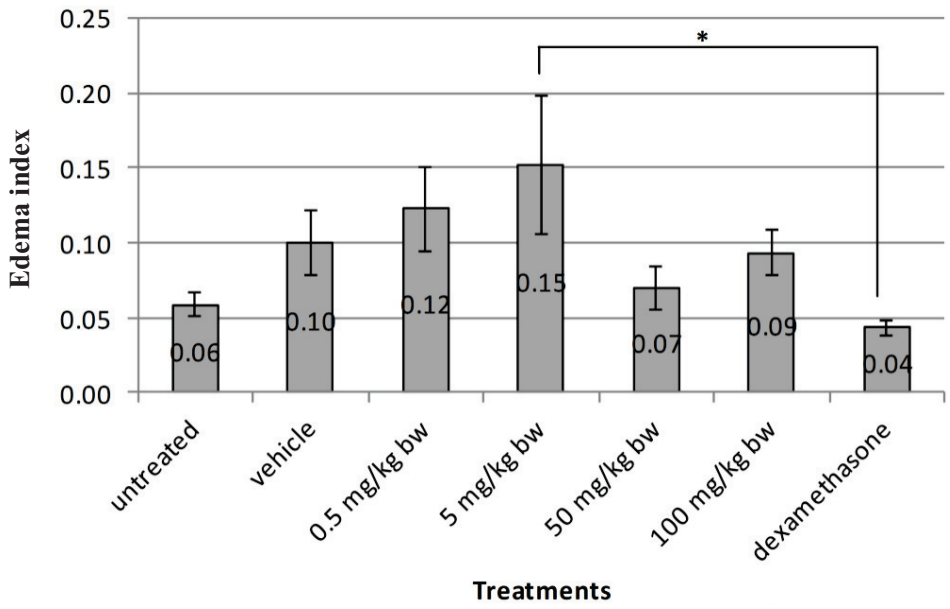

Figure 2. Mean edema indices (standard errors indicated) of mice (n=5) treated for seven days with *V. globosa* ethanolic extract (0.5, 5, 50 and 100 mg/kg bw), phosphate buffered saline (vehicle group) and 0.2 mg/kg bw dexamethasone (immunosuppressed group). Edema index of each mouse was calculated by taking the difference between the right hind footpad thickness (where SRBC was intradermally injected at Day 7) and the left hind footpad thickness and then divide it with the left hind footpad thickness. The asterisk (*) signifies that the 5 mg/kg bw and dexamethasone groups are significantly different with each other ($P < 0.05$).

(0.2 ml) via intraperitoneal (IP) injection. On Day 7, the mice were immunized with 1×10^8 (0.1 ml) SRBC injected intradermally on the right hind footpad. On Day 8, response to the challenge was evaluated through the increase in the paw volume as determined by measuring the foot pad thickness using a dial calliper. DTH reaction was expressed as edema index (EI) calculated as follows:

$$EI = \frac{(\text{Right hind paw thickness} - \text{Left hand paw thickness})}{\text{Left hand paw thickness}}$$

The mice were then weighed and sacrificed by cervical dislocation. The spleen of each mouse was dissected and weighed for the lymphoid organ to body weight ratio. The right hind paw of each mouse was excised and fixed in 10% buffered formalin solution (10% formaldehyde in 28 mM sodium phosphate, pH 7) for histological processing. The paws were decalcified, dehydrated, embedded in paraffin and sections of the tissue were stained with haematoxylin andeosin (HE) for examination. Qualitative assessment under the microscope was based on the inflammatory reaction.

Statistical tests

One-way analysis of variance (ANOVA) and *post hoc* test Duncan's multiple range test were performed. All statistical tests were conducted using the statistical package for social sciences (SPSS) version 15.0 (Chicago, Il, USA). *P* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Immunomodulation could result in either potentiating the immune response, a reaction favoured in cases of immune dysfunction, or it can be suppression of a reaction, a response that is favorable in organ transplant procedures and autoimmune diseases. The present study investigated whether the leaf extract of *V. globosa*, an endemic plant in the Philippines, could modify the immune response of ICR mice. This is a preliminary investigation on the potential of this plant species as an immunomodulator.

Spleen weight and body weight ratio (Figure 1) of mice treated with *V. globosa* extract did not produce any significant difference when compared with the control groups (untreated and vehicle) and with the immuno-suppressed group. However, it was notable that the dexamethasone-treated group (immunosuppressed group) exhibited lower spleen weight to body weight ratio compared to the other groups. The spleen serves a vital role in immune response as it is the site of antibody synthesis and it has been found to contain in its reserve, half of the body's monocytes that turns into dendritic cells and macrophages if the need arises. The spleen is also one of the centers of activity of the reticuloendothelial system. The reduction of its size is indicative of the decreased amount of immune regulatory activity. Thus,

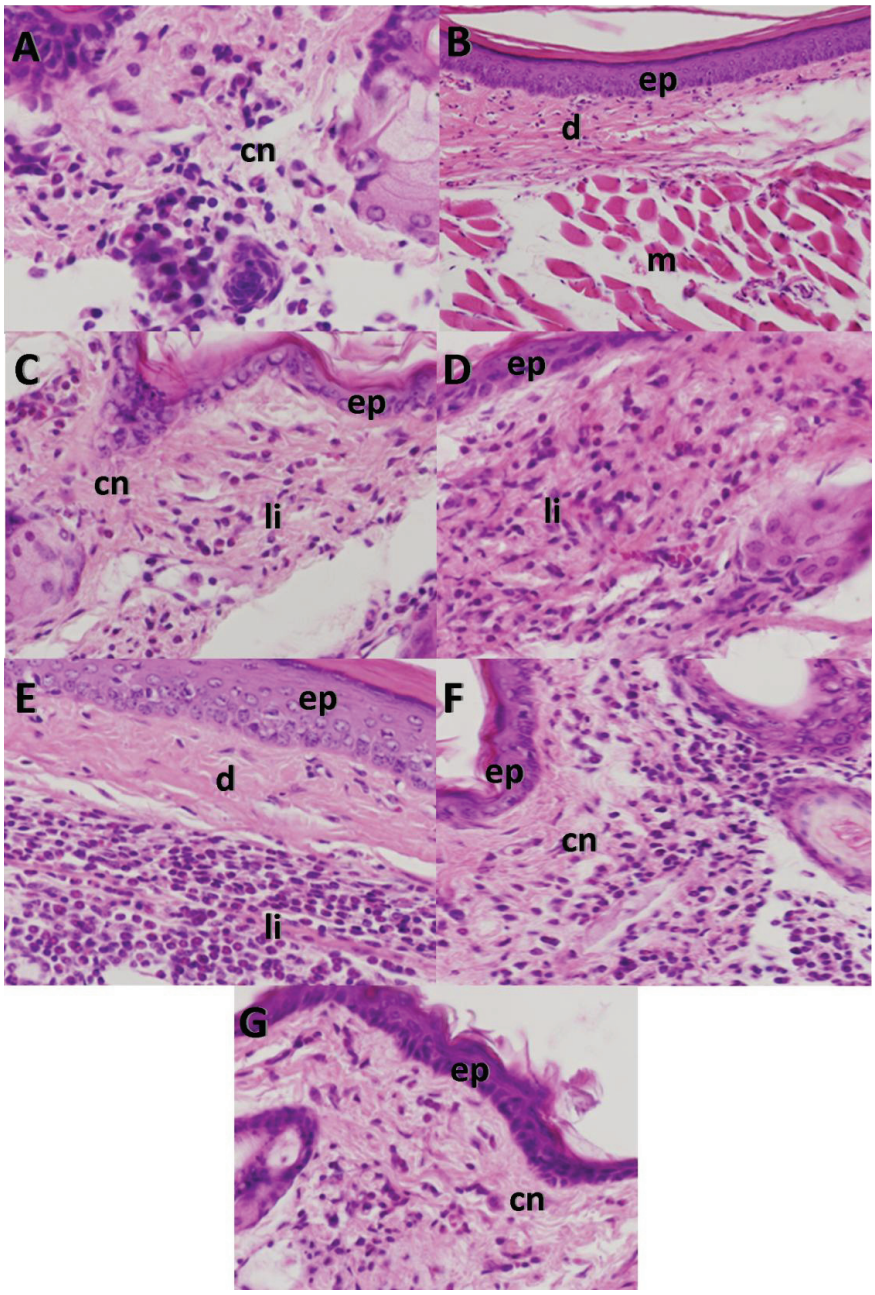

Figure 3. Photomicrographs of excised right hind footpad (day 8) where SRBC was intradermally injected at day 7 (Haematoxylin and Eosin stain). Leukocyte infiltration (li) in untreated (A) and treated mice for seven days with 0.2 mg/kg bw dexamethasone (B), phosphate buffered saline (C) and *V. globosa* ethanolic extract 0.5 mg/kg bw (D), 5 mg/kg bw (E), 50 mg/kg bw (F), and 100 mg/kg bw (G). Treatment with *V. globosa* extract induced remarkable inflammatory cell infiltration compared with the control groups which exhibited very minimal cellular infiltration. (ep) Epidermis, (d) dermis, (m) muscle, (cn) connective tissue.

higher weight indices suggest a higher number of leukocytes residing in the lymph nodes, which could further indicate active boosting of the immune regulatory system (Nishimoto et al., 2009).

A study by Dhabhar et al. (1999) mentioned that immunopotentiating agents promote endothelial “adhesivity” on leukocytes in lymph node compartments that result in a selective retention of leukocytes within lymph nodes. When inflammatory mediator signals act up, stressed animals have more leukocytes that transmigrate through the endothelial lining and infiltrate the site of inflammation. Thus, higher weight indices suggest a higher number

of leukocytes residing in the lymph nodes, suggesting in turn, active boosting of the immune regulatory system.

Delayed type hypersensitivity (DTH) response was then evaluated by calculating the edema index (Figure 2), indicated by the thickness of the paw of each mice injected with *V. globosa* and the control mice. Dexamethasone-injected mice showed the least amount of paw swelling, which was significantly different (p = 0.042) from the edema indices obtained from mice treated with 0.5 and 5 mg/kg bw *V. globosa*. More importantly, edema indices of mice treated with 5 mg/kg bw *V. globosa* were found to be significantly higher compared to those of the untreated, dexamethasone-treated, and 50 mg/kgbw *V. globosa*-treated.

Elevated DTH could be attributed to sensitized T-cells that, when challenged by an antigen sheep red blood cells (SRBC), are converted to lymphoblast attracting more scavenger cells to the site of reaction (Sharififar et al., 2009) or it could be due to activated macrophage function (Akerkar et al., 2009). There are a number of possible mechanisms for elevated DTH, namely activation of complements, releasing of mediators by activated mast cells, kinin, reactive oxygen or nitrogen species by arachidonic acid metabolites and pro-inflammatory cytokines (Manosroi et al., 2005). A high DTH response suggests influences of *V. globosa* leaf extract on any of these immunological mediators.

To validate the data obtained from the DTH reaction, the swollen paws were subjected to histopathological staining (HE) for examination of leukocyte infiltration. Tissue sections of the immunosuppressed group (Figure 3B) exhibited reduced or minimal hypertrophy as muscle cells are found in close proximity to the epidermis, as opposed to the thickened dermal layers in the extract-treated mice. This reinforces the findings in the DTH experiment, with the 5 mg/kg bw *V. globosa* treatment having the most inflammatory infiltrate in the histological sections. Figure 3D to G show the tissue sections of the mice treated with extract showing varying degrees of leukocyte infiltration. Compared with the immunosuppressed group, leukocyte infiltration is very pronounced in the tissue sections, supporting the inflammatory-enhancing effect of the *V. globosa* extract. DTH response is characterized by an immune-inflammatory reaction where activation of T cells by antigen presentation results in the secretion of various cytokines that induce Th1 cell proliferation and recruit macrophages into the inflamed area, promoting secretion of IL-12, TNF-α and IL-1 (Pinto et al., 2007).

The results for the first time demonstrated that the extract of *V. globosa* exert *in vivo* cellular immunomodulatory activities in a dose-dependent, biphasic manner. At 0.5 mg/kg bw, *V. globosa* may not have had enough active components to elicit a stable response but exhibited a pro-inflammatory response at 5 mg/kg bw. Immunostimulation at the low doses can be facilitated by mediating the main actors of celllular immunity, the T-cells, including the cytotoxic T cells and natural killer cells. Activated inflammatory response can kill tumors and produce many lymphocyte factors that increase and enhance macrophage phagocytosis and killing capacity (Lu et al., 2007; Pan et al., 2013). As seen in the results, proliferation of innate immunity response agents increased significantly. An effective pharmacological dose of 5 mg/kg bw is notable, due to its low concentration that is demonstrated to be sufficient to elicit a pro-inflammatory response. In higher doses however (50 and 100 mg/kg bw), the immune functions did not increase significantly and exhibited similar reactions to that of the immunosuppresive control dexamethasone. Suppression of DTH response and lack of immune cell proliferation suggests a parallel mechanism of action. This may indicate a threshold level of dosage effective for activating pro-inflammatory response and a possible shift of mechanism to an anti-inflammatory pathway.

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REFERENCES

Akerkar AS, Ponkshe CA, Indap MM (2009). Evaluation of immuno-modulatory activity of extracts from marine animals. Indian J. Med. 38:22-27.

Auttachoat W, Chitsomboona B, Peacheeb VL, Guob TL, White Jr KL (2004). Immunomodulation by Dok Din Daeng (*Aeginetia indica* Roxb.) extracts in female B6C3F1 mice: II. Int. Immunopharmacol. 4:1381–1390.

Canoy RJC, Lomanta JMJ, Ballesteros PM, Chun EAC, Dator R, Jacinto SD (2011). Cancer chemotherapeutic potential of endemic and indigenous plants of Kanawan, Morong, Bataan Province, Philippines. Asia Life Sci. 20(2):331-339.

Dhabhar FS, McEwen BS (1999). Enhancing versus suppressive effects of stress hormones on skin immune function. PNAS. 96:1059-1064.

Friedl R, Moeslinger T, Kopp B,Spieckermann PG (2001). Stimulation of nitric oxide synthesis by the aqueous extract of *Panax* ginseng root in RAW 264.7 cells. Br. J. Pharmacol. 134:1663–1670.

Hong CH, Hur SK, Oh OJ, Kim SS, Nam KA, Lee SK (2002). Evaluation of natural products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in cultured mouse macrophage cells. J. Ethnopharmacol. 83:153–159.

Im SA, Kim K, Lee CK (2006). Immunomodulatory activity of polysaccharides isolated from *Salicornia herbacea*. Int. Immunopharmacol. 6:1451–1458.

Jacinto SDJ, Ramos EF, Siguan APT, Canoy RJC (2011). Determining the antioxidant property of plant extracts: A laboratory exercise. Asian J. Biol. Educ. 5:22-25.

Lu Y, Fan J, Zhao YP, Chen SY, Zheng XD, Yin YM, Fu CX (2007). Immunomodulatory activity of aqueous extract of *Actinidia macrosperma.* Asia Pac. J. Clin. Nutr. 16:261-265.

Manosroi A, Sarapchanchotiwitthaya A, Manosroi J (2005). *In vivo* immunomodulating activity of wood extracts from Clausena excavate Burm. f. J. Ethnopharmacol. 102:5-9.

Nishimoto S, Kanda K, Okabe M, Akiyama K, Kakinuma Y, Sugahara T (2009). In: abnormal response induced by pesticides on mammalian immune system. Vol 2: Interdisciplinary Studies on Environmental Chemistry Vol. 2: Environmental Research in Asia for Establishing a Scientist's Network pp. 211-218.

Olaleye SB, Oke JM, Etu AK, Omotosho IO, Elegbe RA (2004). Anti-oxidant and anti-inflammatory properties of a flavonoid fraction from the leaves of *Voacanga africana*. Niger. J. Physiol. Sci. 19:69-76.

Pan DD, Wu Z, Liu J, Cao XY, Zeng XQ (2013). Immunomodulatory and hypoallergenic properties of milk protein hydrolysates in ICR mice. J. Dairy Sci. 96:4958-4964.

Pinto A, Rego GC, Siquerra A, Cardoso CC, Reis P, Marques E, Coelho MG, Sabino KC (2007). Immunosuppressive effects of *Echinodorusmacrophyllus* aqueous extract. J. Ethnopharmacol. 111:435-439.

Sharififar F, Pournourmohammadi S, Arabnejad M (2009). Immunomodulatory activity of aqueous extract of *Achillea wilhemsii* C. Koch in mice. Indian J. Exp. Biol. 47:668-671.

Tan PV, Penlap VB, Nyasse B, Nguemo JDB (2000). Anti-ulcer actions of the bark methanol extract of *Voacanga africana* in different experimental ulcer models in rats. J. Ethnopharmacol. 73:423-428.