International Scholars Journals

African Journal of Immunology Research ISSN 9431-5833 Vol. 4 (7), pp. 277-284, July, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Modulatory roles of water extract on various cellular events managed by macrophages and monocytes

Byung Hun Kim [1], Jaehwi Lee [2], Kook Hyun Kim[3] and Jae Youl Cho[1]*

[1]School of Bioscience and Biotechnology and Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea.
[2]College of Pharmacy, Chung-Ang University, Chuncheon 156-756, Korea.
[3]Gapi-Korea, Seoul 153-803, Korea.

Accepted 03 March, 2017

Tabebuia **sp. (Bignoniaceae) is representative traditional herbal plants, found in tropical rain forest areas throughout Central and South America. These plants have been mostly used as a folk medicine to treat bacterial infection, blood coagulation, cancer and inflammatory diseases. In this study, we aimed to demonstrate the modulatory role of *Tabebuia avellanedae* in various macrophage- or monocyte-mediated immune responses using its water extract (Ta-WE). Ta-WE was able to up-regulate cell-cell interaction by decreasing the migration of cells and by enhancing CD29-mediated cell-cell adhesion, and the surface levels of adhesion molecules (CD18, CD29, and CD82) and costimulatory molecules (CD80 and CD86) linked to macrophage stimulation as seen in up-regulation of ROS release. In addition, this fraction also suppressed an alteration in the membrane levels of macrophages such as phagocytic uptake and morphological changes. Therefore, these results suggest that the water extract of *T. avellanedae* can strongly modulate an ability of macrophages and monocytes to manage immune cell-cell interaction in host defence system.**

Key words: *Tabebuia avellanedae* (Bignoniaceae), water extract, macrophages, monocytes, cell-to-cell interaction.

INTRODUCTION

Tabebuia avellanedae Lorentz ex Griseb., a Bignoniaceae, is a representative herbal plant used for treating various skin inflammatory diseases including eczema, psoriasis, fungal infections and even skin cancers for over 1,000 years (Casinovi et al., 1963; de Santana et al., 1968; Woo and Choi, 2005). Recent pharmacological studies have also increased the under-standing of its therapeutic efficacies including astringent, anti-inflammatory, antibacterial, antifungal, diuretic, anti-coagulational and laxative properties as well as an anti-cancer effect (Choi et al., 2003; Machado et al., 2003; Awale et al., 2005; Bohler et al., 2008). So far, the variety of pharmacologically active ingredients with such biological activities from the plant such as naphthoquinones, furanonaphthoquinones, anthrax-quinones, benzoic acid derivatives, benzaldehyde derivatives, iridoids, coumarins and flavonoids (Kreher et al., 1988; Ueda et al., 1994; Choi et al., 2003; Machado et al., 2003; Pereira et al., 2006; Kim et al., 2007; Kung et al., 2007) have been identified.

Previously, we reported that the water extract of this plant can suppress several inflammatory responses including lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin (PG)E_2 production in macrophage-like RAW264.7 cells and arachidonic acid-treated mouse ear edema (Byeon et al., 2008). In addition, other groups proposed that aqueous extract of this plant can display anti-nociceptive and anti-edematogenic properties, linked to its anti-inflammatory and analgesic activities (de Miranda et al., 2001; Awale et al., 2005). In this study, we aimed to investigate the modulatory roles of the water extract on various cellular events managed by macrophages and monocytes, major

*Corresponding author. E-mail: jaecho@kangwon.ac.kr. Tel: +82-33-250-6488. Fax: +82-33-241-6480.

inflammatory cells.

MATERIALS AND METHODS

Materials

The water extract from the inner bark of *T. avellanedae*, was prepared as previously reported (de Miranda et al., 2001; Byeon et al., 2008). FITC-dextran, Sodium nitroprusside (SNP), (3 - 4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole (MTT) and LPS (*Escherichia coli* 0111:B4) were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum and RPMI1640 were obtained from GIBCO (Grand Island, NY). RAW264.7 and U937 cells were purchased from ATCC (Rockville, MD). All other chemicals were of Sigma grade. Aggregation-inducing antibodies to CD29 (MEM 101A), CD43 (161-46) were used for these studies (Diaz et al., 1997; Cho et al., 2003). Antibodies to CD69, CD80, CD82, and CD86 were purchased from BD Bioscience (San Jose, CA).

Cell culture

RAW264.7, a murine macrophage cell line and U937 cells, a human promonocytic cell line, were maintained in RPMI1640 supplemented with 100 U/ml of penicillin, 100 µg/ml of streptomycin and 10% fetal bovine serum. Cells were grown at 37°C and 5% CO_2 in humidified air.

Quantitative homotypic cell-cell assay

For quantitative aggregation assays (Diaz et al., 1997), 50 µl of cells were resuspended in RPMI1640 medium supplemented 10% FCS at 2×10^6 cells/ml and plated in a flat bottom microwell with an equal volume of various concentrations of test compounds. The plates were cultured at 37°C for 30 to 60 min. Twenty microliter cell suspension was placed into round bottom microplate well followed with 20 µl of mAb solution added. Cells were then incubated for a further 3 h in the same conditions. Thereafter, the numbers of unaggregated and total cells were counted in a haemocytometer and percentage of cells in aggregates was determined by the following equation:

% of cells in aggregates = [total cells-free cells/total cells] × 100.

In some experiments, the results were expressed as % of control, showing aggregation in presence of inhibitors as a % of aggregation in the presence of aggregating mAb only.

Cell-extracellular matrix protein (fibronectin) adhesion assay

For the cell-fibronectin adhesion assay, U937 cells (5×10^5 cells/well) were seeded on a fibronectin (50 µg/ml)-coated plate and incubated for 3 h (Larrucea et al., 1998). After removing unbound cells with PBS, the attached cells were treated with 0.1% of crystal violet for 15 min. The OD value at 540 nm was measured by a Bio-TEK EL800 reader (Winooski, VT).

Cell migration assay

RAW264.7 cells (2×10^5 cells/ml) were incubated with indicated concentrations of Ta-WE for 30 min. After scratching the cultured cells with a pipette, the cells were further incubated for 12 h. The images of the cells in culture were obtained using an inverted phase contrast microscope attached to a video camera.

Flow cytometric analysis

Surface levels of CD18, CD29, CD69, CD80, CD82, and CD86 on U937 or RAW264.7 cells were determined by flow cytometric analysis as reported previously (Cho et al., 2001). The fluorescence level of stained cells was analyzed by a FACScan device (Becton-Dickinson, San Jose, CA).

ROS determination

The level of intracellular ROS was measured by determining fluorescence levels generated from oxidation of the probe DHR123 (Bai et al., 2005). Briefly, 5×10^5 cells/well were incubated with Ta-WE for 30 min and then with SNP (125 µM) for an additional 6 h. After a final incubation with 2.5 µM DHR123 for 1 h, the intracellular ROS level was determined using flow cytometry.

LPS-induced morphological change

RAW264.7 cells (1×10^5) were incubated with indicated concentrations of Ta-WE in the presence or absence of LPS for 24 h. The images of the cells in culture were obtained using an inverted phase contrast microscope attached to a video camera.

Phagocytic uptake

To measure the phagocytic activity of RAW264.7 cells, a previously reported method was used with slight modifications (Lee et al., 2007). RAW 264.7 cells (1×10^6 cells/ml) were preincubated with or without Ta-WE for 30 min, and further incubated for 6 h. Finally, the cells were further incubated with FITC-dextran (1 mg/ml) for 30 min at 37°C. The incubation was stopped by addition of 2 ml of ice-cold PBS, and the cells were washed four times with cold PBS. After fixing the cells with 3.7% formaldehyde, phagocytic uptake was analyzed using a FACScan device (Becton-Dickinson, San Jose, CA, USA).

Statistical analysis

The student's *t*-test and one-way ANOVA were used to determine the statistical significance between values of the various experimental and control groups. Data (Figures 1, 2A, 3A, 3C and 4B) expressed as means ± standard errors (SEM) are taken from at least three independent experiments performed in triplicate (Figures 1, 2A, 3A, 3C and 4B). The data (Figures 2B, 3B, 4A and 4C) are representative of three different experiments with similar results. P values of 0.05 or less were considered to be statistically significant.

RESULTS AND DISCUSSION

Taheebo has been used in South American area for a long time to treat skin inflammatory diseases such as eczema and psoriasis and anti-fungal diseases such as candidiasis, pyorrhea and athlete's foot (Burnett and Thomson, 1968). Recently, since its anti-cancer activity has been demonstrated, water extract of the inner bark of *T. avellanedae* is now being directly used to cancer

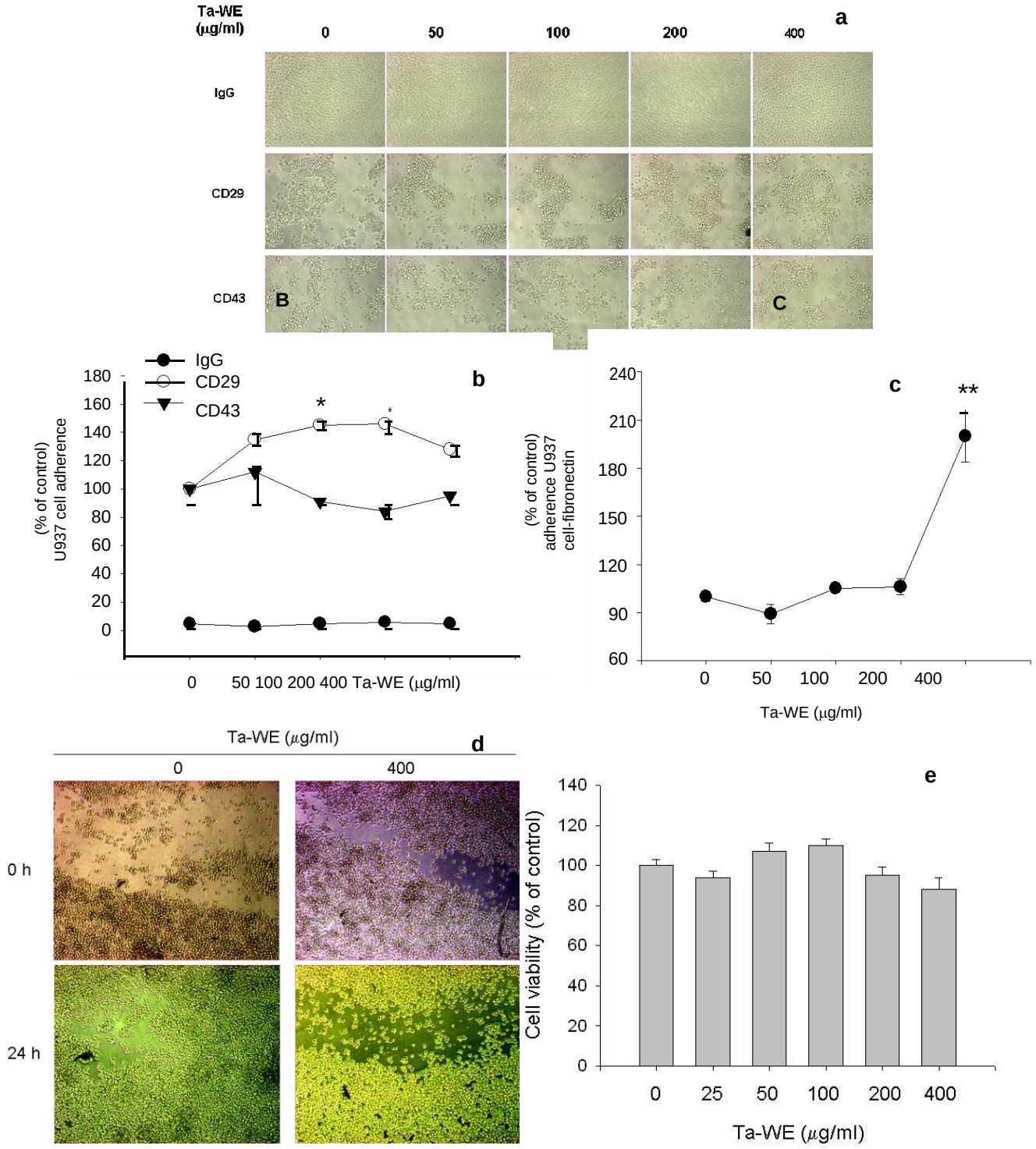

Figure 1. Effect of Ta-WE on cell-cell and cell-fibronectin adhesion. (A and B) U937 cells (1×10^6 cells/ml) pretreated with Ta-WE were incubated either in the presence or absence of pro-aggregative (agonistic) antibodies (1 µg/ml each) to CD29 (MEM 101A), CD98 (ANH-18) or CD43 (161-46) for 3 h. Images of the cells in culture (A) were obtained using an inverted phase contrast microscope attached to a video camera. Quantitative analysis of cell-cell clusters (B) was assessed by quantitative cell-cell adhesion assay as described in Materials and methods. (C) U937 cells (1×10^6 cells/ml) pretreated with Ta-WE were seeded on fibronectin (50 µg/ml)-coated plates and further incubated for 3 h. Attached cells were determined by crystal violet assay, as described in Materials and Methods. (D) RAW24.7 cells (1×10^5 cells/ml) pre-treated with Ta-WE were scratched with pipette and further incubated for 24 h. (E) RAW264.7 cells (1×10^6 cells/ml) were treated with Ta-WE for 24 h. Cell viability was determined by the MTT assay Data (B, C, D and E) represent mean ± SEM of three independent observations performed in triplicate. *: $p < 0.05$, **: $p < 0.01$ compared to control.

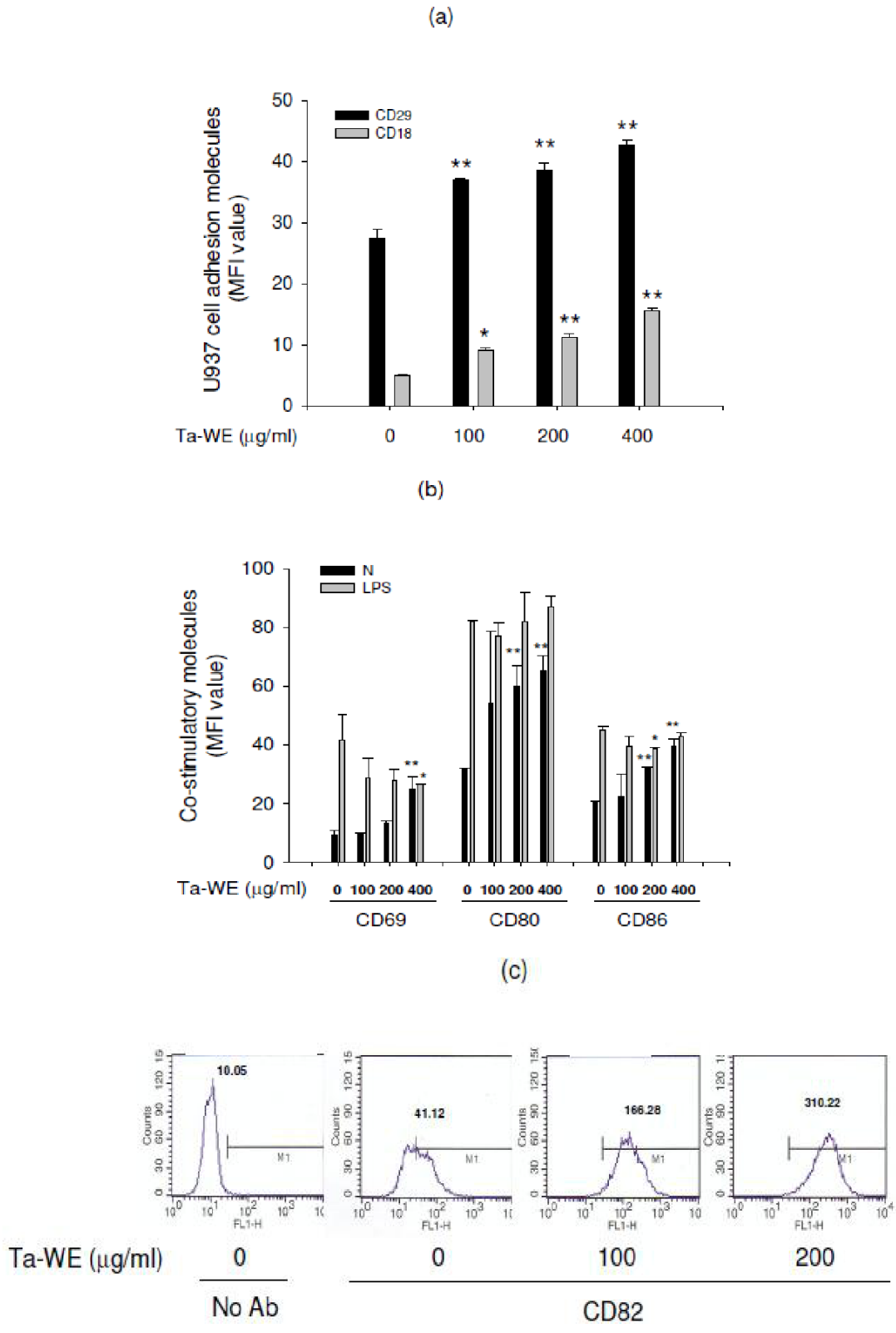

Figure 2. Effects of Ta-WE on surface level of adhesion and costimulatory molecules. (A and C) U937 cells (1×10^6 cells/ml) were treated with Ta-WE for 12 h. The surface levels of CD29, CD18, and CD82 in U937 cells were determined by flow cytometry as described in Materials and Methods. (B) RAW264.7 cells (1×10^6 cells/ml) were treated with Ta-WE for 12 h in the presence or absence of LPS (1 μg/ml). The surface levels of CD69, CD80, and CD86 were determined by flow cytometry. Data represent mean ± SEM of three independent observations performed in triplicate. *: $p < 0.05$ compared to control.

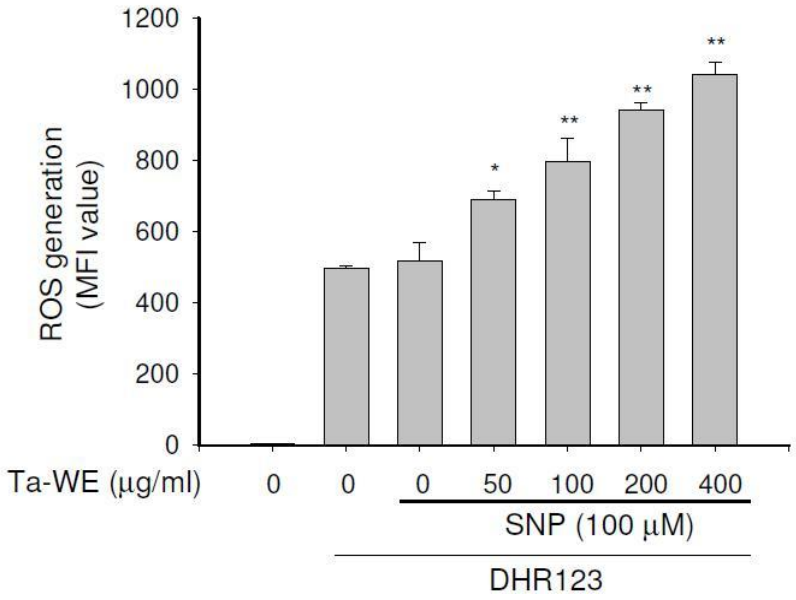

Figure 3. Effect of Ta-WE on the production of ROS in SNP-treated RAW264.7 cells. RAW264.7 cells (1 x 10^6) were pretreated with various concentrations of Ta-WE in the presence or absence of SNP (100 μM) for 30 min. The level of generated ROS was determined by flow cytometric analysis as described in Materials and Methods. Data represent mean ± SEM of three independent observations performed in triplicate. *: $p < 0.05$ and **: $p < 0.01$ compared to control.

patients (Mukherjee et al., 2009). Previously, we have demonstrated that the anti-inflammatory effect of this medicinal plant could be due to blockade of NO and PGE_2 generation in LPS-activated macrophage-like RAW264.7 cells (Byeon et al., 2008). In this study, we tried to further characterize the modulatory effect of macrophage and monocyte functions by Ta-WE to understand its immunoregulatory potentials.

Ta-WE significantly up-regulated: 1) The functional activity of CD29 under treatment of its function-activating antibody and fibronectin, an extracellular matrix protein (Martin et al., 1998) interacting with CD29 (Figure 1 and 2). The normal surface expression levels of adhesion molecules (CD29 and CD18) (Figure 2A) and costimulatory molecules (CD80, CD82 and CD86) (Figures 2B and 2C); and 3) The generation of ROS induced with SNP (100 μM) (Figure 3). In contrast, this herb negatively regulated; 1) Normal cell migration events observed after scratching RAW264.7 cells with pipette (Figure 1D); 2) LPS-induced morphological changes (Figure 4A); and 3) Phagocytic uptake of FITC-labelled dextran (Figure 4). Therefore, these results suggest that the extract seems to clearly affect cellular responses occurred in macrophages and monocytes.

Immune responses are mediated by various types of immune cells such as antigen presenting cells (APC) (such as dendritic cells, macrophages, and monocytes), lymphocytes, neutrophils and NK cells (Friedl et al., 2005). One of recent important issues is how to interact between these immune cells performing their immunological activity. Some cases (e.g. between APC and lymphocytes) are now greatly understood on their interaction mechanism (Friedl et al., 2005), but others (e.g. between macrophages and NK cells) are still unelucidated yet. Such an interaction is known to be mediated by adhesion molecules such as β1-integrins (CD29) and β2-integrins (CD18) (Cho et al., 2001). The activation of these molecules could lead the cells to close interaction between cells to be immunologically up-regulated (Ohkawara et al., 2000; Gasic et al., 2004). By the reason, various soluble factors such as cytokines and inflammatory mediators can be more produced and subsequent tremendous immune responses are maintained (Brown et al., 2003; Sabatos et al., 2008). The facts that; 1) Ta-WE treatment strongly enhanced the cell-cell adhesion events up to 35 to 40% at 50 to 400 μg/ml (Figure 1B); 2) U937 cell-fibronectin adhesionevent mediated by activated CD29 was also strongly up-regulated by 2-fold at 400 μg/ml (Figure 1C); 3) The migration of scratched cells has been completely blocked; 4) Surface expression levels of two representative adhesion molecules (CD29 and CD18) as well as CD82 were increased up to 2-fold level at 400 μg/ml (Figures 2A and C); and Ta-WE simply maintained the cluster formation of RAW264.7 cells even after LPS treatment (Figure 4A) seem to indicate an assumption that this extract may play an important role in up-regulating cell-cell interaction through stimulation of cell

Fig. 4

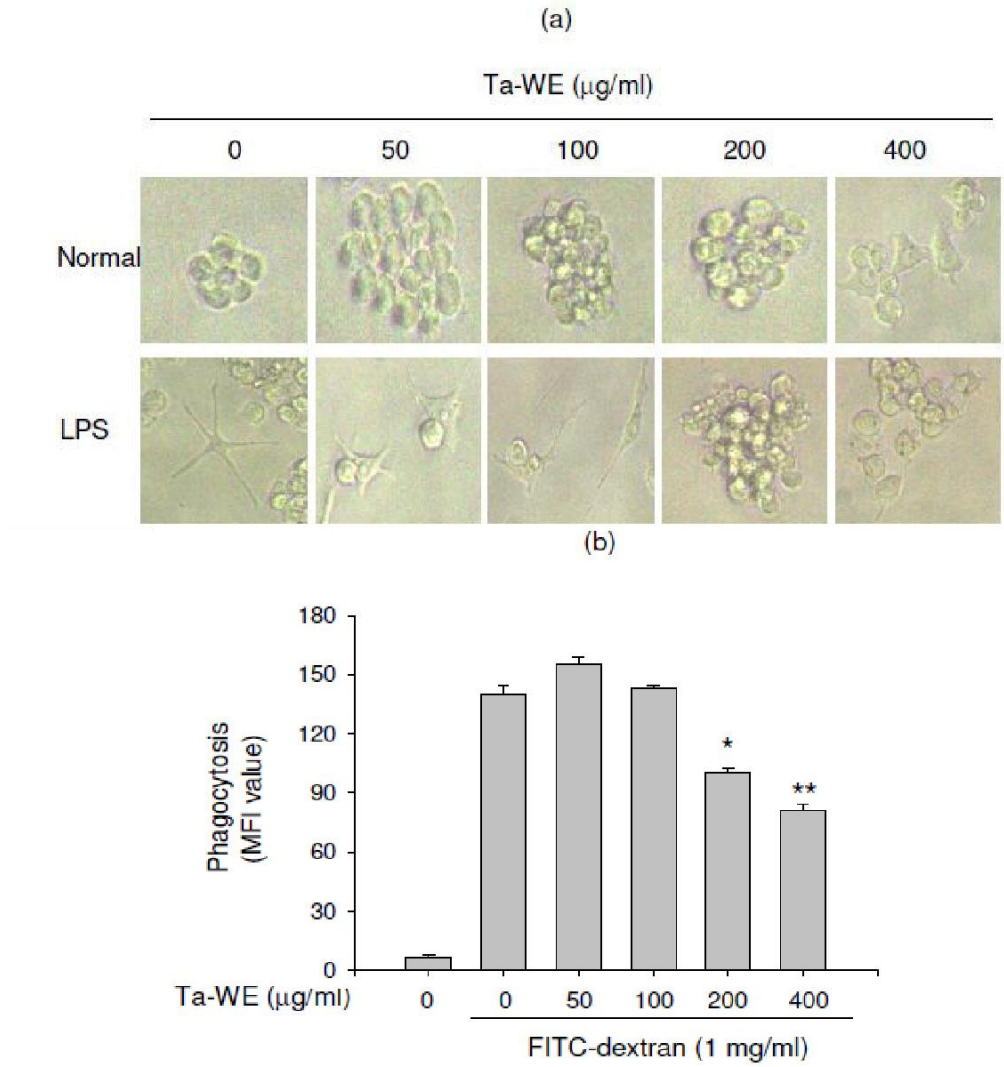

Figure 4. Effect of Ta-WE on the alteration of membrane levels in RAW264.7 cells. (A) in RAW264.7 cells (1×10^5) were incubated with indicated concentrations of Ta-WE in the presence or absence of LPS (1 μg/ml) for 12 h. The images of the cells (in culture were obtained using an inverted phase contrast microscope attached to a video camera. (B) RAW264.7 cells (2×10^5 cells/ml), pretreated with Ta-WE, were stimulated with FITC-dextran (1 mg/ml) for 6 h. The extent of the phagocytic uptake was determined by flow cytometric analysis, as described in Materials and Methods. Data (B) represent mean ± SEM of three independent observations performed in triplicate. *: $p < 0.05$, **: $p < 0.01$ compared to control.

clustering formation. Furthermore, the enhancement of cell-cell adhesion seemed to block the migration of macrophages or monocytes, since scratched area in Ta-WE-treated cells was not filled by the cells. We have not yet measured further immunological responses such as cytokine production under cell-cell adhesion conditions. However, the facts that CD29 activation is capable of enhancing ROS generation in U937 cells (Wang et al., 2000; Hunt et al., 2002), and that Ta-WE strongly increased both CD29 activation (Figure 1) and ROS generation (Figure 3) may imply the up-regulatory roles of this extract on immune cell-cell interaction. Furthermore, the effect on the expression of costimulatory molecules such as CD69, CD80 and CD86 in RAW264.7 cells also appears to suggest that Ta-WE may up-regulate the interaction between APC and T lymphocytes, since these costimulatory molecules are involved in cellular interaction between these cells (Dong et al., 2008). Detailed studies will be further followed to prove this possibility.

The major active compounds identified in the hot water extract include naphthoquinones, uranonaphthoquinones, anthraquinones, benzoic acid derivatives, benzaldehyde derivatives, iridoids, coumarins, and flavonoids

(Kreher et al., 1988; Ueda et al., 1994; Choi et al., 2003; Machado et al., 2003; Pereira et al., 2006; Kim et al., 2007; Kung et al., 2007). So far, regulatory effects of these compounds on macrophage functions are not fully elucidated, while anti-cancer and anti-microbacterial effects of these compounds were reported previously (Tobe et al., 1982; Mukherjee et al., 2009). Therefore, which components can be involved in such modulatory activities on macrophage- or monocyte-mediated cell-cell interaction should be followed in the next project.

In summary, we have demonstrated that the water extract of *T. avellanedae* is able to up-regulate cell-cell interaction by decreasing the migration of cells and by enhancing the functional activation of adhesion molecules such as CD29, and the surface levels of their proteins (CD18, CD29, and CD82) and costimulatory molecules (CD80 and CD86) linked to macrophage stimulation such as increased ROS release. In addition, this fraction suppressed an alteration in the membrane structure levels such as phagocytic uptake and morphological changes. Considering all the data, these results suggest that water extract can impose an ability of macrophages and monocytes to modulate immune cell-cell interaction in host immune responses.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Education, Science Technology (MEST) and Korea Industrial Technology Foundation (KOTEF) through the Human Resource Training Project for Regional Innovation.

REFERENCES

Awale S, Kawakami T, Tezuka Y, Ueda JY, Tanaka K, Kadota S (2005). Nitric oxide (NO) production inhibitory constituents of *Tabebuia avellanedae* from Brazil. Chem. Pharm. Bull. (Tokyo) 53: 710-713.

Bai, XC, Lu D, Liu AL, Zhang ZM, Li XM, Zou ZP, Zeng WS, Cheng BL, Luo SQ (2005). Reactive oxygen species stimulates receptor activator of NF-kappaB ligand expression in osteoblast. J. Biol. Chem. 280: 17497-17506.

Bohler, T, Nolting J, Gurragchaa P, Lupescu A, Neumayer HH, Budde K, Kamar N, Klupp J (2008). *Tabebuia avellanedae* extracts inhibit IL-2-independent T-lymphocyte activation and proliferation. Transpl. Immunol. 18: 319-323.

Brown, RA, Lever R, Jones NA, Page CP (2003). Effects of heparin and related molecules upon neutrophil aggregation and elastase release in vitro. Br. J. Pharmacol. 139: 845-853.

Burnett, AR, Thomson RH (1968). Lapachol. Chem. Ind. 50: 1771.

Byeon, SE, Chung JY, Lee YG, Kim BH, Kim KH, Cho JY (2008). In vitro and in vivo anti-inflammatory effects of taheebo, a water extract from the inner bark of *Tabebuia avellanedae*. J. Ethnopharmacol. 119: 145-152.

Casinovi, CG, Marini Bettolo GB, Limaog DA, Daliamaia ME, D AI (1963). on Quinones Isolated from the Wood of *Tabebuia Avellanedae* Lor. Ex Griseb.. Rend. Ist. Sup. Sanit. 26: 5-10.

Cho, JY, Chain BM, Vives J, Horejsi V, Katz DR (2003). Regulation of CD43-induced U937 homotypic aggregation. Exp. Cell. Res. 290: 155-167.

Cho, JY, Fox DA, Horejsi V, Sagawa K, Skubitz KM, Katz DR, Chain B (2001). The functional interactions between CD98, beta1-integrins, and CD147 in the induction of U937 homotypic aggregation. Blood 98: 374-382.

Choi, BT, Cheong J, Choi YH (2003). beta-Lapachone-induced apoptosis is associated with activation of caspase-3 and inactivation of NF-kappaB in human colon cancer HCT-116 cells. Anticancer Drugs 14: 845-850.

de Miranda, FG, Vilar JC, Alves IA, Cavalcanti SC, Antoniolli AR (2001). Antinociceptive and antiedematogenic properties and acute toxicity of *Tabebuia avellanedae* Lor. ex Griseb. inner bark aqueous extract. BMC Pharmacol. 1: 6.

de Santana, CF, de Lima O, d' Albuquerque IL, Lacerda AL, Martins DG (1968). Antitumoral and toxicological properties of extracts of bark and various wood components of Pau d'arco (*Tabebuia avellanedae*). Rev. Inst. Antibiot. (Recife) 8: 89-94.

Diaz, LA, Jr., Friedman AW, He X, Kuick RD, Hanash SM, Fox DA (1997). Monocyte-dependent regulation of T lymphocyte activation through CD98. Int. Immunol. 9: 1221-1231.

Dong, L, Wang S, Chen M, Li H, Bi W (2008). The activation of macrophage and upregulation of CD40 costimulatory molecule in lipopolysaccharide-induced acute lung injury. J. Biomed. Biotechnol. 852571.

Friedl, P, den Boer AT, Gunzer M (2005). Tuning immune responses: diversity and adaptation of the immunological synapse. Nat. Rev. Immunol. 5: 532-545.

Gasic, S, Vucevic D, Popovic P, Vasilijic S, Colic M (2004). R-MC46 monoclonal antibody stimulates adhesion and phagocytosis by rat macrophages. Vojnosanit. Pregl. 61: 581-588.

Hunt, MJ, Aru GM, Hayden MR, Moore CK, Hoit BD, Tyagi SC (2002). Induction of oxidative stress and disintegrin metalloproteinase in human heart end-stage failure. Am. J. Physiol. Lung Cell Mol. Physiol. 283: L239-245.

Kim, SO, Kwon JI, Jeong YK, Kim GY, Kim ND, Choi YH (2007). Induction of Egr-1 is associated with anti-metastatic and anti-invasive ability of beta-lapachone in human hepatocarcinoma cells. Biosci. Biotechnol. Biochem. 71: 2169-2176.

Kreher, B, Lotter H, Cordell GA, Wagner H (1988). New Furanonaphthoquinones and other Constituents of *Tabebuia avellanedae* and their Immunomodulating Activities in vitro. Planta Med. 54: 562-563.

Kung, HN, Chien CL, Chau GY, Don MJ, Lu KS, Chau YP (2007). Involvement of NO/cGMP signaling in the apoptotic and anti-angiogenic effects of beta-lapachone on endothelial cells in vitro. J. Cell Physiol. 211: 522-532.

Larrucea, S, Gonzalez-Rubio C, Cambronero R, Ballou B, Bonay P, Lopez-Granados E, Bouvet P, Fontan G, Fresno M, Lopez-Trascasa M (1998). Cellular adhesion mediated by factor J, a complement inhibitor. Evidence for nucleolin involvement. J. Biol. Chem. 273: 31718-31725.

Lee, YG, Kim JY, Lee JY, Byeon SE, Hong EK, Lee J, Rhee MH, Park HJ, Cho JY (2007). Regulatory effects of *Codonopsis lanceolata* on macrophage-mediated immune responses. J. Ethnopharmacol. 112: 180-188.

Machado, TB, Pinto AV, Pinto MC, Leal IC, Silva MG, Amaral AC, Kuster RM, Netto-dosSantos KR (2003). In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents 21: 279-284.

Martin, CA, el-Sabban ME, Zhao L, Burakoff R, Homaidan FR (1998). Adhesion and cytosolic dye transfer between macrophages and intestinal epithelial cells. Cell Adhes. Commun. 5: 83-95.

Mukherjee, B, Telang N, Wong GY (2009). Growth inhibition of estrogen receptor positive human breast cancer cells by Taheebo from the inner bark of *Tabebuia avellandae* tree. Int. J. Mol. Med. 24: 253-260.

Ohkawara, Y, Tamura G, Iwasaki T, Tanaka A, Kikuchi T, Shirato K (2000). Activation and transforming growth factor-beta production in eosinophils by hyaluronan. Am. J. Respir. Cell Mol. Biol. 23: 444-451.

Pereira, EM, Machado Tde B, Leal IC, Jesus DM, Damaso CR, Pinto AV, Giambiagi-deMarval M, Kuster RM, Santos KR (2006). *Tabebuia avellanedae* naphthoquinones: activity against methicillin-resistant staphylococcal strains, cytotoxic activity and in vivo dermal irritability analysis. Ann. Clin. Microbiol. Antimicrob. 5: 5.

Sabatos, CA, Doh J, Chakravarti S, Friedman RS, Pandurangi PG,

Tooley AJ, Krummel MF (2008). A synaptic basis for paracrine interleukin-2 signaling during homotypic T cell interaction. Immunity 29: 238-248.

Tobe, H, Yoshimoto A, Ishikura T, Naganawa H, Takeuchi T, Umezawa H (1982) New anthracyclinone metabolites from two blocked mutants of *Streptomyces galilaeus* MA144-M1. J. Antibiot. (Tokyo) 35: 1641-1645.

Ueda S, Umemura T, Dohguchi K, Matsuzaki T, Tokuda H, Nishino H, Iwashima A (1994). Production of anti-tumour-promoting furanonaphthoquinones in *Tabebuia avellanedae* cell cultures. Phytochemistry 36: 323-325.

Wang, JJ, Liu TY, Yin PH, Wu CW, Chern YT, Chi CW (2000). Adamantyl maleimide induced changes in adhesion molecules and ROS are involved in apoptosis of human gastric cancer cells. Anticancer Res. 20: 3067-3073.

Woo, HJ, Choi YH (2005). Growth inhibition of A549 human lung carcinoma cells by beta-lapachone through induction of apoptosis and inhibition of telomerase activity. Int. J. Oncol. 26: 1017-1023.