Journal of Medicinal Plants Research Vol. 5(8), pp. 1418-1424, 18 April, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

The inhibitory effect of *Opuntia humifusa* Raf. ethyl acetate extract on platelet aggregation

Won-Jun Oh[1], Mehari Endale[1], Ji-Young Park[1], Yi-Seong Kwak[2], Suk Kim[3], Gon-Sup Kim[3*] and Man Hee Rhee[1*]

[1]College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Republic of Korea.
[2]Korea Ginseng Corporation Central Research Institute, Daejeon 305-805, Republic of Korea.
[3]College of Veterinary Medicine, Gyeongsang National University, Jinju 302-305, Republic of Korea.

Accepted 1 November, 2010

This study was designed to investigate the activity of ethyl acetate extract from *Opuntia humifusa* Raf. (OH-EAE) in ligand-activated platelet aggregation. Platelet aggregation was induced either by ADP, a potent agonist to platelet G protein-coupled P2Y receptor, by collagen, a potent ligand that activates platelet integrin α2β1 and glycoprotein VI, or thrombin, a platelet protease-activated receptors subtype I and IV. The OH-EAE inhibited platelet aggregation induced by ADP (10 μM) in a dose dependent manner. In addition, OH-EAE significantly and dose-dependently inhibited collagen (2.5 μg/ml)- and thrombin (0.05 U/ml)-induced platelet aggregation. Moreover, the downstream signaling analysis revealed that the extract potently inhibited ADP-induced intracellular calcium mobilization ($[Ca^{2+}]_i$). Since degranulation is a marker of platelet activation, the extract effect on the dense granule secretary activity was evaluated. As such, OH-EAE strongly suppressed ADP-induced ATP release. This preliminary result suggests that *O. humifusa* may be taken as a candidate lead natural compound to be considered in the search for natural products with beneficial effects on aberrant platelet activation mediated cardiovascular disorders.

Key words: *Opuntia humifusa* extract, calcium, rat platelets, platelet agonists.

INTRODUCTION

It is well known that platelets play an important role in the physiology of primary hemostasis and in pathophysiological processes such as thrombosis (Shattil et al., 1998; Stouffer and Smyth, 2003). Aberrant intravascular thrombosis is the main cause of a wide variety of cardiovascular diseases (Grenache et al., 2003; Huo and Ley, 2004).

Upon the activation of a platelet receptor on plasma membrane, bioactive substances (e.g. calcium, growth factor, and aggregation-related secretory biomolecules in granules are released in an energy-dependent process that requires ionized calcium (Leclerc, 2002; Savage et al., 2001). The signaling of these endogenous and exogenous molecules is the main factor of platelet aggregation (Huo and Ley, 2004). *In vitro* platelet aggregation can be induced by platelet-activating ligands, such as ADP, collagen, and thrombin. ADP can mainly bind to G protein-coupled P2Y1 receptor and activates phospholipase C, and thus resulting in the elevation of intracellular calcium concentration $[Ca^{2+}]_i$. Collagen, the subendothelial matrix, can bind to integrin α2β1 and glycoprotein VI (GP VI). GP VI and α2β1 binding to collagen lead to an increase in cytosolic calcium levels and protein kinase C activation. Thrombin, a serine protease, also takes a critical role in both coagulation cascade and platelet activation. The activation of platelet by thrombin is mediated through two protease-activating receptors (PAR), PAR 1 and PAR 4, belonging to G protein-coupled receptors. Both PAR1 and PAR4 couple

*Corresponding author. E-mail: rheemh@knu.ac.kr, gonskim@gnu.ac.kr. Tel: +82-53-950-5967, +82-55-751-5823. Fax: +82-53-950-5955, +82-55-751-5803.

Abbreviations: OH-EAE, Ethyl acetate extract from *Opuntia humifusa* Raf.; **GP VI,** glycoprotein VI; **PLCβ,** Phospholipase Cβ; **DAG,** diacylglycerol; **PKC,** protein kinase C; **PAR,** Protease-activated receptor; **ATP**, Adenosine triphosphate.

to phospholipase Cβ (PLCβ) via Gq in human platelets. Upon activation, PLCβ hydrolyses phosphatidylinositol 4, 5-bisphosphate to inositol-3-phosphate, which contributes to calcium release from internal stores, and diacylglycerol (DAG), which activates protein kinase C (PKC). PAR1 and PAR4 also couple to G12/13 to activate Rho/Rho kinase (Woulfe, 2005). The activation of downstream signaling by these ligands leads to shape change and granule secretion. Thus, the activation of integrin $\alpha_{IIb}\beta_3$ results in complete platelet aggregation.

On the other hand, *in vivo* platelet aggregation can be a cause of serious cardiovascular diseases, including atherosclerosis, ischemia, thrombosis, infarction, stroke (Huo and Ley, 2004). On the other hand, the search for natural products with the potential of having anti-platelet activity while reducing adverse side effects is currently the main target of research in the field of food and medicine science.

Opuntia humifusa Raf. *(O. humifusa* Raf.*)* is a member of the Cactaceae family, and is widely distributed in semiarid countries throughout the world, especially in the Mediterranean and Central America (Acuna et al., 2002; Goldstein and Nobel, 1994; Lee et al., 2002). In Korea, *O. humifusa* Raf. has been cultivated for a long time. Concerning the pharmacological profile of *Opuntia* spp., the total phenols in an ethanolic extract from South Korea's *O. ficus-indica* var. *saboten* has been responsible for the radical scavenging activity toward superoxide and hydroxyl anions (Hyang et al., 2003; Lee et al., 2002). In addition, an ethanol extract of *O. ficus-indica* var. *saboten* was reported to have analgesic effects in a writhing test which were induced by acetic acid and anti-inflammatory effect against gastric lesions (Park et al., 1998).

Whereas *O. ficus-indica* var. *saboten* and other *Opuntia* spp. have been extensively studied for their biological effects, such as therapeutic properties against arthritis and cancer (Butera et al., 2002; Hyang et al., 2003; Galati et al., 2002; Park and Chun, 2001), little is known about the pharmacological effect of *O. humifusa* Raf. In our previous studies, we reported that solvent-extracted fractions have anti-oxidative and anti-inflammatory properties (Cho et al., 2006). Since it is known that the natural products with potent anti-oxidative characteristics can be potential sources of anti-platelet agens (Hung et al., 2005; Kang et al., 2001; Lee et al., 2005; Olas et al., 2005; Son et al., 2004), we examined whether the ethyl acetate extract of (OH-EAE) modulates agonist-induced platelet aggregation. We here report a preliminary *in vitro* result on anti-platelet activity of OH-EAE in agonist-induced rat platelets activation.

MATERIALS AND METHODS

Materials

ADP, thrombin and fura-2/AM were obtained from the Sigma Co (St. Louis, MO, USA). Collagen was procured from the Chronolog Co (Havertown, PA, USA). All other chemicals were of reagent grade.

Preparation of the extract

O. humifusa Raf. was collected in October 2005 from the province of Asan (Korea). The voucher specimen (PLOH-1001) is deposited in the herbarium of Kyungpook National University, Laboratory of Physiology and cell signaling. The preparation of ethyl acetate extract was described previously (Cho et al., 2006). The crude extracts were stored in –20°C until use.

Animals

Male Sprague-Dawely rats weighing from 240 to 250 g were obtained from Orient Co. (Seoul, Korea) and maintained in a standard laboratory animal facility with free access to feed, water and acclimated for at least two weeks before use. The experiments were carried out in accordance to internationally accepted guidelines on the use of laboratory animals and the protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Kyungpook National University.

Platelet preparation and aggregation assay

The preparation of the platelets has been described previously (Kim et al., 2006). Rat blood (8 ml) was collected from abdominal aorta via the venipuncture, using a 23-g needle, and transferred to a 15 ml test tube containing 1 ml of a citrate phosphate dextrose solution (CPD; 90 mM of $Na_3C_6H_5O_7 \cdot 2H_2O$, 14 mM of $C_6H_8O_7 \cdot H_2O$, 128.7 mM of $NaH_2PO_4 \cdot H_2O$, 2.55 g/100 ml dextrose). Blood was centrifuged at 1,000 rpm for 7 min in order to achieve platelet-rich plasma. In order to remove residual erythrocytes, the PRP samples were again centrifuged at 500 rpm for 7 min. Again, to remove the CPD solution, PRP was centrifuged twice at 2,500 rpm for 10 min and the supernatant was allocated to platelet-poor plasma (PPP), which is used as a reference solution for aggregation assay. The platelets of the precipitance were adjusted to the proper number [10^8/ml for aggregation assay with a Tyrode buffer (137 mM of NaCl, 12 mM of $NaHCO_3$, 5.5 mM of glucose, 2 mM of KCl, 1 mM of $MgCl_2$, 0.3 mM of $NaHPO_4$, pH 7.4)].

Platelet aggregation was performed as previously described (Kim et al., 2006). Aggregation was monitored by measuring light transmission via an aggregometer (Chronolog Co, Havertown, PA). The washed platelets were preincubated at 37°C for 2 min with either ethyl acetate extract or vehicles. The reaction mixture was further incubated for 5 min, with stirring, at 1,200 rpm. The concentration of the vehicle was kept at less than 0.5% so as to exclude the artificial effect.

Determining the $[Ca^{2+}]_i$

The $[Ca^{2+}]_i$ was determined with fura-2/AM as described previously (Kamruzzaman et al., 2010). Briefly, the platelet-rich plasma was incubated with 5 μM of fura-2/AM for 60 min at 37°C. The fura-2-loaded washed platelets (10^8/ml) were then pre-incubated with OH-EAE for 2 min at 37°C in the presence of 1 mM $CaCl_2$. Next, the platelets were stimulated with ADP for 3 min. Fura-2 fluorescence was measured in a spectrofluorometer (F-2500, Hitachi, Japan) with an excitation wavelength that ranges from 340 to 380 nm, changing every 0.5 s, and with the emission wavelength of 510 nm. The $[Ca^{2+}]_i$ was calculated by the method of (Schaeffer and Blaustein, 1989): $[Ca^{2+}]_i$ in cytosol = 224 nM × $(F-F_{min})/(F_{max}-F)$, where 224 nM is the dissociation constant of the fura-2-Ca^{2+} complex, and

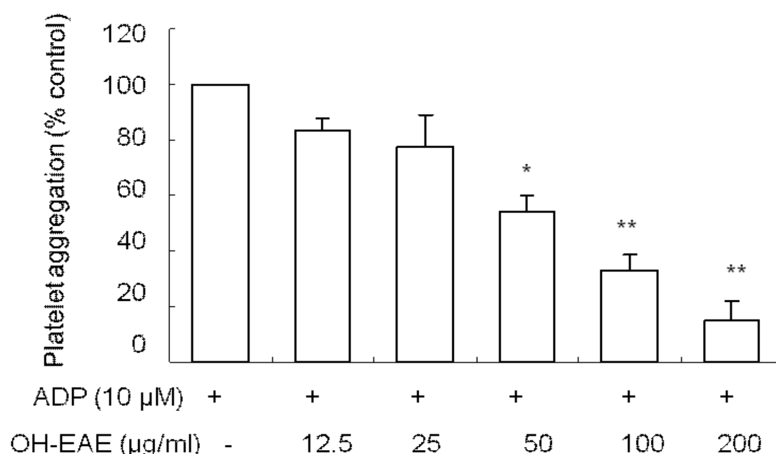

Figure 1. The ethyl acetate extract of *O. humifusa* Raf. (OH-EAE) inhibited ADP (10 µM)–induced rat platelet aggregation. Platelets were pre-incubated with indicated-concentration levels of OH-EAE for 2 min. and then, ADP was added to trigger aggregation for a further 5 min. The data are presented as means ± SEM (n=4). * : P < 0.05, ** : P < 0.01.

F_{min} and F_{max} represent the fluorescence intensity levels at very low and very high Ca^{2+} concentrations, respectively. In our experiment, F_{max} is the fluorescence intensity of the fura-2-Ca^{2+} complex at 510 nm after the platelet suspension containing 1 mM $CaCl_2$ had been solubilized by Triton X-100 (0.1%). F_{min} is the fluorescence intensity of the fura-2-Ca^{2+} complex at 510 nm, after the platelet suspension containing 20 mM Tris/3 mM of EGTA had been solubilized by Triton X-100 (0.1%). F represents the fluorescence intensity of the fura-2-complex at 510 nm after the platelet suspension was stimulated by ADP, with OH-EAE or vehicle, in the presence of 1 mM $CaCl_2$.

ATP release assay

ATP assay was determined as described by (Lee et al., 2010). Briefly, washed platelets were pre-incubated for 3 min at 37°C with indicated concentrations of the ethyl acetate extracts and then stimulated with ADP. The reaction was terminated, samples were centrifuged and supernatants were used for the assay. ATP release was measured in a luminometer (GloMax 20/20, Promega, Madison, USA) using ATP assay kit (Biomedical Research Service Center, Buffalo, NY, USA).

Statistical analysis

Data were analyzed by a one–way analysis of variance, followed by a post-hoc Dunnett's test in order to determine the statistical significance of the differences. All data are presented as means ± SEM. P values of 0.05 or less were considered to be statistically significant.

RESULTS

The effects of *O. humifusa* Raf. on ADP-induced platelet aggregation

Since we have previously reported that the ethyl acetate extracts of *O. humifusa* Raf. displayed potent anti-oxidative and anti-inflammatory properties (Cho et al., 2006), we intended to determine whether this extract affects platelet function. Washed rat platelets were preincubated with OH-EAE and then, exposed to ADP (10 µM) in order to examine the inhibitory effect of *O. humifusa* Raf. on rat platelet aggregation. Under ADP treatment, we also obtained an appropriate amount of platelet aggregation up to 98.0 ± 4.5%, which was effectively inhibited by U73122 (10 µM), a specific phospholipase C inhibitor (data not shown). As shown in Figure 1, the extract inhibited the platelet aggregation induced by ADP in a concentration-dependent manner. Its maximal inhibition rate at the dose of 200 µg/ml was 85.2%.

Effects of *O. humifusa* Raf. on collagen-induced platelet aggregation

We next determined whether the OH-EAE affects the collagen (2.5 µg/ml)-induced rat platelet aggregation. As shown in (Figure 2), the ethyl acetate extracts inhibited platelet aggregation in a dose-dependent manner with IC_{50} values of 75.3 ± 4.4 µg/ml.

Effects of *O. humifusa* Raf. on thrombin-induced platelet aggregation

To further investigate the anti-aggregatory activity of the extract on protease activated receptor- mediated platelet activation, we examined the response of platelets to the extract pretreatment in thrombin-stimulated platelets. OH-EAE dose dependently attenuated thrombin-induced

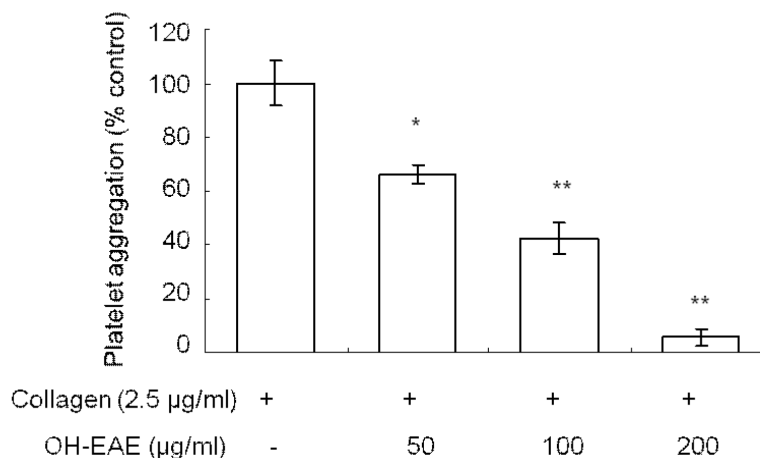

Figure 2. The OH-EAE suppressed collagen (2.5 Og/ml)–induced rat platelet aggregation. Platelets were pre-incubated with indicated-concentration levels of the extract for 2 min. and then, collagen was added to trigger aggregation for a further 5 min. Data are presented as means ± SEM (n=3). * : $P < 0.05$, ** : $P < 0.01$.

platelet aggregation (Figure 3).

Effects of *O. humifusa* Raf. on cytosolic Ca^{2+} levels

Free cytosolic Ca^{2+} concentrations in rat platelets were measured by the fura-2/AM loading method. As shown in (Figure 4), ADP (10 μM) evoked a marked increase in $[Ca^{2+}]_i$, and this increase was markedly inhibited in the presence of the extract. At maximal dose of the extract (100 μg/ml), cytosolic calcium levels reversed to the basal levels.

The effects of *O. humifusa* Raf. on ATP granule secretion

Similar to the extent of platelet aggregation, ADP provoked the release of endogenous ATP, from platelet dense granules. The amount of ATP released from platelets, determined at 5 min after application of ADP, was increased by four folds from resting state. As shown in (Figure 5), the ethyl acetate extracts of the plant significantly suppressed the ADP-induced ATP release, which was comparable to the treatment of U73122 (4 μM).

DISCUSSION

Natural products of plant origin with anti-platelet activity can be important sources of lead compounds and novel therapeutics. Because of their relative effectiveness, limited side effects, and low cost, natural products are widely prescribed even when their biologically active compounds are unknown. The active constituent(s) of some herbs with nutritional and medicinal values are still unknown. Further studies should be carried out to elucidate these compounds and determine their pharmacological activities as they may represent major but yet largely unknown source of new pharmaceutical products.

Feugang et al. reviewed that cactus pear (*Opuntia* spp.) have not only been used as a source of food but also as medicinal resource (Feugang et al., 2006). The medicinal properties of cactus have been confirmed through an intensive research conducted worldwide. The extractable ingredients of cactus were incorporated in products and were claimed to improve biological function of human body (Feugang et al., 2006). Previous studies suggested that flavonoids such as quercetin, (+)-dihydroquercetin isolated from *Opuntia ficus-indica* var. *saboten* possess neuroprotective effects (Hyang et al., 2003), and attenuates neuronal injury in *in vitro* and *in vivo* models of cerebral ischemia (Kim et al., 2006a). In addition, antioxidant activities of *Opuntia ficus indica* fruit extracts (Butera et al., 2002) and radical scavenging effect of flavonol glycosides from *Opuntia dillenii* have been reported. Moreover, Sreekanth et al. reported apoptotic effects of betanin purified from fruits of *Opuntia ficus-indica* in human chronic myeloid leukemia cell line-K562 (Sreekanth et al., 2007). As a result, studies on medicinal *Opuntia* spp. have attracted special attention in recent years due to their potential biological and pharmacological activities including neuroprotective, antitumor and immunomodulating, and antioxidant effects (Feugang et al., 2006). However, anti-platelet activity of *Opuntia* spp is not reported yet.

We previously reported that *O. humifusa* Raf extract exhibited anti-oxidant, free radical scavenging and

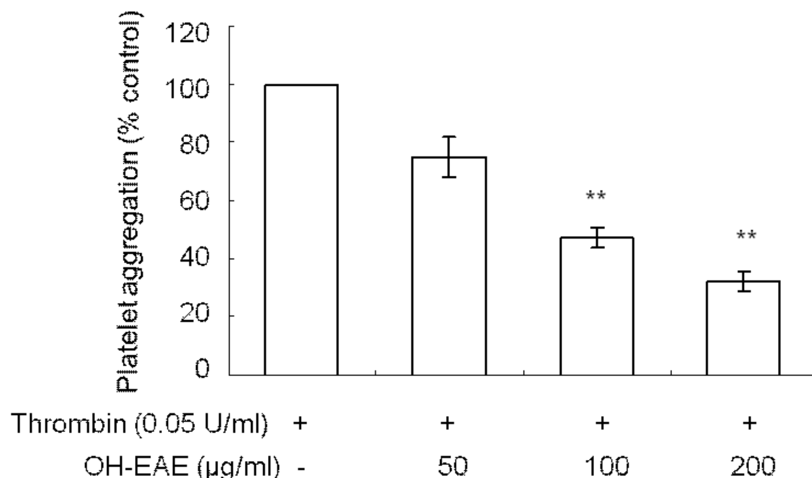

Figure 3. The OH-EAE inhibited thrombin (0.05 U/ml)–induced rat platelet aggregation. Platelets were pre-incubated with indicated-concentration levels of the ethyl OH-EAE for 2 min. and then, thrombin was added to trigger aggregation for a further 5 min. Data are presented as means ± SEM (n=3). ** : P < 0.01.

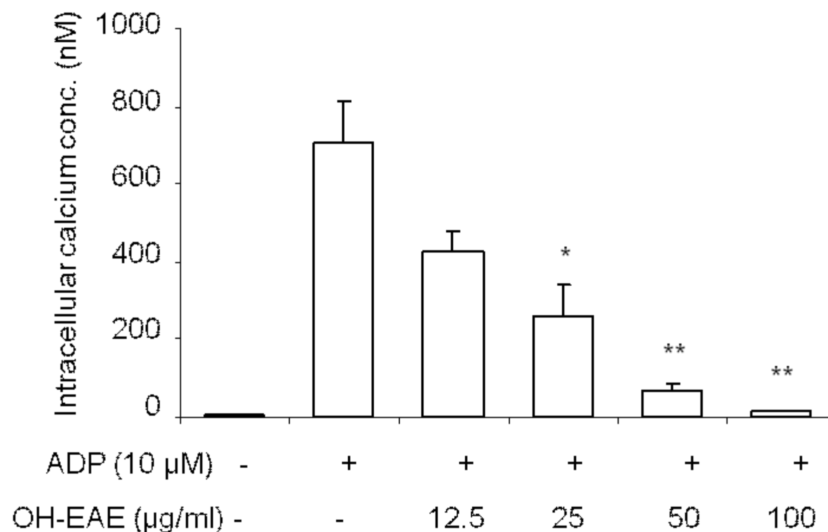

Figure 4. OH-EAE attenuated ADP-activated platelets intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. Platelets were loaded with Fura-2/AM as described in 'Materials and Methods'. The platelets (10^8/ml) were pre-incubated with or without the ethyl acetate extract in the presence of 1 mM $CaCl_2$ for 2 min at 37℃. The platelets were stimulated with ADP (10 μM) for 3 min at 37℃. $[Ca^{2+}]$i levels were determined as described in 'Materials and Methods'. Bar graphs show mean ±SEM of at least 3 independent experiments performed. *: P < 0.05 or **: P < 0.01 vs agonist activated control.

anti-inflammatory activities (Cho et al., 2006). However, information on the effect of this plant on platelet function is limited.

In this study, we report the preliminary *in vitro* findings on anti-platelet activity of *O. humifusa* ethyl acetate extract in agonist-induced platelet activation. In the present study, the decrease in agonist-induced platelet aggregations by OH-EAE suggests that the extract influenced the signaling pathways triggered by both agonists. Therefore, the OH-EAE mediated inhibition of platelet aggregation induced by various agonists in the present study suggests that the extract effect is

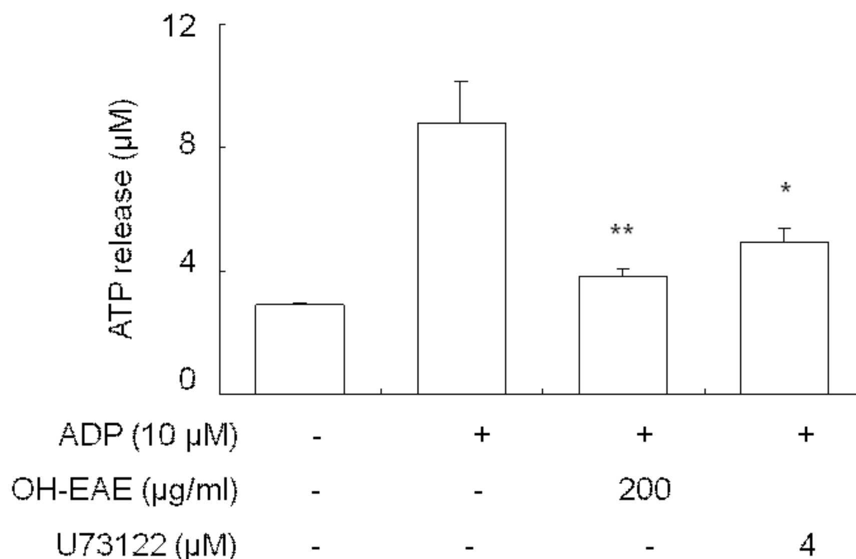

Figure 5. Effects of the OH-EAE on ADP activated platelet granule secretion. Washed platelets were pre-incubated with the OH-EAE (at the concentrations indicated), and stirred in an aggregometer for 2 min prior to ADP stimulation for 5 min, and then the reactions were terminated followed by granule secretion assay. ATP release in response to agonist stimulation was performed as described in the 'Materials and Methods' and the OH-EAE significantly suppressed ADP (10 μM) stimulated platelet ATP release. Bar graphs show mean ± SEM of 3 independent experiments performed. *: $P < 0.05$ or **: $P < 0.01$vs.agonist activated control.

downstream of the agonist-receptor interactions. In addition, the extract inhibited $[Ca^{2+}]_i$, suggesting that it may involve inhibition of cytoplasmic calcium increase. This conclusion is supported by the fact that calcium plays a central role for granule secretions such as ATP release, which is inhibited by the extract in this study. More importantly, platelet $\alpha_{IIb}\beta_3$ is activated downstream of adhesion receptors GPVI and GPIb-IX–V, or G-protein-coupled receptors, for example, thrombin (PAR-1 or PAR-4) or ADP receptors (P_2Y_1 or P_2Y_{12}) that reinforce $\alpha_{IIb}\beta_3$-dependent platelet aggregation and inside–out activation. The integrin $\alpha_{IIb}\beta_3$ activation is Ca^{2+}-dependent and involves changes in the conformation of both the ligand-binding extracellular region and the cytoplasmic tails (Xiong et al., 2003). Following ligand binding, outside–in signals and altered interactions with cytoskeletal proteins, such tyrosine kinases (Calderwood et al., 2002), control post-adhesion events, such as spreading and contraction in platelet aggregation.

Conclusion

In *in vitro* assays using freshly isolated rat platelets, the OH-EAE showed significant inhibition of collagen, thrombin and ADP-induced platelet aggregation, ATP secretion and $[Ca^{2+}]$i mobilization. The main findings of this study suggest that the inhibitory effects of OH-EAE in platelet aggregation possibly involve $[Ca^{2+}]_i$ inhibition in activated platelets. Thus, these results suggest that anti-platelet activity of this plant extract may be considered as a lead source candidate in the search of anti-platelet agents. Further study using isolated compound(s) of the extract together with the possible mechanisms of action is required.

ACKNOWLEDGEMENTS

The authors wish to thank "Yours Cheonnyuncho Co." for providing *O. humifusa* Raf. This work was supported from Ministry of Food, Agriculture, Forestry and Fisheries (No 20080439), Republic of Korea.

REFERENCES

Acuna UM, Atha DE, Ma J, Nee MH, Kennelly EJ (2002). Antioxidant capacities of ten edible North American plants. Phytother. Res., 16: 63-65.

Butera D, Tesoriere L, Di GF, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea MA (2002). Antioxidant activities of sicilian prickly pear (Opuntia ficus indica) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J. Agric. Food Chem., 50: 6895-6901.

Calderwood DA, Yan B, De PJM, Alvarez BG, Fujioka Y, Liddington RC, Ginsberg MH (2002). The phosphotyrosine binding-like domain of talin activates integrins. J. Biol. Chem., 277: 21749-21758.

Cho JY, Park SC, Kim TW, Kim KS, Song JC, Kim SK, Lee HM, Sung HJ, Park HJ, Song YB, Yoo ES, Lee CH, Rhee MH (2006). Radical scavenging and anti-inflammatory activity of extracts from Opuntia

humifusa Raf. J. Pharm. Pharmacol., 58: 113-119.

Hyang DG, Lee KH, Kim HJ, Lee EH, Lee J, Song YS, Lee YH, Jin C, Lee YS, Cho J (2003). Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from Opuntia ficus-indica var. saboten. Brain Res., 965: 130-136.

Feugang JM, Konarski P, Zou D, Stintzing FC, Zou C (2006). Nutritional and medicinal use of Cactus pear (Opuntia spp.) cladodes and fruits. Front Biosci., 11: 2574-2589.

Galati EM, Tripodo MM, Trovato A, Miceli N, Monforte MT, (2002). Biological effect of Opuntia ficus indica (L.) Mill. (Cactaceae) waste matter. Note I: diuretic activity. J. Ethnopharmacol., 79: 17-21.

Goldstein G, Nobel PS (1994). Water Relations and Low-Temperature Acclimation for Cactus Species Varying in Freezing Tolerance. Plant Physiol., 104: 675-681.

Grenache DG, Coleman T, Semenkovich CF, Santoro SA, Zutter MM (2003) Alpha2beta1 integrin and development of atherosclerosis in a mouse model: assessment of risk. Arterioscler. Thromb. Vasc. Biol., 23: 2104-2109.

Hung CC, Tsai WJ, Kuo LM, Kuo YH (2005). Evaluation of caffeic acid amide analogues as anti-platelet aggregation and anti-oxidative agents. Bioorg. Med. Chem., 13: 1791-1797.

Huo Y, Ley KF (2004). Role of platelets in the development of atherosclerosis. Trends Cardiovasc. Med., 14: 18-22.

Kamruzzaman SM, Endale M, Oh WJ, Park SC, Kim KS, Hong JH, Kwak YS, Yun BS, Rhee MH (2010). Inhibitory effects of Bulnesia sarmienti aqueous extract on agonist-induced platelet activation and thrombus formation involves mitogen-activated protein kinases. J. Ethnopharmacol., 130: 614-620.

Kang WS, Chung KH, Chung JH, Lee JY, Park JB, Zhang YH, Yoo HS, Yun YP (2001). Antiplatelet activity of green tea catechins is mediated by inhibition of cytoplasmic calcium increase. J. Cardiovasc. Pharmacol., 38: 875-884.

Kim JH, Park SM, Ha HJ, Moon CJ, Shin TK, Kim JM, Lee NH, Kim HC, Jang KJ, Wie MB (2006). Opuntia ficus-indica attenuates neuronal injury in in vitro and in vivo models of cerebral ischemia. J. Ethnopharmacol., 104: 257-262.

Kim SD, Park SK, Cho JY, Park HJ, Lim JHIYH, Park SC, Lee KY, Kim SK, Rhee MH, (2006). Surfactin C inhibits platelet aggregation. J. Pharm. Pharmacol., 58: 867-870.

Leclerc JR (2002) Platelet glycoprotein IIb/IIIa antagonists: lessons learned from clinical trials and future directions. Crit. Care Med., 30: S332-340.

Lee HS, Kim SD, Lee WM, Endale M, Kamruzzaman SM, Oh WJ, Cho JY, Kim SK, Cho HJ, Park HJ, Rhee MH (2010). A noble function of BAY 11-7082: Inhibition of platelet aggregation mediated by an elevated cAMP-induced VASP, and decreased ERK2/JNK1 phosphorylations. Eur. J. Pharmacol., 627: 85-91.

Lee JC, Kim HR, Kim J, Jang YS (2002). Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. J. Agric. Food Chem., 50: 6490-6496.

Lee YY, Jang DS, Jin JL, Yun CHS (2005). Anti-platelet aggregating and anti-oxidative activities of 11-O-(4'-O-methylgalloyl)-bergenin, a new compound isolated from Crassula cv. 'Himaturi'. Planta Med., 71: 776-777.

Olas B, Wachowicz B, Majsterek I, Blasiak J (2005). Resveratrol may reduce oxidative stress induced by platinum compounds in human plasma, blood platelets and lymphocytes. Anticancer Drugs, 16: 659-665.

Park EH, Chun MJ (2001). Wound healing activity of Opuntia ficus-indica. Fitoterapia, 72: 165-167.

Park EH, Kahng JH, Paek EA (1998). Studies on the pharmacological action of cactus: identification of its anti-inflammatory effect. Arch. Pharm. Res., 21: 30-34.

Savage B, Cattaneo M, Ruggeri ZM (2001). Mechanisms of platelet aggregation. Curr. Opin. Hematol., 8: 270-276.

Schaeffer J, Blaustein MP (1989). Platelet free calcium concentrations measured with fura-2 are influenced by the transmembrane sodium gradient. Cell Calcium, 10: 101-113.

Shattil SJ, Kashiwagi H, Pampori N (1998). Integrin signaling: the platelet paradigm. Blood, 91: 2645-2657.

Son DJ, Cho MR, Jin YR, Kim SY, Park YH, Lee SH, Akiba S, Sato T, Yun YP (2004). Antiplatelet effect of green tea catechins: a possible mechanism through arachidonic acid pathway. Prostaglandins Leukot Essent Fatty Acids, 71: 25-31.

Sreekanth D, Arunasree MK, Roy KR, Chandramohan RT, Reddy GV, Reddanna P (2007). Betanin a betacyanin pigment purified from fruits of Opuntia ficus-indica induces apoptosis in human chronic myeloid leukemia Cell line-K562. Phytomedicine, 14: 739-746.

Stouffer GA, Smyth SS (2003). Effects of thrombin on interactions between beta3-integrins and extracellular matrix in platelets and vascular cells. Arterioscler. Thromb. Vasc. Biol., 23: 1971-1978.

Woulfe DS (2005). Platelet G protein-coupled receptors in hemostasis and thrombosis. J. Thromb. Haemost., 3, 2193-2200.

Xiong JP, Stehle T, Goodman SL, Arnaout MA (2003). New insights into the structural basis of integrin activation. Blood, 102: 1155-1159.