Journal of the Korea Academia-Industrial cooperation Society
Vol. 20, No. 12 pp. 298-305, 2019

https://doi.org/10.5762/KAIS.2019.20.12.298
ISSN 1975-4701 / eISSN 2288-4688

# *Cudrania Tricuspidata* root extract (CTE) has an anti-platelet effect *via* cGMP-dependent VASP phosphorylation in human platelets

Ju-Ye Ro, Hyun-Jeong Cho*
Department of Biomedical Laboratory Science, Konyang University

# 꾸지뽕나무 뿌리 추출물의 cGMP에 의한 VASP 인산화 기전을 통한 항혈소판 효과

**노주예, 조현정***
**건양대학교 의과학대학 임상병리학과**

**Abstract** Cudrania tricuspidata has been reported to have many biological activities, including anti-inflammatory, anti-cancer, and antioxidant properties. However, the effects of C. tricuspidata root extract (CTE) on human platelet aggregation induced by collagen as well as the signaling pathways involved remain unknown. In the present study, we investigated the effect of CTE on human platelets. CTE inhibited platelet aggregation via down-regulation of thromboxane $A_2$ ($TXA_2$) by blocking cyclooxygenase-1 (COX-1) activity and intracellular $Ca^{2+}$ mobilization in collagen-induced platelets. CTE also reduced the phosphorylation of phospholipase C (PLC) $\gamma 2$ and syk. CTE regulated platelet aggregation via cyclic guanosine monophosphate (cGMP)-dependent phosphorylation of vasodilator-stimulated phosphoprotein (VASP) $Ser^{239}$. In addition, administration of CTE (50 and 100 mg/kg) significantly reduced hyper-aggregated platelet aggregation by collagen (5 $\mu$g/mL) without hepatotoxicity in HFD (high fat diet)-fed rats. Taken together, these results suggest that CTE has anti-platelet effects both in vitro and in vivo. Therefore, CTE may be an effective therapeutic and preventive agent for cardiovascular disease, and is a safe and natural product.

**요 약** 꾸지뽕나무 뿌리 추출물은 항염, 항암, 항산화와 같은 효과를 갖는 많은 생리활성 물질을 포함하고 있다고 알려져 있다. 그러나 꾸지뽕나무 뿌리 추출물(이하 CTE)의 사람 혈소판 응집 억제 기전에 관하여는 알려진 바 없다. 본 연구에서는 CTE가 혈소판에 미치는 영향을 확인하고자 하였다. CTE는 collagen으로 유도한 혈소판 응집 반응에서 cyclooxygenase-1 활성을 억제하고, 세포 내 칼슘 농도를 낮추는 방식으로 thromboxane $A_2$ 생성을 억제하였다. 또한, phospholipase C$\gamma 2$와 syk의 인산화 반응을 억제하였으며, guanosine monophosphate (cGMP)에 의존적인 방식으로 vasodilator-stimulated phosphoprotein(VASP)의 $Ser^{239}$ 위치를 인산화하여 항혈소판 효과를 나타내었다. 또한, 고지방 식이로 혈소판 활성화를 유도한 랫드에서 CTE를 경구 투여 하였을 때, 간독성 없이 콜라겐으로 유도한 혈소판 응집반응을 thromboxane $A_2$ 생성을 억제함으로써 항혈소판 효과를 보였다. 이는 *in vivo*와 *in vitro*에서 같은 결과를 제시하고 있다. 결론적으로, CTE는 항혈소판 작용 및 심혈관계 질환 예방을 위한 천연물 유래의 안전하고, 새로운 물질임을 제시하는 바이다.

**Keywords :** *Cudrania Tricuspidata*, Cyclic Guanosine Monophosphate, Cyclooxygenase-1 Activity, Platelet Aggregation, Phospholipase C$\gamma 2$, Syk, Thromboxane $A_2$, Vasodilator-stimulated Phosphoprotein

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0024028 & 2016R1C1B2007025).
*Corresponding Author : Hyun-Jeong Cho(Konyang University)
e-mail: hjcho@konyang.ac.kr
Received October 31, 2019 Revised December 3, 2019
Accepted December 6, 2019 Published December 31, 2019

## 1. Introduction

*Cudrania tricuspidata*, which belongs to the Moraceae family, is used as a traditional remedy in East Asia [1]. Its root and cortex have been used as a treatment for cancer in Korea and China [2]. In previous studies, it has been shown to have many biological activities, such as anti-inflammatory, $\alpha$-glucosidase inhibitory, and anti-oxidative properties [3,4]. In particular, catecholic xanthones from *C. tricuspidata* help to prevent chronic diseases related to reactive oxygen species, and have anti-atherosclerotic activity against low-density lipoprotein oxidation [5]. Despite these biological studies, pharmacological investigation into the mechanism of platelet aggregation by of *C. tricuspidata* root extract is not well understood [6]. Platelets express several its receptors. Of these receptors, glycoprotein (GP) VI is required for collagen-induced platelet activation [7]. GP VI is coupled to Fc Receptor $\gamma$ chain (FcR $\gamma$) [7]. Upon crosslinking of GP VI by its ligand, immunoreceptor tyrosine-based activation motif (ITAM) (originally found in T cell antigen receptor signaling) has been shown to result in phosphorylation of two tyrosine residues [8,9]. ITAM phosphorylation leads to binding tyrosine kinase Syk, which can activate initiating further signaling including LAT and phospholipase C$\gamma$2 (PLC$\gamma$2) [10]. The activation of PLC catalyzes the hydrolysis of phosphatidylinositol biphosphate ($PIP_2$) to inositol trisphosphate ($IP_3$) and diacyglycerol (DAG). DAG is hydrolyzed to arachidonic acid (20:4). Platelets synthesize thromboxane $A_2$ ($TXA_2$) from 20:4 released from membrane phospholipids through rapid, coordinated activation of cyclooxygenase (COX)-1 and $TXA_2$ synthase [11]. Meanwhile, intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are known to reduce intracellular calcium levels [12]. The anti-platelet effects of cAMP and cGMP are mediated by cAMP/A-kinase and cGMP/G-kinase-dependent protein kinase, which phosphorylate substrate proteins, vasodilator-stimulated phosphoprotein (VASP). VASP is phosphorylated at various sites, and these are phosphorylated with differing kinetics. In A-kinase, $Ser^{157}$ is preferred site of phosphorylation. In G-kinase, on the other hand, $Ser^{239}$ is the preferred phosphorylation site, in vitro [13]. Phosphorylation of VASP involves in the decrease of VASP affinity for protein filamentous actin to inhibit platelet aggregation [14]. Therefore, inhibiting the $[Ca^{2+}]_i$ or increasing the level of cyclic nucleotides and phosphorylation of VASP is very useful for the evaluating the anti-platelet effects of substances. In this study, we investigated the anti-platelet effect of *C. tricuspidata* root extract (CTE) and demonstrated inhibition of $TXA_2$ production via reduction of COX-1 activity and $[Ca^{2+}]_i$ reduction via increase of cGMP.

## 2. Methods

### 2.1 Preparation of CTE

*C. tricuspidata* roots were collected from Yeosu, which is located in Jeollanamdo, South Korea. The roots were washed in tap water twice and dried at room temperature. They were then heated in boiling water with 70 % ethanol for 1 hour and distilled water for 4 hours. The mixture was centrifuged at 2,000 × g for 10 min and the clear supernatant was collected. Collected CTE was freeze-dried with a freeze dryer (Ilshin BioBase Co., Ltd., South Korea) and re-suspended in distilled water.

### 2.2 Analysis of the contents of CTE with high-performance liquid chromatography

Quercetin and chlorogenic acid were obtained from Sigma Corp. (St. Louis, MO, USA). CTE and standard samples were dissolved in 80 % methanol and then analyzed by HPLC. An

Alliance HPLC e2695 Separations Module (Waters Corporation, UK) was equipped with a 2998 photodiode array detector. A phenomenex Luna C18 (2) (250 × 4.6 mm, 5 $\mu$m) was used at a column temperature of 40°C. The mobile phase consisted of water with 0.1 % trifluoroacetic acid (A) and acetonitrile (B) over a period of 0 - 60 min. The flow rate was set at 1.0 mL/min and the sample injection volume was 10 $\mu$L. UV detection was done at 210 nm.

### 2.3 Measurement of human platelet aggregation and $TXB_2$

Human platelet-rich plasma (PRP) anticoagulated with acid-citrate-dextrose solution (0.8 % citric acid, 2.2 % sodium citrate, 2.45 % glucose) were obtained from Korean Red Cross Blood Center (Daejeon, Korea). Washed platelets ($10^8$ cells/mL) were prepared according to previously published methods [15]. Washed platelets were pre-incubated for 3 min at 37°C in the presence of 2 mM exogenous $CaCl_2$ with or without CTE, then stimulated with 10 $\mu$g/mL of collagen for 5 min. Aggregation was monitored using a Chrono-Log aggregometer (Havertown, PA, USA). Platelet aggregation reactions were terminated by the addition of ice-cold EDTA (5 mM) and indomethacin (0.2 mM). The amount of $TXB_2$ (a stable metabolite of $TXA_2$) was determined using a $TXB_2$ EIA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

### 2.4 COX-1 and $TXA_2$ synthase activity assays

Platelets in a suspension buffer containing 1 % protease inhibitor were sonicated. The platelet lysates (10 $\mu$g-protein) were incubated with or without various concentrations of CTE for 30 min at 37°C. COX-1 activity was measured according to previously published methods [15]. For measurement of $TXA_2$ synthase, the platelet lysates were pre-incubated with or without CTE at 37°C for 5 min and then measurement was performed according to previously published methods [15]. $TXA_2$ synthase activity was assessed by measuring $TXB_2$ formation after 1 min incubation.

### 2.5 Measurement of cyclic nucleotides and determination of $[Ca^{2+}]_i$

After platelet aggregation reactions were terminated by the addition of 80 % ice-cold ethanol, cAMP and cGMP were measured using cAMP and cGMP EIA kits (Biovision, Milpitas, CA, USA). To measure $[Ca^{2+}]_i$, fura 2-AM (Sigma Corp.)-loaded washed platelets were stimulated with collagen (10 $\mu$g/mL) for 5 min. Fura 2 fluorescence was measured with an RF-5301 spectrofluorometer (Shimadzu Corp., Kyoto, Japan) with an excitation wavelength that changed every 0.5 sec from 340 to 380 nm; the emission wavelength was set at 510 nm. $[Ca^{2+}]_i$ values were calculated using the method published by Bush *et al.* [16].

### 2.6 Western blot analysis

After platelet aggregation reactions were terminated by the addition of an equal volume (250 $\mu$L) of lysis buffer, platelet lysates containing the same protein (10 $\mu$g) were used for analysis. The effects of substances on Syk, PLC$\gamma$2 and VASP-phosphorylations were analyzed by western blotting. Blots were incubated with primary antibodies, including Syk, PLC$\gamma$2, phospho-Syk, phospho-PLC$\gamma$2, VASP, phospho-VASP ($Ser^{239}$) (Cell Signaling Technology, Beverly, MA, USA). After incubation with secondary antibodies, blots were analyzed by using Fusion SL imaging systems (Vilber Lourmat, France).

### 2.7 Cell toxicity assay

To assess whether CTE is cytotoxic, we examined the effect of CTE on lactate dehydrogenase release. Washed platelets ($10^8$ cells/mL) were incubated for 5 min at 37°C with CTE (~ 500 $\mu$g/mL). Platelets were

centrifuged at 2000 × g at 25°C, and then the supernatant was measured using a lactate dehydrogenase (LDH) assay kit (Cayman Chemical) according to the manufacturer's instructions.

### 2.8 Animals and diets

Six-week-old male Sprague-Dawley rats were purchased from Taconic Farm., Inc. (Hudson, NY, USA). They were fed a diet consisting of 45 % fat; the high fat diet (HFD) (TD.06415, Harlan Laboratories Ltd., Indianapolis, USA) was used to rapidly induce obesity. The rats were divided into 4 groups of 8 animals each (normal diet, Group I; HFD, Group II; 2 groups with CTE administration (Groups III and IV). Groups II, III, and IV were maintained on a HFG for 6 weeks in order to induce obesity. CTE was dissolved in mineral water and administered orally to HFD-induced obese rats for 2 weeks at doses of 50 and 100 mg/kg of body weight/day. The rats had ad libitum access to food and water. After treatment with CTE for 2 weeks, the rats were sacrificed after a 12-hour starvation period. For histological examination of the liver, after blood was drained, the liver was fixed in 3.7 % formaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin. Images were viewed with a Zeiss 20 × lens, and digital images were captured with a Zeiss Axiocam MRC digital camera and Axiovision software. All animal experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Konyang University (P-15-08-A-01) (Daejeon, south Korea).

### 2.9 Statistical analysis

Experimental results are expressed as the mean ± S.D. Statistical analysis was performed by a two tailed-unpaired Student's t-test or analysis of variance (ANOVA), as appropriate. If this analysis indicated significant differences among the group means, each group was compared by post hoc tests [17].

## 3. Results and Discussion

### 3.1 CTE possess chlorogenic acid and quercetin

*Cudrania tricuspidata* has been reported to contain various flavonoids and phenolic compounds, including quercetin. HPLC revealed that CTE contains quercetin and chlorogenic acid (Fig. 1a). The retention time of chlorogenic acid and quercetin were 12.6 and 35.0 min, respectively (data not shown). The concentrations of chlorogenic acid and quercetin in CTE were 4.93 ± 0.2 mg/g (0.49 %) for chlorogenic acid and 0.36 ± 0.1 mg/g (0.03 %) for quercetin. In previous studies, we investigated the anti-platelet effects of quercetin and chlorogenic acid on

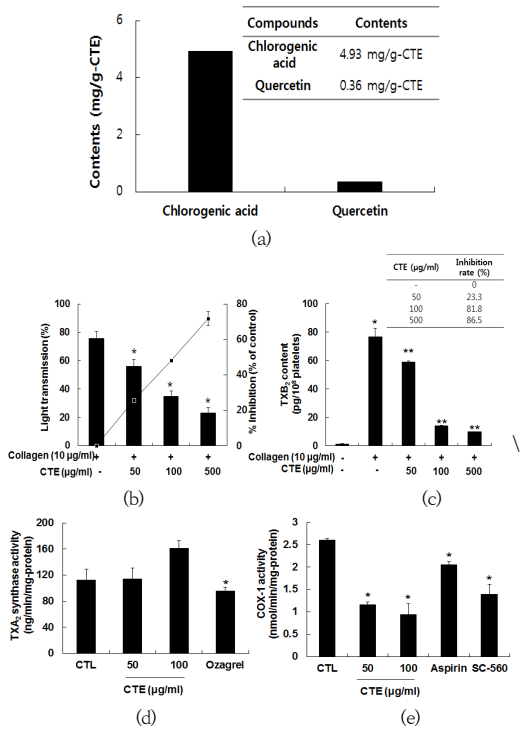


Fig. 1. The contents of CTE and the effects of CTE on collagen-induced platelet aggregation and $TXA_2$ generation.
(a) Chlorogenic acid and quercetin contents in CTE. (b) Effect of CTE on collagen-induced platelet aggregation. (c) Effect of CTE on $TXA_2$ generation. Effects of CTE on $TXA_2$ synthase (d) and COX-1 (e) activities. Data are expressed as mean ± S.D. (n = 4). $^{*}p < 0.05$ as compared with control. $^{**}p < 0.05$ as compared with collagen-treated control.

collagen-induced rat platelet aggregation [12,15]. These results showed that the anti-platelet effect of CTE is clearly linked to quercetin and chlorogenic acid, although other peaks were not confirmed.

### 3.2 Effects of CTE on platelet aggregation and $TXA_2$ generation *via* COX-1 activity

The rate of platelet aggregation induced by collagen was 75.5 ± 5.3 %, but CTE significantly inhibited platelet aggregation (Fig. 1b). $TXA_2$ is one of the most important stimulators of platelet aggregation, and it plays an important role in the mechanism of platelet activation. As shown in Fig. 1c, CTE significantly reduced the levels of $TXB_2$ in a dose-dependent manner. $TXA_2$ is synthesized by activated platelets from AA through the COX and $TXA_2$ synthase pathway [18,19]. Since CTE suppressed collagen-stimulated platelet aggregation *via* a decrease in $TXA_2$ levels, we determined whether CTE affects COX-1 or $TXA_2$ synthase activity (the metabolic enzyme responsible for $TXA_2$ production). As Fig. 1d shows, CTE does not affect $TXA_2$ synthase activity. Ozagrel (11 nM) was used as an inhibitor; it effectively decreased $TXA_2$ levels. However, CTE (50 and 100 $\mu$g/ml) inhibited COX-1 activity from 2.6 ± 0.1 (control) to 1.2 ± 0.1 and 0.9 ± 0.2 nmol/min/mg protein, respectively (Fig. 1e). Aspirin (500 $\mu$M) and SC-560 (330 nM) were used as positive controls, and selectively inhibited COX-1 activity. These results suggest that the inhibitory activity of CTE on $TXA_2$ production was the result of a reduction in COX-1 activity, not $TXA_2$ synthase.

### 3.3 Effects of CTE on cyclic nucleotides and $[Ca^{2+}]_i$

Cyclic nucleotides are anti-platelet second messengers in platelet aggregation. Therefore, we investigated whether CTE increases the cellular levels of cAMP or cGMP. As Fig. 2b shows, CTE significantly increased cGMP levels in a dose-dependent manner (34.6 ± 3.5, 37.4 ± 4.2 and 56.5 ± 6.5 pmol/$10^8$ platelets, respectively). However, CTE did not affect the production of cAMP (Fig. 2a). $Ca^{2+}$ is known to be essential for platelet aggregation and adhesion. Elevated cAMP or cGMP levels decrease the concentration of intracellular $Ca^{2+}$ [20]. We investigated the effect of CTE on intracellular $Ca^{2+}$ mobilization. As Fig. 2c shows, $[Ca^{2+}]_i$ was significantly decreased by treatment with CTE in a dose-dependent manner (554.5 ± 67.4, 371.9 ± 56.7, and 332.2 ± 11.3 nM, respectively). Cyclic nucleotides stimulate cAMP-dependent protein kinase and cGMP-dependent protein kinase to phosphorylate substrate proteins [20]. Such phosphorylation results in the inhibition of $Ca^{2+}$ release from intracellular stores [20,21]. Therefore, these results indicate that CTE inhibits $[Ca^{2+}]_i$ owing to up-regulation of cGMP. In addition, in a cytotoxicity assay, significant LDH release was not observed when platelets were incubated with the concentrations of CTE used in this study (Fig. 2d).

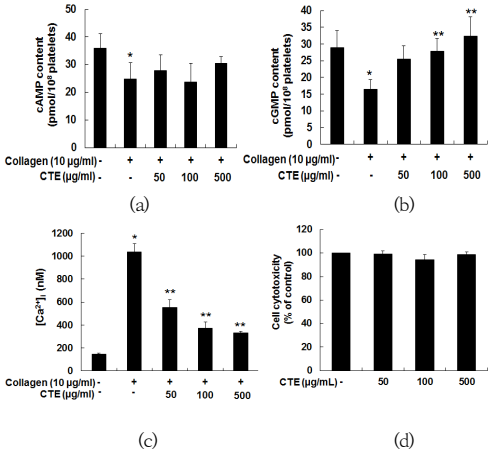


Fig. 2. Effects of CTE on $[Ca^{2+}]_i$ mobilization *via* cyclic nucleotide production and cytotoxicity.
(a) Effect of CTE on cAMP production. (b) Effect of CTE on cGMP production. (c) Effect of CTE on $[Ca^{2+}]_i$ mobilization. (d) Effect of CTE on cytotoxicity. Data are expressed as mean ± S.D. (n = 4). $^*p < 0.05$ as compared with resting platelets, and $^{**}p < 0.05$ as compared with collagen-stimulated platelets.

### 3.4 Effects of CTE on phosphorylation of PLC, Syk and VASP $Ser^{239}$

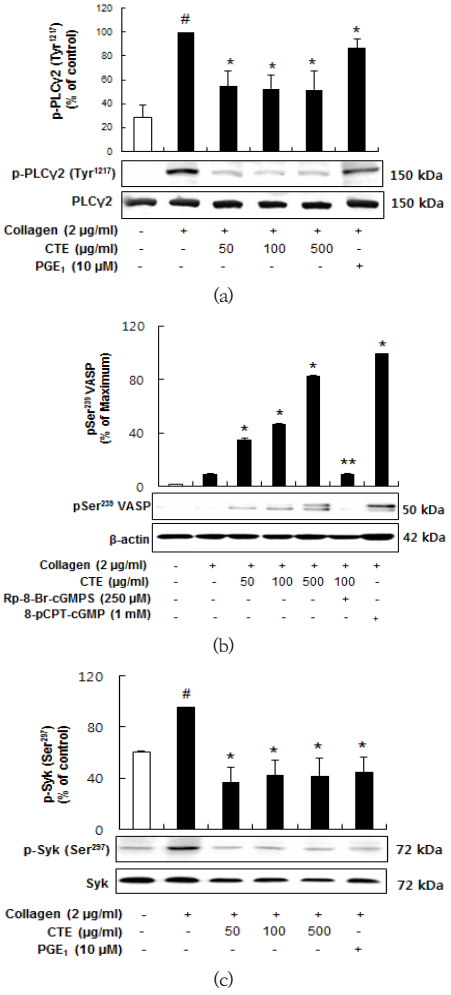


Fig. 3. Effects of CTE on the phosphorylation of PLCγ2, Syk and VASP.
(a) Effect of CTE on PLCγ2 phosphorylation. (b) Effect of CTE on Syk phosphorylation. (c) Effect of CTE on VASP phosphorylation. Data are expressed as mean ± S.D. (n = 4). $^{\#}p < 0.05$ as compared with resting platelets, $^{*}p < 0.05$ as compared with collagen control, and $^{**}p < 0.05$ as compared with collagen + CTE 100 μg/ml-treated platelets.

Platelet activation by collagen is mediated by the sequential tyrosine phosphorylation of the Fc receptor gamma-chain (FcR gamma-chain), which is part of the collagen receptor glycoprotein VI, the tyrosine kinase Syk and PLCγ2 [8]. Thus, it was investigated whether CTE regulates platelet activation by modification of PLCγ2 and/or Syk phosphorylation induced by collagen. Treatment with CTE abolished phosphorylation of PLCγ2 and Syk stimulated by collagen (Figs. 3a and b). PGE1 markedly diminished phosphorylation of PLCγ2 as compared to collagen control. These results indicate that CTE inhibits platelet activation *via* decrease of phosphorylation of PLCγ2 and Syk. The $Ser^{239}$ phosphorylation of VASP is mediated by cGMP/G-kinase pathway. As shown in Fig. 2b, CTE increased the level of cGMP. Therefore, it was investigated whether CTE can induce VASP $Ser^{239}$ phosphorylation on human platelets. As illustrated in Fig. 3c, CTE activated the phosphorylation of VASP ($Ser^{239}$), a cGMP/G-kinase substrate. A selective activator of cGMP-dependent protein kinase (8-pCPT- cGMP) induced VASP $Ser^{239}$ phosphorylation. Also, VASP phosphorylation of $Ser^{239}$ was reversed in the presence of Rp-8-Br-cGMPs, an inhibitor of cGMP-dependent protein kinase, indicating that $Ser^{239}$ phosphorylation by CTE is achieved by stimulating cGMP/G-kinase pathway.

### 3.5 Effects of CTE on HFD-induced obese rats

Obese rats are prone to vascular dysfunction associated with hyper-aggregation of platelets [22]. In this study, the obese rat model was used to evaluate the anti-platelet effect of CTE *ex vivo*. After six weeks of being on a HFD, the body weights in the HFD-fed groups (groups II, III, and IV) were significantly higher than in group I (Data not shown). As Fig 4a shows, HFD increased platelet aggregation by 70.6 ± 4.2 % (group I) to 81.5 ± 2.2 % (group II). However, consumption of CTE for 2 weeks (groups III and IV) significantly decreased platelet aggregation (77.3 ± 0.9 % and 69.1 ± 2.7 %, respectively). Consistent with the results shown in Fig. 1b, CTE decreased collagen-stimulated $TXA_2$ generation in HFD-induced obese rats (Fig. 4b). These results demonstrate that consumption of CTE has an anti-platelet effect that involves regulation of

$TXA_2$ production. In histological examination of liver specimens, a mild degree of hepatic steatosis was found in Group II (Fig. 4d). The formation of hepatic steatosis in the HFD-fed control group was correlated with a significant increase in liver weight (9.6 ± 0.1 g in group I *vs.* 10.7 ± 0.4* g in group II, $^{*}p < 0.05$, n = 8, data not shown). The administration of 50 and 100 mg/kg CTE resulted in a reduction in hepatic fatty deposition in hepatocytes (Figs. 4e and f).

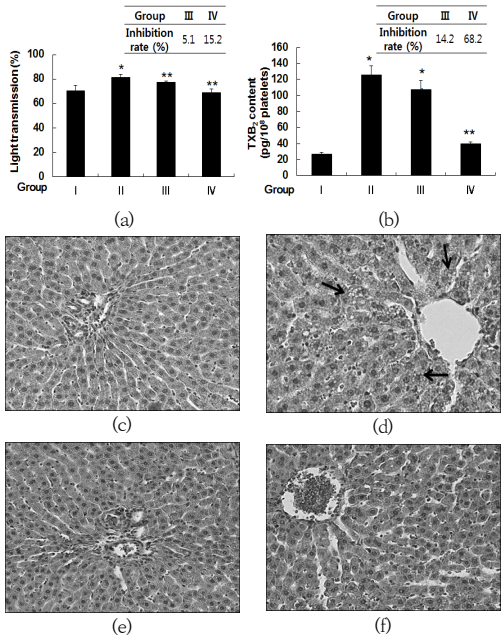


Fig. 4. Effects of CTE on HFD-induced obese rats. (a) Effect of CTE on platelet aggregation in HFD-induced obese rats. (b) Effect of CTE on $TXA_2$ production in HFD-induced obese rats. (c) Normal hepatocytes from group I. (d) Mild hepatic fatty deposition seen in group II hepatocytes. (e) Prevention of hepatic fatty deposition in group III. (f) Prevention of hepatic fatty deposition in group IV. Sections were stained with hematoxylin-eosin (H&E). All magnifications: 200 ×. Arrows indicate fatty hepato- cytes. Group I, normal diet; group II, HFD; group III, 50 mg/kg CTE; group IV, 100 mg/kg CTE. Data are expressed as mean ± S.D. (n = 8). $^{*}p < 0.05$ as compared with group I, and $^{**}p < 0.05$ as compared with group II.

## 4. Conclusion

CTE inhibited platelet aggregation *via* reducing phosphorylation of PLC$\gamma$2 and syk by regulation of cGMP-dependent phosphorylation of VASP $Ser^{239}$. We suggest that CTE has anti-platelet effects and may be an effective therapeutic and preventive agent for cardiovascular diseases, and is a safe and natural product.

## References

[1] M. Marin, S. Manez, "Recent trends in the pharma-cological activity of isoprenyl phenolics", *Curr Med Chem*, Vol.20, No.2, pp.272-279, 2013.
DOI: https://doi.org/10.2174/092986713804806676

[2] X. Li, Z. Yao, X. Jiang, J. Sun, G. Ran, X. Yang, Y. Zhao, Y. Yan, Z. Chen, L. Tian, W. Bai, "Bioactive compounds from *Cudrania tricuspidata*: A natural anticancer source", *Crit Rev Food Sci Nutr*, Vol.22, pp.1-21, Dec, 2018.
DOI: https://doi.org/10.1080/10408398.2018.1541866

[3] E. J. Seo, M. J. Curtis-Long, B. W. Lee, H. Y. Kim, Y. B. Ryu, T. S. Jeong, W. S. Lee, K. H. Park, "Xanthones from *Cudrania tricuspidata* displaying potent alpha-glucosidase inhibition", *Bioorg Med Chem Lett*, Vol.17, No.23, pp.6421-6424, Dec, 2007.
DOI: https://doi.org/10.1016/j.bmcl.2007.10.00

[4] H. Y. Joo, K. T. Lim, "Glycoprotein isolated from *Cudrania tricuspidata* Bureau inhibits iNO and COX-2 expression through modulation of NF-kappaB in LPS-stimulated RAW 264.7 cells", *Environ Toxicol Pharmacol*, Vol.27, No.2, pp.247-252, Mar, 2009.
DOI: https://doi.org/10.1016/j.etap.2008.10.014

[5] T. S. Jeong, Y. B. Ryu, H. Y. Kim, M. J. Curtis-Long, S. J. An, J. H. Lee, W. S. Lee, K. H. Park, "Low density lipoprotein (LDL)-antioxidant flavonoids from roots of Sophora flavescens", *Biol Pharm Bull*, Vol. 31, No. 11, pp. 2097-2102, Nov, 2008.
DOI: https://doi.org/10.1248/bpb.31.2097

[6] H. Yoo, S. K. Ku, W. Lee, S. Kwak, Y. D. Baek, B. W. Min, G. S. Jeong and J. S. Bae, "Antiplatelet, anticoagulant, and profibrinolytic activities of cudratricus xanthone A", *Arch Pharm Res*, Vol.37, No.8, pp.1069-1078, Aug, 2014.
DOI: https://doi.org/10.1007/s12272-013-0290-4

[7] S. Geue, B. Walker-Allgaier, D. Eißler, R. Tegt- meyer, M. Schaub, F. Lang, M. Gawaz, O, Borst, P. Münzer, "Doxepin inhibits GPVI-dependent platelet $Ca^{2+}$ signaling and collagen-dependent thrombus formation", *Am J Physiol Cell Physiol*, Vol.312, No.6, pp.C765-C774, Apr, 2017.
DOI: https://doi.org/10.1152/ajpcell.00262.2016

[8] W. Bergmeier, L. Stefanini, "Platelet ITAM signaling", *Curr Opin Hematol*, Vol.20, No.5, pp.445-450, Sep, 2013.
DOI: https://doi.org/10.1097/MOH.0b013e3283642267

[9] R. H. Lee, W. Bergmeier, "Platelet immunoreceptor tyrosine-based activation motif (ITAM) and hemITAM signaling and vascular integrity in inflammation and development", *J Thromb Haemost*, Vol.14, No.4, pp.645-654, Apr, 2016.
DOI: https://doi.org/10.1111/jth

[10] C. Feng, C. B. Post, "Insights into the allosteric regulation of Syk association with receptor ITAM, a multi-state equilibrium", *Phys Chem Chem Phys*, Vol.18, No.8, pp.5807-5818, Feb, 2016.
DOI: https://doi.org/10.1039/c5cp05417f

[11] J. H. Ryu, J. Y. Ro, H. J. Park, H. J. Cho, "Anti-platelet effect of Ginkgolide A from *Ginkgo biloba*", *J Korean Soc Appl Biol Chem*, Vol.57, No.2, pp.221-228, Apr, 2014.
DOI: http://doi.org/10.1007/s13765-013-4275-2

[12] H. J. Cho, H. J. Kang, Y. J. Kim, D. H. Lee, H. W. Kwon, Y. Y. Kim, H. J. Park, "Inhibition of platelet aggregation by chlorogenic acid *via* cAMP and cGMP-dependent manner", *Blood Coagul Fibrinolysis*, Vol.23, No.7, pp.629-635, Oct, 2012.
DOI: https://doi.org/10.1097/MBC.0b013e3283570846

[13] J. H. Shin, H. W. Kwon, H. J. Cho, M. H. Rhee, H. J. Park, "Vasodilator-stimulated phosphoprotein-phosphorylation by ginsenoside Ro inhibits fibrinogen binding to aIIb/b3 in thrombin-induced human platelets", *J Ginseng Res*, Vol.40, pp.359-365, 2016.
DOI: http://doi.org/10.1016/j.jgr.2015.11.003

[14] Y. Wu, S. J. Gunst, "Vasodilator-stimulated phosphoprotein (VASP) regulates actin polymerization and contraction in airway smooth muscle by a vinculin-dependent mechanism", *J Biol Chem*, Vol.290, No.18, pp.11403-11416, May, 2015.
DOI: https://doi.org/10.1074/jbc.M115.645788

[15] J. Y. Ro, J. H. Ryu, H. J. Park, H. J. Cho, "Onion (Allium cepa L.) peel extract has anti-platelet effects in rat platelets", *SpringerPlus*, Vol.4, No.7, pp.1-8, Jan, 2015.
DOI: https://doi.org/10.1186/s40064-015-0786-0

[16] D. S. Bush, R. L. Jones, "Measurement of cytoplasmic calcium in aleurone protoplasts using indo-1 and fura-2; Progress and Prospects", *Cell Calcium*, Vol.93, No.3, pp.841-845, Jul, 1990.
DOI: https://doi.org/10.1104/pp.93.3.841

[17] K. Anders, "Resolution of Students t-tests, ANOVA and analysis of variance components from intermediary data", *Biochem Med (Zagreb)*, Vol.27, No.2, pp.253-258. Jun, 2017.
DOI: https://doi.org/10.11613/BM.2017.026

[18] J. H. Shin, H. W. Kwon, M. H. Rhee, H. J. Park, "Inhibitory effects of thromboxane $A_2$ generation by ginsenoside Ro due to attenuation of cytosolic phospholipase $A_2$ phosphorylation and arachidonic acid release", *J Ginseng Res*, Vol.43, No.2, pp.236-241, Apr, 2019.
DOI: https://doi.org/10.1016/j.jgr.2017.12.007

[19] D. H. Lee, Y. J. Kim, H. H. Kim, H. J. Cho, J. H. Ryu, M. H. Rhee, H. J. Park, "Inhibitory effects of epigallocatechin-3-gallate on microsomal cyclooxygenase-1 activity in platelets", Vol.21, no.1, pp.54-59, Jan, 2013.
DOI: https://doi.org/10.4062/biomolther.2012.075

[20] R. Lorenz, D. Betinetti, F. W. Herberg, "cAMP-Dependent Protein Kinase and cGMP-Dependent Protein Kinase as Cyclic Nucleotide Effectors", *Handb Exp Pharmacol*, Vol.238, pp.105-122, 2017.
DOI: https://doi.org/10.1007/164_2015_36

[21] H. Jahn, W. Nastainczyk, A. Rohrkasten, T. Schneider, F. Hofmann, "High-Throughput $Ca^{2+}$ Flux Assay To Monitor CyclicNucleotide-Gated Channel Activity and Characterize Achromatopsia Mutant Channel Function", *ACS Chem Neurosci*, Vol.10, No.8, pp.3662-3670, Aug, 2019.
DOI: https://doi.org/10.1021/acschemneuro.9b00231

[22] P. F. Monteiro, R. P. Morganti, M. A. Delbin, M. C. Calixto, M. E. Lopes-Pires, S. Marcondes, A. Zanesco, E. Antunes, "Platelet hyperaggregability in high-fat fed rats: a role for intraplatelet reactive-oxygen species production", *Cardiovasc Diabetol*, Vol.11, pp.5, Jan, 2012.
DOI: https://doi.org/10.1186/1475-2840-11-5

Ju-Ye Ro [Regular member]

- Feb. 2012 : Konyang Univ., Biomed. Lab. Sci., BS
- Feb. 2014 : Konyang Univ., Biomed. Lab. Sci., MS
- Mar. 2014 ~ Aug. 2017 : Konyang Univ., Dept. of Public Health, PhD

〈Research Interests〉
Hematology, Neuroscience

Hyun-Jeong Cho [Regular member]

- Feb. 2002 : Inje Univ., Biomed. Lab. Sci., MS
- Feb. 2005 : Inje Univ., Biomed. Lab. Sci., PhD
- Mar. 2005 : Pharmafoods LtD., Japan, Post-doc
- Feb. 2008 ~ current : Konyang Univ., Dept. of Biomed. Lab. Sci., Professor

〈Research Interests〉
Hematology, Neuroscience