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Evaluation of the Effects of *Acorus gramineus* and *Acorus tatarinowii* Extracts on a Rat Model of Arterial Thrombosis induced by Ferric Chloride

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Abstract – We investigated the antithrombotic effect of *Acorus gramineus* Soland (*A. gramineus*) from Korea and *Acorus tatarinowii* Schott (*A. tatarinowii*) from China in a rat model of arterial thrombosis induced by ferric chloride ($FeCl_3$). Thirty minutes prior to a 35% $FeCl_3$ application, Sprague Dawley rats were intraperitoneally injected with saline, *A. gramineus* and *A. tatarinowii* (100 mg/kg), respectively. Occlusion time of rats injected with *A. gramineus* was delayed significantly compared to that of the vehicle and *A. tatarinowii*. Thrombus weight was meaningfully decreased in rats injected with *A. gramineus* compared to the vehicle. Additionally, *A. gramineus* inhibited collagen fiber damage in vessel compared to the vehicle, but *A. tatarinowii* did not show a significant effect. Our results show that *A. gramineus* and *A. tatarinowii* from the same genus have different antithrombotic effects, and especially *A. gramineus* has a better antithrombotic effect than *A. tatarinowii.*
Keywords – Acori Rhizoma, *Acorus gramineus* Soland, *Acorus tatarinowii* Schott, $FeCl_3$- induced carotid arterial rat model, Thrombosis

Introduction

A thrombus is a blood clot that forms in blood vessels and interrupts blood circulation. A thrombus may cause cardiovascular or cerebrovascular diseases such as myocardial infarction, stroke, or cerebral infarction due to the discontinuity in oxygen supply (Yoo *et al*., 2010). A thrombus occurs because of abnormal blood clotting function, which is an imbalance between the blood coagulation systems, fibrin formation, and the thrombolysis system involved in fibrin elimination; if vascular endothelial cells are damaged, certain changes occur in platelets (Lyu, 2011). When blood vessels are damaged, platelets attach to the damaged surface and aggregate with each other, thereby becoming a mass with uncertain boundary (Erhardt *et al*., 2006). Adenosine diphosphate (ADP) and serotonin may induce aggregation within platelets. Moreover, a platelet plug is formed by a reaction among collagen, fibrin, serotoin, ADP, and epinephrine with fibrinogen and mucopolysaccharides, and a thrombus is generated. In particular, powerful vasoconstrictors (epinephrine, levonordefrin, and phenylephrine), and the platelet agglutinogen (thromboxane A_2) are produced by platelets, and a thrombosis is induced where the vascular endothelium is damaged (Eddy *et al*., 1986). As the diseases caused by thrombus can be prevented by managing vascular risk factors, fibrinolytic enzymes, thrombolysis agents, anticoagulants, and antiplatelet agents have been utilized in clinical studies to prevent and treat thrombus (Choi *et al*., 2001, Hirsh *et al*., 2003, Honda *et al*., 2006). However, such drug therapies possess disadvantages such as high cost, intravenous injection, diarrhea, and difficulties predicting the antithrombotic effects due to the non-specific effects of the proteins involved in thrombus formation (Choi *et al*., 2001, Hirsh *et al*., 2003, Honda *et al*., 2006). Thus, many studies have investigated antithrombotic agents or candidates from various herbal medicines to prevent thrombus, which are possibly safer and less expensive alternatives to drug therapies (Sohn *et al*., 2006, Kim *et al*., 2010).

The origins of Acori Rhizoma species differ by countries (Table 1); Although the Chinese characters of the names of *Acorus gramineus* Soland (*A. graminus*) and *Acorus tatarinowii* Schott (*A. tatarinowii*) are the same, the Acori Rhizoma that originated from Korea is officially Acori Graminei Rhizoma in Korean Herbal Pharmacopoeia, whereas that from China is Acori (Tatariinowii) Rhizoma in Chinese Pharmacopoeia meaning that each country

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defines and classifies Acori Rhizoma differently (Choi *et al*., 2006). According to China's first pharmacology book '*Sinnongbonchokyeong*', Acori Rhizoma "Mainly treats skeletal pain due to stagnation of blood Gi due to the combination of three kinds of Gi such as wind, cold and dampness; helps cough with asthma; circulates the nine orifices to prevent blocking; strengthens five organs (heart, liver, spleen, lungs, and kidneys), clears the eyes and ears and makes voice". That book only describes treatments for certain diseases but there were no explanations with regard to origins. Further, it did not subcategorize Acori Rhizoma and *Acorus calamus*, but only named as '*Acorus calamus var. angustatus BESS*' (Choi *et al*., 2004). Acori Rhizoma, has a known effect on GaeGyu (opening the orifices), removes phlegm by opening the orifices when phlegm is abundant and patients lose consciousness, make Gi movement smooth after drying damp, eliminates and disperse pathogenic wind and treats paralysis, cures asthma, awakens the spirit, and improves depressed Qi circulation due to wet phlegm, indicating that it is likely to be related with systematic circulation. Furthermore, Acori Rhizoma has been used for symptoms such as faint, calenturish and epilepsy, cold limbs and headache, and cures symptoms when the patient is unable to open the mouth to eat or speak because of closed teeth caused by apoplexy or other symptoms, and wet stroke, as well as paralysis accompanying pain ((National College of Oriental Medicine, Joint Textbook Compilation Committee., 2004; Choi *et al*., 2004). Although the two species have different origins and scientific names, they have been used and named as Acori Rhizoma; thus, multiple investigations have been conducted with regard to differentiating the origins of these two species (Chio *et al*., 2006). Acori Rhizoma is difference of morphology and constituents but also the expression of specific gene makes a difference. (Monn *et al*., 2011). Additionally, differences in the main components such as β-asarone and α-asarone have been reported for the hot-water and methanol extracts of *A. gramineus* and *A. tatarinowii* (Greca *et al*., 1989). Additionally, neuroprotective effects (Kim *et al*., 2000), antimicrobial activity (Jung and Kim, 2008), antitumor activity (Oh *et al*. 2007), improvement of memory and cognitive function (Choi *et al*., 2011), and thrombosis and elevated blood viscosity effects (Cho *et al*., 1994) have been reported for patients administered Acori Rhizoma. But, Acori Rhizoma is to comparison of the biological activity from little research. Recently study in four species of Acorus Rhizoma by comparing the antioxidant have been reported (Chio *et al*., 2011). And, according to our previous study regarding the effects of Acori Rhizoma water extracts on human whole blood, *A. gramineus* provides excellent platelet aggregation inhibitory effects (Chio *et al*., 2010). In preliminary data, we found that the efficacy of *A. gramineus* was better than that of *A. tatarinowii*. The objective of this study was to determine whether *A. gramineus* and *A. tatarinowii* have different antithrombotic effects, and to categorize the different effects in a $FeCl_3$-induced carotid arterial thrombosis model. As a result, apparent differences in potency of each Acori Rhizoma species depending on the country of production were showed; the *A. gramineus* produced from Korea showed a greater antithrombotic effect than that of *A. tatarinowii* produced from China in a ferric chloride ($FeCl_3$) induced animal model.

Experimental

Materials – Identical medicinal herbs were utilized in the present study as in other studies conducted by our research group (Chio *et al*., 2010). A batch of Acori Rhizoma originating from either China or Korea in a standard packaged form or cut fresh plants were purchased from Gwangmyeongdan Medicine Inc. (Ulsan, Korea); these samples were used after identification based upon morphological criteria (Table 1).

Animals – The study was carried out in the Animal Study Laboratory of the Korea Institute of Oriental Medicine. Ten week old male Sprague–Dawley rats (220 - 250 g) were obtained from Orientbio Inc, (Gyeinggi do, Korea). This study protocol was approved by the experimental animal ethics committee of the Korea Institute of Oriental Medicine (approval number 12-033). Once the rats were obtained, their appearance was examined and general symptoms were observed for 7 days during the adaptation period. Animals were

Table 1. Information of two herbal materials used in this study

Herbal medicine	Scientific name	Origin	Hot water extracts		
			Weight (g)	Extract (g)	Yield (%)
Acori Tatarinowii Rhizoma	*Acorus tatarinowii* Schott	China	20.00	2.81	14.1
Acori Gramineus Rhizoma	*Acorus gramineus* Soland	Korea	20.00	3.25	16.3

randomly grouped by weight ranges. Solid food and water were provided *ad libitum* during the entire period; the temperature (25 ± 5 ºC), humidity (50 ± 10%), and a 12 h light/dark cycle were maintained in the animal room. Rat management, utilization, and treatment followed the National Institutes of Health (Bethesda. MD, USA) criteria and animal experiment guideline established by Korean Academy of Medical Sciences (2000).

Preparation of hot water extracts from the different Acori Rhizoma – The medicinal herbs were pulverized in a blender and reflux extracted to prepare the extract. The herbs (20.0 g) and solvent (400 mL distilled water) were mixed in a 1 L round flask and hot water extraction was carried out on a heating mantle equipped with a reflux condenser for 2 h. Then, the samples were cooled down to room temperature and filtered via cotton wool, followed by evaporation. The samples were dried in a dying oven (No-600M frying oven, Jeio Tech, Korea) at 105 ºC for 10 h to remove all moisture. The obtained samples were powdered and kept in the refrigerator until further analysis. Table 1 was shown that the samples were diluted in physiological saline solution before being utilized (Jeon *et al*., 2008).

$FeCl_3$-induced carotid arterial thrombus model preparation and drug injection – Based upon previous methods (Robinson *et al*., 2003, Soni *et al*., 2008, Wang *et al*., 2006), the optimized conditions for $FeCl_3$ induced thrombus in the rat model were established (Lee *et al*., 2011). Eighteen male rats (300 ± 50 g) were adapted for 1 week, and then utilized in the study. Thirty minutes prior to the induction of thrombus with $FeCl_3$, rats were given vehicle (saline), *A. tatarinowii* Schott (100 mg/kg), or *A. gramineus* Soland (100 mg/kg), via intraperitoneal injection. Once anesthetized with isoflurane, body temperature of the animals was maintained at 37 ºC using a temperature controller; the left carotid artery was exposed and placed on the laser tissue blood flowmeter. Filter paper was immersed in 35% $FeCl_3$ and placed on the carotid artery, 0.8 mm in front of the Doppler probe and applied for 3 min. The filter paper was removed, washed with saline, and the blood stream was measured using LDF for up to 30 min.

Blood vessel tissue sample preparation and staining – A carotid arterial thrombus was induced in all rats and thrombus formation time was measured; rats were sacrificed and 3 - 4 mm of the carotid artery was isolated followed by 24 h fixation in 4% neutral paraformaldehyde. Paraffin blocks and tissue samples were prepared by placing the 4 μm thick slice of tissue block in a rotating microtome. Once deparaffinized, samples were dehydrated through a grade series of 50% alcohol to anhydrous alcohol and then with xylene followed by staining with hematoxylin & eosin (H&E) and Masson's trichrome solution. Stained sample tissues were observed for morphological changes with an optical microscope (Olympus Bx 51, Tokyo, Japan)

Quantification of collagen fiber tissue – Once blood vessel endothelial cells are damaged, collagen, which is located beneath the cells, is exposed and platelets attach; serotonin and ADP leak, and the coagulated platelet causes a thrombus (Yang *et al*., 2007). Due to endothelial cell damage of the blood vessels, collagen fibers are exposed and thrombus formation is initiated; thus, quantifying the remaining collagen fibers would determine the potency of the samples with regard to blood vessel protection. Cross sectional slices of blood vessel tissue were stained with Masson's trichrome staining, which is specific for collagen fibers; samples were observed with an optical microscope and damage severity to the collagen fibers in the blood vessels was analyzed using an image analysis program (Meta Imaging Series 7.7, USA). The area ratio between the collagen fibers in the blood vessels and the entire blood vessel tissue was measured and quantified.

Statistical analysis – All results are expressed as mean ± standard deviation. All data were analyzed using one-way ANOVA followed by the Tukey's post-hoc test. All analyses were performed using the Statistical Package for Social Science (SPSS, version 12.0, USA) and R package version 3.0.0 for Windows. p-values < 0.05 were considered significant.

Results and Discussion

Along with changes in diet and social development, the average life span in Korea has been increasing every year, which is accompanied by increases in geriatric diseases (National Statistical Office). Geriatric, cerebrovascular, and heart diseases exhibit high mortality rates, making such diseases critical factors to determine the quality of life of the elderly; thus, the significance of prevention has been emerging rather than treatment after disease onset (Ok and Cho, 2005). Thrombus is known major etiological factor for vascular diseases and a kind of blood clotting in blood vessels or the heart that causes blood circulation disorders, decreased blood circulation speed, and abnormal blood viscosity thereby inducing various pathological symptoms in tissues and organs (Ryn *et al*., 2010). Vascular diseases caused by thrombus are generally due to the accumulation of body waste in blood vessels; this

Table 2. Effects of Acori Rhizoma water extracts on $FeCl_3$-induced arterial thrombosis

Herbal medicine	Dose (mg/kg)	n	TTO (min)	TTO reduction (ratio)	TW (mg/mm)	TW inhibition (%)
Vehicle	Saline	6	7.67 ± 0.52	–	0.79 ± 0.02	–
Acori Tatarinowii Rhizoma	100	6	8.67 ± 1.03	1.08 ± 0.13	0.77 ± 0.02	1.29 ± 1.39
Acori Gramineus Rhizoma	100	6	13.17 ± 1.33$^{***, ###}$	1.80 ± 0.20	0.73 ± 0.01$^{***, ##}$	8.35 ± 3.19

$^{***}p < 0.001$ compared with than those vehicle group
$^{##}p < 0.01$and $^{###}p < 0.001$ compared with than those Acori Tatarinowii Rhizoma group.

hinders the blood circulation, and the supply of nutrients and oxygen to tissues eventually causing cerebrovascular diseases, hemorrhage, stroke, heart attack, and heart failure and heart diseases (National Statistical office). Atherosclerotic blood vessels due to thrombus are most likely because of the exacerbation of platelet aggregation (Park *et al*., 2009). As cerebrovascular and heart diseases can be prevented by controlling risk factors such as obesity, hypertension, and diabetes, the drug therapies utilizing platelet aggregation inhibitors (aspirin, dipyridamole), thrombolytic enzymes (streptokinase, urokinase, and aminocaproic acid) and anticoagulants (heparin and wafarin) are used to prevent and treat thrombus formation (Choi and Kim, 2001, Hirsh *et al*., 2003, Honda *et al*., 2006). In addition, anti-platelet agents (aspirin, dipuydanol, and cilostazol) are being used as well (Greca *et al*., 1989, Park, 2011, Kim *et al*., 2000, Jung and Kim, 2008, Oh *et al*., 2007). Such products to treat and prevent of thrombus are not specific for thrombus and possess some side effects such as systematic bleeding and short half life. Furthermore, these drugs have high costs and long-term use is difficult. Therefore, there are multiple ongoing investigations utilizing various herbal medicines to identify safer and inexpensive thrombus preventive agents to overcome such disadvantages (Sohn *et al*., 2006, Kim and Jeon, 2010). Investigations regarding the antithrombus effects of herbal medicines have been conducted using various approaches, as thrombus is believed to be related with pathological mechanisms of stasis and abnormal Qi distribution in Oriental Medicine. Various herbal medicines have been utilized for the antithrombus effects such as *Salvia miltiorrhiza* Bunge for anti-blood stasis effects (Yang *et al*., 2007), *Paeoniae Radix* Alba for blood circulation (Ju *et al*., 2008), modified-Jehotang (Kim and Jeon, 2010) and *Lonicera japonica* Thunberg (Yang *et al*., 2011) for relieving heat, and *Syzygium aromaticum* (Baek and Roh, 2011) and persimmon tree stem (Kurz *et al*., 1990) for treating stagnant and inverse Qi. The antithrombus effects have been approached based upon multiple mechanisms, meaning that such medicines are not only directly related to stasis symptoms but also associated with others manifestations including abnormal Qi distribution, inverse Qi, and relieving heat with cold medicines. Acori Rhizoma opens holes and possess efficacy to relieve phlegm through opening holes, controlling moisture, treating stroke, paralysis, swelling and pain, relieving dampness, and supplementing the functions of stomach and the intestines, opening holes and stopping phlegm, awaking spirit and making patients smarter; it has also been used to treat fever and coma, fainting, calenturish (e.g., malaria), epilepsy, bad memory, tinnitus, deafness, abdominal pain, and symptoms in which the patient is unable to open their mouth to eat or speak because of closed teeth caused by apoplexy or other symptoms, wet stroke, and paralysis accompanying pain (National College of Oriental Medicine, Joint Textbook Compilation Committee., 2004). We hypothesized that such effects (i.e., relieving phlegm, and circulating Qi) would be protective against thrombus and delay of the blood stream. A previous study conducted by our research group indicated that *A. gramineus* was more effective with regard to antiplatelet activity compared to that of *A. tatarinowii* (Chio *et al*., 2010). Hence, in the present study, we selected Acori Rhizoma from China and Korea and verified their antithrombotic efficacy. The $FeCl_3$-induced carotid arterial thrombus animal model was established to identify the mechanisms of thrombus formation (Kurz *et al*., 1990); we chose the same animal model as it was suggested in our previous investigation that an animal model of administering 35% $FeCl_3$ is the most stable and promising to determine the potency of samples (Lee *et al*., 2011). As a result, Table 2, we found significant differences in thrombus formation time, decreased thrombus weight, and inhibition of damage to carotid artery blood vessels between groups administered either Acori Rhizoma from Korea or China. Fig. 1. the results of the Laser tissue blood flowmeter, to determine the antithrombus potency, the group administered *A. gramineus* showed delayed the $FeCl_3$ induced thrombus formation time by 1.80 ± 0.20% (***$p < 0.001$) and 1.54 ± 0.28% ###$p < 0.001$) compared to that in the vehicle and the group administered *A. tatarinowii*, respectively.

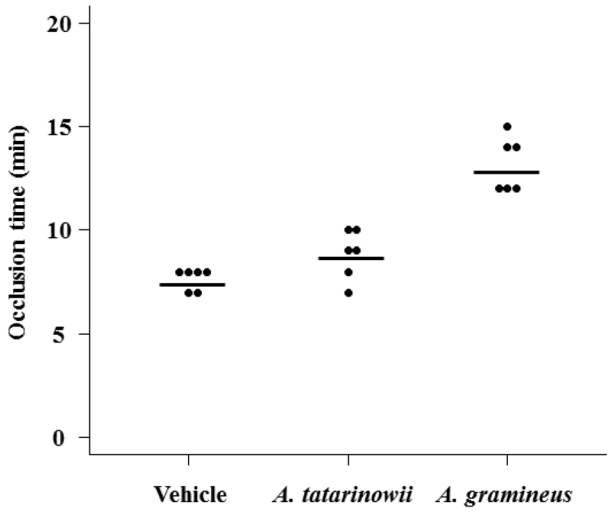

Fig. 1. Effects of a Acori Rhizoma hot water extract on occlusion time in $FeCl_3$-induced carotid arterial thrombosis rat from vehicle (saline), *A. tatarinowii* (100 mg/kg) and *A. gramineus* (100 mg/kg). Results are expressed mean ± standard deviation (n = 6). $^{***}p < 0.001$ vs. vehicle group, $^{###}p < 0.001$ vs. *A. tatarinowii* group. *A. tatarinowii*; *Acorus tatarinowii* Schott, *A. gramineus*; *Acorus gramineus* Soland.

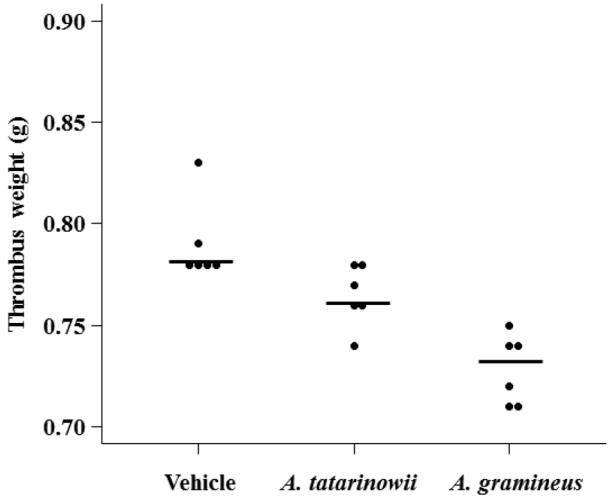

Fig. 2. Thrombus weight of *Acorus tatarinowii* and *Acorus gramineus* hot water extract on $FeCl_3$-induced carotid arterial thrombosis rat. Results are expressed mean ± standard deviation (n = 6). $^{***}p < 0.001$ vs. vehicle group, $^{##}p < 0.01$ vs. *A. tatarinowii* group. *A. tatarinowii*; *Acorus tatarinowii* Schott, *A. gramineus*; *Acorus gramineus* Soland.

Furthermore, the results (Fig. 2.) showed that thrombus weight of the *A. gramineus* group 8.35 ± 3.19% ($^{***}p < 0.001$), and 4.68 ± 2.15% ($^{##}p < 0.01$) reduction in thrombus weight compared to those in the vehicle and the group administered *A. tatarinowii*. The H&E stain, which shows structural changes in vascular tissues, was utilized to determine whether vascular endothelium and smooth muscle cells proliferated and whether the blood vessels

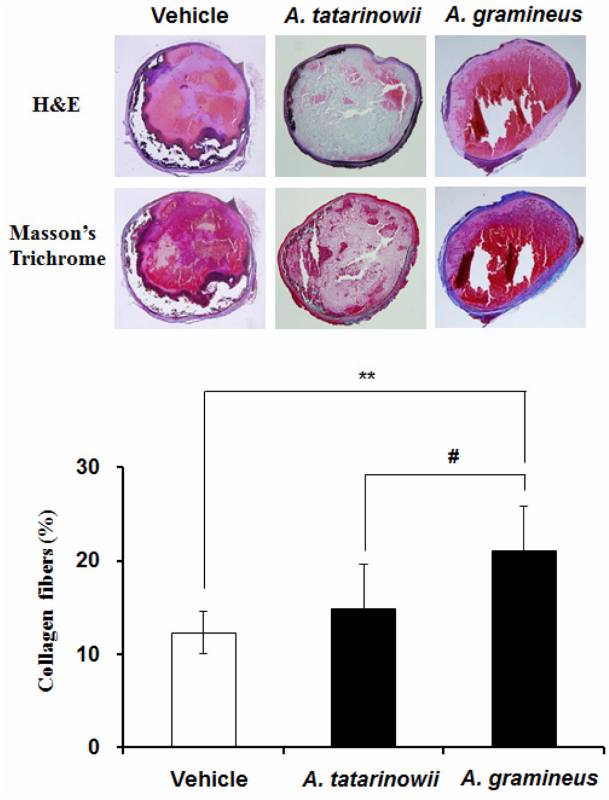

Fig. 3. Histological examination of $FeCl_3$-induced thrombus from the vehicle, *Acorus tatarinowii*, and *Acorus gramineus* groups. These groups were given the Acori Rhizoma extracts intraperitoneally 30 min prior to ferric chloride. All sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome. Results are mean ± standard deviation (n = 6). $^{**}p < 0.01$ vs. vehicle group, $^{#}p < 0.05$ vs. *A. tatarinowii* group. *A. tatarinowii*; *Acorus tatarinowii* Schott, *A. gramineus*; *Acorus gramineus* Soland.

were shielded by the thrombus. As a result, we confirmed that the thrombus was generated by $FeCl_3$. Additionally, Masson's trichrome staining was performed to determine sample potency with regard to the collagen fibers that were exposed when blood vessels were damaged. Fig. 3. the results showed that protection of blood vessels damage, as measured by the ratio between collagen fiber tissue and all blood vessels tissue, increased significantly by 1.61 ± 0.41 fold ($^{**}p < 0.01$) and 1.49 ± 0.27 fold ($^{#}p < 0.05$) in the *A. gramineus* group compared to those of the vehicle and the group administered *A. tatarinowii*. Although significant collagen fiber damage was observed in blood vessels of the vehicle group, *A. gramineus* administration significantly inhibited the damage to collagen fibers and reduced thrombus formation. Thus, we confirmed that *A. gramineus* showed more potent protection for blood vessels compared to that of *A. tatarinowii*.

Overall, we confirmed the antithrombus effects of

Acori Rhizoma in this study. In particular, *A. gramineus* showed more potent effects compared to that from China. These results agree with those from our previous studies (Chio *et al*., 2010) as well as investigations concerning the different composition of active compounds in extracts based on the origins of Acori Rhizoma (Chio *et al*., 2006, Chio *et al*., 2010). Such differences are likely due to origins. Furthermore, it can be speculated that the efficacy of the medicine might be lost as time elapsed from the harvest. However, we were unable to confirm the storage duration during the distribution processes of Acori Rhizoma. Thus, further investigations regarding the effects of distribution processes and the contents of unstable volatile (aromatic) compounds and their efficacy are warranted. In addition, future studies are necessary to identify the different profiles of active components in these Acori Rhizoma species, depending upon the origin, using the simultaneous analysis method. In summary, the *A. gramineus* extract possessed better potency than that of *A. tatarinowii* with regard to anti-thrombus effects. Therefore, the efficacy of *A. gramineus* against cerebrovascular and cardiovascular diseases is warranted.

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References

Baek, K.M. and Roh, S.S., Effects of Aqueous extract of *Diospyros kaki* calyx on anti-thrombotic activity in vitro and in vivo. *Korean. J. Herbol.,* **26**, 139-147 (2011).

Chio, G.Y., Kim, S.K., Lee, I.S., Baek, J.S., and Jeon, W.K., Effects of Acori Rhizoma extract on the in vitro anti-platelet activity in human whole blood. *Korean. J. Herbol.,* **25**, 91-95 (2010).

Cho, K.H., Kim, Y.S., Bae, H.S., Lee, K.S., and Kwon, J.N., Effects of Gyuntongdodamtang on thrombosis and elevated blood viscosity in rats. *J. Kyung Hee. Univ. Med. Cent.,* **10**, 134-149 (1994).

Choi, C.H., Kim, S.H., and Chung, D.K., Studies on the nootropic and anti-amnestic and anxiolytic-like effects of G.J.D-P.P.A. in mice. *J. Orient. Neuropsychiatry.,* **22**, 85-105 (2011).

Choi, G.Y., Kim, H.J., and Ju, Y.S., Study on internal-external morphological analysis in Acori Graminei Rhizoma. *Korean. J. Orient. Med.,* **12**, 91-100 (2006).

Choi, GY., Ko, B.S., Lee, M.Y., Chae, S.W., Kim, Y.H., Ryuk, J.A., Baek, J.J., and Lee, H.W., Comparative Study of Changpo (Chângpú) species on Antioxidant Activity and HPLC Pattern Analysis *Kor. J. Herbology.*, **26**, 13-19 (2011).

Choi, H.S., Aroma evaluation of an aquatic herb, changpo (*Acorus calamus* Var. *angustatus* Bess), by AEDA and SPME. *J. Agric. Food. Chem*., **52**, 8099-8104 (2004).

Choi, N.S. and Kim, S.H., The effect of sodium chloride on the serine-type fibrinolytic enzymes and the thermostability of extracellular protease from *Bacillus amyloliquefaciens* DJ-4. *Bio. and Mol. Biol.,* **34**, 134-138 (2001).

Eddy, C.A., Laufe, L.E., Dunn, R.L., and Gibson, J.W., The use of prostacyclin analogue-containing suture for the prevention of postperative venous thrombosis in the rat. *Plastic. Reconstructive. Surgery.,* **78**, 504-512 (1986).

Erhardt, J.A., Toomey, J.R., Douglas, S.A., and Johns, D.G., $P2X_1$ stimulation promotes thrombin receptor-mediated platelet aggregation. *J. Thromb. Haemost.,* **4**, 882-890 (2006).

Greca, M.D., Monaco, P., Previtera, L., Aliotta, G., Pinto, G., and Pollio, A., Allelochemical activity of phenylpropanes from *Acorus gramineus*. *Phytochem.,* **28**, 2319-2321 (1989).

Hirsh, J., Fuster, V., Ansell, J., and Halperin, J.L., American Heart Association; American College of Cardiology Foundation. American heart Association/American College of Cardiology Foundation guide to warfarin therapy. *Circul.,* **107**, 1692-1711 (2003).

Honda, F., Imai, H., Ishikawa, M., Kubota, C., Shimizu, T., Fukunaga, M., and Saito, N., Cilostazol attenuates gray and white matter damage in a rodent model of focal cerebral ischemia. *Stroke.,* **37**, 223-228 (2006).

Jeon, W.K., Kim, Y.E., Park, S.O., Kwon, D.Y., Ahn, S.W., Lee, J.H., Ji, M.S., and Ko, B.S., The modified Je-Ho-Tang, korean herbal medicine, inhibits whole-bllod aggregation and platelet adhesion to collagen under flow. *Thromb. Res.,* **122**, 804-809 (2008).

Ju, S.T., Lee, M.J., Lee, H.S., Jung, H.J., Kim, H., Kim, J.E., Park, S.D., and Park, W.H., Screening of anti-atherosclerotic effect of Lonicera Flower by antioxidative and anti-thrombotic mechnism. *Korean. J. Orient. Physiol. Pathol.,* **22**, 1509-1517 (2008).

Jung, E.R. and Kim, Y.B., The effects of *Acorus gramineus* on changes of nasal tissue in allergic rhinitis model. *J. Orient. Med. Ophthalmol. Otolaryngol. Dermatol.,* **21**, 16-25 (2008).

Kim, S.K., and Jeon, W.K., Effects of modified Je-Ho-Tang on ferric chloride-induced thrombosis in a rat model and of peripheral circulatory disturbance in a mouse model. *J. Korean. Soc. Appl. Biol. Chem.,* **53**, 842-846 (2010).

Kim, Y.O., Kim, Y.S., Leem, K.H., Suk, K.H., Kim, S.Y., Park, J.Y., Ahn, D.K., and Kim, H.C., Neuroprotective effects of Acori Graminei Rhizoma on the brain ischemia induced by four-vesel occlusion in rats. *Korean. J. Herbol.,* **15**, 79-85 (2000).

Kurz, K.D., Main, B.W., and Sandusky, G.E., Rat model of arterial thrombosis induced by ferric chloride. *Thromb. Res.,* **60**, 269-280 (1990).

Lee, I.S., Choi, S.G., and Jeon, W.K., Optimization of ferric chloride induced carotid artery thrombosis model in a rat: Effect of *Ginko biloba* extracts. *Korean. J. Clin. Lab. Sci.,* **43**, 179-187 (2011).

Lyu, C.J., Introduction to coagulation system. *J. Kor. Soc. Neonatol.,* **18**, 1-5 (2011).

Monn, B.C., Ji, Y.U., Lee, Y.M., Chun, J.M., Lee, A.Y., Choo, B.K., and Kim, H.K., Development of SCAR Markers for the Authentication of Acori Rhizoma Based on the Analysis of RAPD and Multiplex-PCR. *Korea. J. Medicinal Crop sci*., **19**, 162-169 (2011).

National College of Oriental Medicine, Joint Textbook Compilation Committee., (2004). Herbology. p565-566. YoungLimSa. Seoul.

National Statistical office. http://www.index.go.kr/egams/stts/jsp/potal/stts/PO_ STTS_IdxMain.jsp?idx_cd=1012&bbs=INDX_001&clas_div=C&rootKey=1.48.0 (2012)

Oh, C.H., Kim, N.S., Lee, K.H., Kim, T.H., Bae, J.B., Kim, S.G., Jeon, H., Lim, J.P., Shin, T.Y., Lee, C.H., Jeong, S.I., and Kwon, J., Immuno-regulatory and anti-cancer effect of *Acorus gramineus* solander. *Korean. J. Orient. Physiol. Pathol.,* **21**, 869-873 (2007).

Ok, M. and Cho, Y.S., Screening of Fibrinolytic Enzyme Producing from

Microorgansms in Korean Fermented Soybean Paste and Optimum Conditions of Enzyme Production. *Korean. J. Food. Presev.,* **12**, 643-649 (2005).

Park, C.H., Phytochemical constituents of *Acorus gramineus*. *Arch. Pharmacal. Res.,* **34**, 1289-1296 (2011).

Park, S.G., Lee, M.J., Jung, H.J., Lee, H.S., Kim, H., Na, S.T., Park, S.D., and Park, W.H., Preventive effects of peony foot extracts on oxidative stress, thrombosis and atherosclerosis. *J. Korean. Oriental. Med.,* **30**, 88-103 (2009).

Robinson, M.A., Welsh, D.C., Bickel, D.J., Lyncj, J.J., and Lyle, E.A., Differential effects of sodium nitroprusside and hydralazine in a rat model of topical FeCl3-induced carotid artery thrombosis. *Thromb. Res.,* **111**, 59-64 (2003).

Ryu, H.Y., Ahn, S.M., Kim, J.S., and Sohn, H.Y., Evaluation of In-vitro Anticoagulation Activity of 33 Different Medicinal Herbs. *J. Life. Sci.,* **20**, 922-928 (2010).

Sohn, H.Y., Kwon, C.S., Son, K.H., Kwon, G.S., Rye, H.Y., and Kun, E.J., Antithrombin and Thrombosis Prevention Activity of Buckwheat Seed, *Fagopyrum esculentum* Moench. *J. Korea. Soc. Food. Sci. Nutr.,* **35**, 132-138 (2006).

Soni, H., Sharma, A., Bhatt, S., Jain, M.R., and Patel, P.R., Antithromotic effects due to pharmacological modulation of thrombin-activable fibrinolysis inhibitor in rats. *Pharmacol.,* **82**, 304-309 (2008).

Wang, X., Smith, P.L., Hsu, M.Y., Gailani, D., Schumacher, W.A., Ogletree, M.L., and Seiffert, D.A., Effects of factor deficiency on ferric chloride-induced vena cava thrombosis in mice. *J. Thromb. Haemo.,* **4**, 1982-1988 (2006).

Yang, S.A., Im, N.K., and Lee, I.S., Effect of methanolic extract from *Salvia miltiorrhiza* bunge on in vitro antithrombotic and antioxidative activities. *Korean. J. Food. Sci. Technol.,* **39**, 83-87 (2007).

Yang, Y.Y., Lee, M.J., Lee, H.S., and Park, W.H., Screening of antioxidative, anti-platelet aggregation and anti-thrombotic effects of Clove extracts. *Korean. J. Oriental. Physiology & Pathology.,* **25**, 471-481 (2011).

Yoo, J.H., Tea, J.B., and Kil, G.J., Anticoagulant activity of gelditsiae spina extract. *Korean. J. Herbol.,* **25**, 39-43 (2010).

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