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The Pharmacological Effects of Herbs on Catecholamine Signaling

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Abstract

Herbs have many biologically and pharmacologically active compounds such as flavonoids and stilbenes. They have been used in remedies for various disorders. Here we review the effects of herbs on catecholamine synthesis and secretion in cultured bovine adrenal medullary cells. Ikariside A (1.0–100 μM), a flavonol glycoside, inhibited the catecholamine secretion induced by acetylcholine (0.3 mM). This inhibition was associated with the suppression of $^{22}\text{Na}^+$ and $^{45}\text{Ca}^{2+}$ influx induced by acetylcholine. The ethanol extract (0.0003–0.005%) of matsufushi (extract of pine nodules) inhibited the catecholamine secretion induced by acetylcholine. SJ-2, one of the stilbene compounds isolated from matsufushi, inhibited acetylcholine-induced catecholamine secretion. Matsufushi extract and SJ-2 reversibly inhibited acetylcholine-induced Na^+ currents in *Xenopus* oocytes expressed with $\alpha 3\beta 4$ nicotinic acetylcholine receptors. Sweet tea is the processed leaves of *Hydrangea macrophylla*. The extract of sweet tea (0.3–1.0 mg/ml) suppressed catecholamine secretion induced by acetylcholine (0.3 mM). Moreover, sweet tea (0.1–1.0 mg/ml), ikariside A (1.0–100 μM), and matsufushi (0.001–0.003%) or SJ-2 (10–30 μM) inhibited acetylcholine-induced ^{14}C -catecholamine synthesis from ^{14}C -tyrosine. These findings indicate that ikariside A, matsufushi (or SJ-2), and sweet tea inhibit the catecholamine secretion and synthesis induced by acetylcholine in cultured bovine adrenal medullary cells and probably in sympathetic neurons.

Keywords: adrenal medullary cells, catecholamine secretion, ikariside A, matsufushi, sweet tea

1. Introduction

Since herbs have many biologically and pharmacologically active compounds such as flavonoids and stilbenes, they have been used in remedies for various disorders. A high dietary intake of herbs has become a focus of research because of herbs' potential to reduce the risks of diseases such as hypertension, coronary heart disease, diabetes, and cancers [1, 2]. Flavonoids are a group of plant secondary metabolites with variable phenolic structures, and they are found in plants, fruits, vegetables, roots, stems, flowers, wine, and tea [3, 4]. Over 5000 individual flavonoids have been reported [5], and 6 principal groups of flavonoids

(flavones, flavonols, flavanones, flavanols, isoflavones, and anthocyanidins) are relatively common in human diets [1]. Polyphenol stilbenes have attracted scientific attention. For example, resveratrol (*trans*-3,4',5-trihydroxy- stilbene) is a natural phytoestrogen found in grapes, berries, and red wine [6, 7] that was reported to be implicated in the beneficial effect of red wine, i.e., the lower incidence of coronary artery disease in certain populations such as the French and the Greeks, despite diets rich in saturated fat and a rate of high smoking, which has been dubbed the “French paradox” [8].

In the human body, the most abundant catecholamines are adrenaline, nor-adrenaline, and dopamine, all of which are produced from phenylalanine and/or tyrosine. Catecholamines are biosynthesized mainly in the adrenal medulla, the postganglionic fibers of the sympathetic nervous system, and the central nervous system [2, 9, 10]. Catecholamines play very important roles in aspects of the cardiovascular system such as heart rate and blood pressure, blood glucose levels, and the general functions of the central and peripheral sympathetic nervous system [9].

Adrenal medullary cells derived from embryonic neural crests are functionally homologous to sympathetic postganglionic neurons [2, 10]. Our research demonstrated that in cultured bovine adrenal medullary cells, at least three distinct types of ionic channels participate in catecholamine secretion, including nicotinic acetylcholine receptor (nAChR)-ion channels, voltage-dependent Na^+ channels, and voltage-dependent Ca^{2+} channels [2, 11]. In these cells, the Na^+ influx induced by acetylcholine (ACh) via nAChR-ion channels or by veratridine via voltage-dependent Na^+ channels is a prerequisite for Ca^{2+} influx via the activation of voltage-dependent Ca^{2+} channels and subsequent catecholamine secretion; in contrast, high K^+ directly gates voltage-dependent Ca^{2+} channels to increase the Ca^{2+} influx without increasing the Na^+ influx [10, 11] (**Figure 1**). ACh-induced Ca^{2+} influx is also a prerequisite for the stimulation of catecholamine synthesis associated with the activation of tyrosine hydroxylase [2, 12–15]. The mechanisms underlying the stimulation of catecholamine synthesis and secretion mediated by these ion channels in adrenal medullary cells are thought to be similar to those of

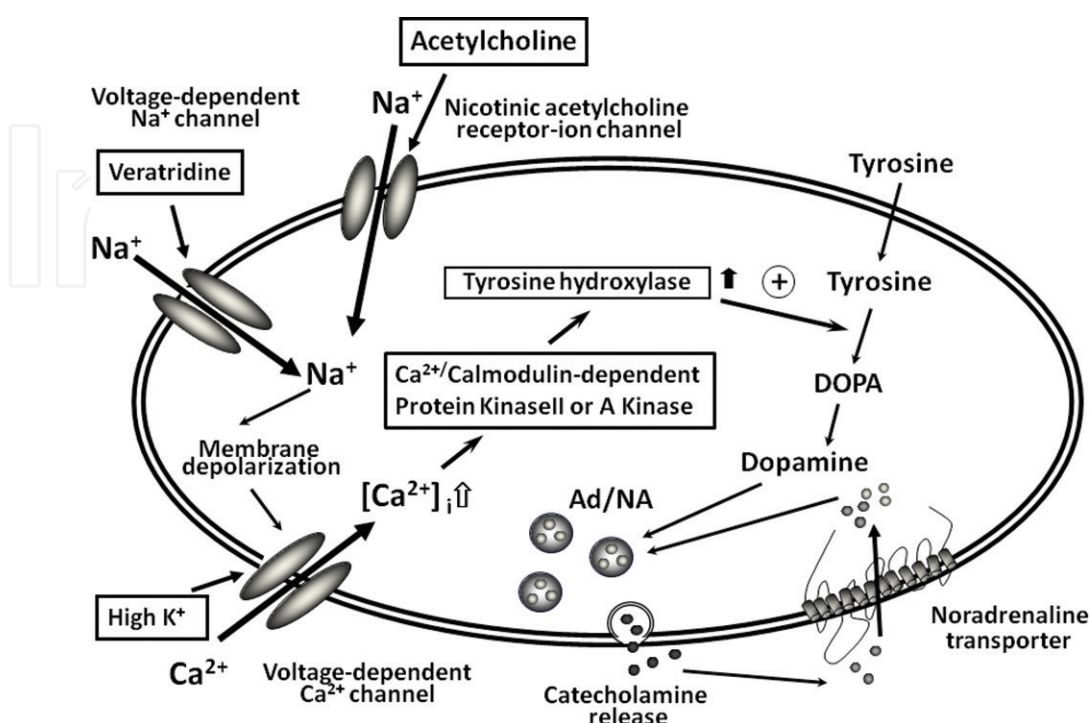


Figure 1. The mechanism underlying the regulation of catecholamine synthesis, secretion, and reuptake in bovine adrenal medullary cells.

noradrenaline in the sympathetic neurons and brain noradrenergic neurons [2]. Thus, adrenal medullary cells have provided a good model for the detailed analysis of antipsychotic [16], cardiovascular [17], and analgesic [18] drugs that act on catecholamine synthesis, secretion, and reuptake [2].

We have demonstrated the effects of several flavonoids and polyphenol stilbenes on catecholamine synthesis and secretion. For example, the treatment of bovine adrenal medullary cells with daidzein (an isoflavone derived from soy beans) stimulated basal catecholamine synthesis but inhibited the catecholamine synthesis and secretion induced by ACh [2, 19]. Genistein (another isoflavone in soy beans) but not daidzein stimulated the function of noradrenaline transporter in a human neuroblastoma cell line, SK-N-SH cells [2, 20]. Nobiletin (a compound of polymethoxy flavone in citrus fruits) stimulated the basal synthesis and secretion of catecholamines, but it suppressed both the ACh-induced synthesis of catecholamines and ACh-induced secretion of catecholamines [2, 21]. Resveratrol also inhibited the catecholamine synthesis and secretion induced by ACh [2, 22].

The present review summarizes our recent and current studies of the pharmacological effects of herbs and their components, i.e., ikarisoside A (a flavonol glycoside); matsufushi (extract of pine nodules), one of matsufushi's stilbene components (SJ-2); and sweet tea on the catecholamine signaling induced by ACh in cultured bovine adrenal medullary cells and on ACh-induced Na^+ current in *Xenopus* oocytes expressing $\alpha 3\beta 4$ nAChRs.

2. Inhibitory effects of ikarisoside A, but not its aglycon, on the catecholamine secretion and synthesis induced by ACh

Ikarisoside A is a natural flavonol glycoside derived from plants of the genus *Epimedium*, which have been used in traditional Chinese medicine as tonics, antirheumatics, and aphrodisiacs [10, 23, 24], and is used as a tonic supplement in Japan. Ikarisoside A has antioxidant and anti-inflammatory effects [23] and anti-osteoporosis effects [10, 25]. Icaritin, another flavonoid in the genus *Epimedium*, has an anti-stress effect in the forced swimming test in mice [26].

We observed that ikarisoside A (1–100 μM) concentration dependently inhibited the secretion of catecholamines induced by ACh (0.3 m) (**Figure 2A**), but not the secretion of catecholamines induced by veratridine and 56 mK^+ [10]. Ikarisoside A also suppressed the $^{22}\text{Na}^+$ influx and $^{45}\text{Ca}^{2+}$ influx induced by ACh in a concentration-dependent manner similar to that of catecholamine secretion (**Figure 2B, C**) [10]. Ikarisoside A is a flavonol glycoside with one rhamnose at the 3 position in the chemical structure. The aglycon of ikarisoside A is 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)-4H-chromen-4-one (**Figure 3A**). It is interesting to note that the aglycon of ikarisoside A had little effect on catecholamine secretion induced by ACh (0.3 m) (**Figure 3B**), suggesting that the rhamnose moiety at the 3 position of ikarisoside A is essential to inhibit the function of nAChR-ion channels [10]. Ikarisoside A (1.0–100 or 10–100 μM) inhibited ACh (0.3 mM)-induced ^{14}C -catecholamine synthesis from ^{14}C -tyrosine and tyrosine hydroxylase activity [10].

3. Inhibitory effects of matsufushi and its stilbene component, SJ-2, on the catecholamine synthesis and secretion induced by ACh

Pine nodules of *Pinus tabulaeformis* or *Pinus massoniana* are formed by pine bark proliferation at places on the trunk or limbs that have undergone damage, either

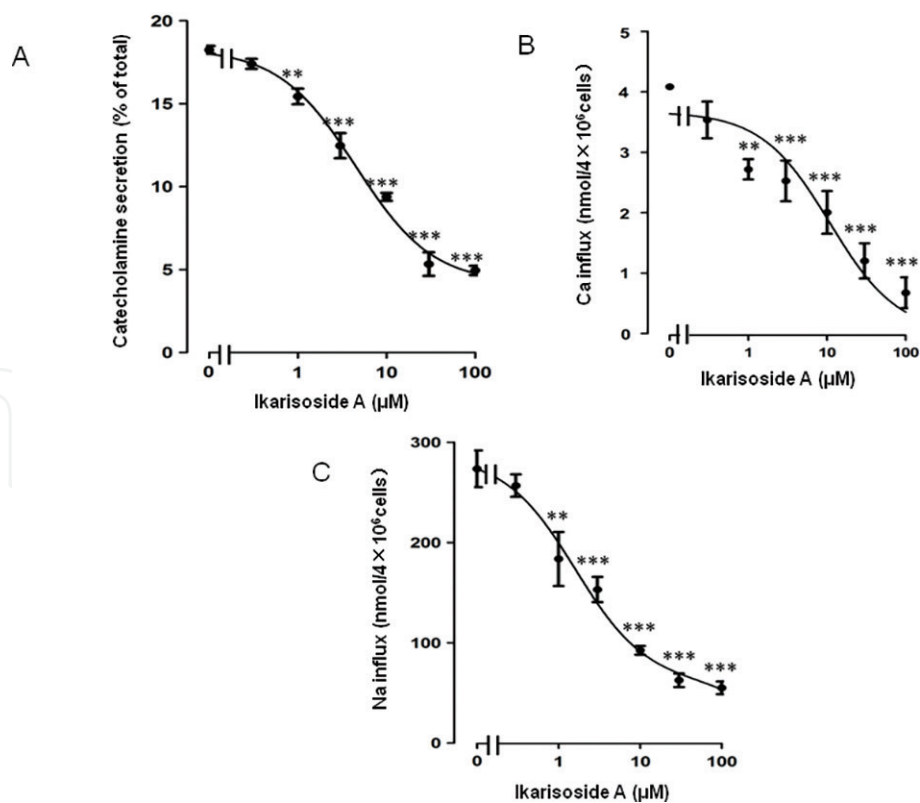


Figure 2. Effects of ikarisoside A on ACh-induced catecholamine secretion (A), ⁴⁵Ca²⁺ influx (B), and ²²Na⁺ influx (C). (A) Cultured bovine adrenal medullary cells (10⁶/well) were stimulated with ACh (300 μM) in the presence or absence of ikarisoside A (0.3–100 μM) for 10 min at 37°C. Catecholamine secretion is expressed as a percentage of the total catecholamines in the cells. (B and C) Cells (4 × 10⁶/well) are stimulated with ACh (300 μM) and 1.5 μCi of ⁴⁵CaCl₂ (B) or ²²NaCl (C) in the presence or absence of ikarisoside A (0.3–100 μM) for 5 min at 37°C. ⁴⁵Ca²⁺ influx and ²²Na⁺ influx were expressed as nmol/4 × 10⁶ cells. Data are means ± SEM from three separate experiments carried out in triplicate. *P < 0.01 and ***P < 0.001 vs. ACh alone (by one-way ANOVA with Dunnett's multiple comparison post hoc test) (cited from [25]).

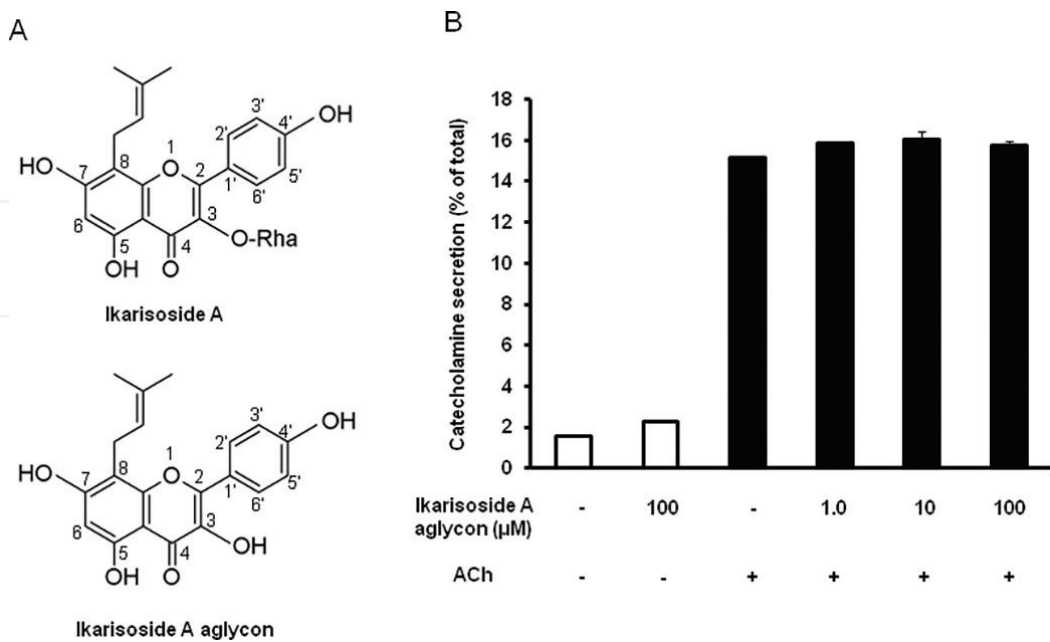


Figure 3. Structures of ikarisoside A and its aglycon (A) and the effect of aglycon of ikarisoside A on the catecholamine secretion induced by ACh (0.3 mM) (B). (A) Chemical structures of ikarisoside A and its aglycon (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)-4H-chromen-4-one). (B) Cells (10⁶/well) were incubated with or without aglycon of ikarisoside A (1–100 μM) and ACh (300 μM) for 10 min at 37°C. Catecholamine secretion is expressed as a percentage of the total. Data are means ± SEM from three separate experiments carried out in triplicate (cited from [25]).

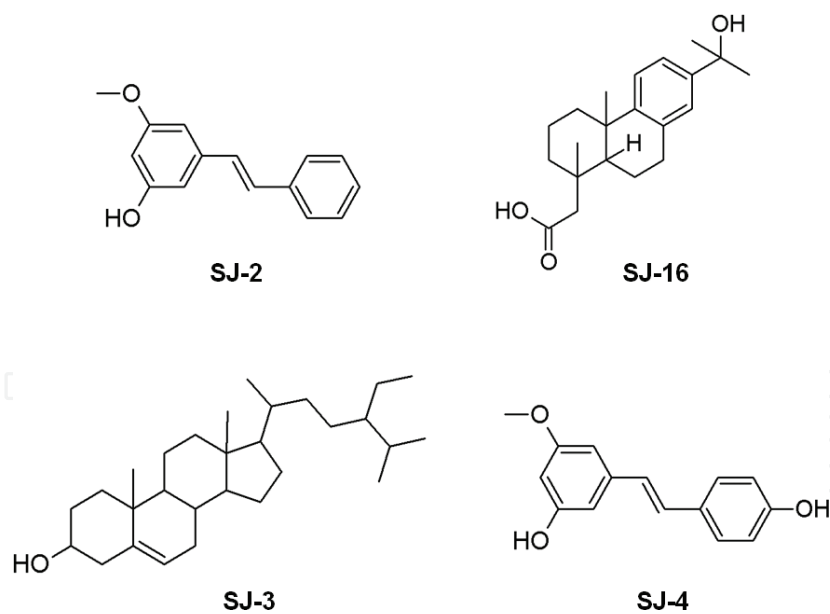


Figure 4.
Chemical structures of SJ-2, SJ-3, SJ-4, and SJ-16 (cited from [28]).

by pests or physical injury [27]. The effective curative components in pine nodule extract (matsufushi) have been used as an analgesic for joint pain, rheumatism, neuralgia, dysmenorrhea, and other complaints in traditional Chinese medicine [27–29]. Matsufushi is used as a healthy supplement in Japan.

Matsufushi ethanol extract (0.0003–0.005%) concentration dependently inhibited the catecholamine secretion and $^{45}\text{Ca}^{2+}$ influx induced by ACh (0.3 mM) and veratridine (0.1 mM), but not 56 mM K^{+} in cultured bovine adrenal medullary cells [27]. Four compounds (SJ-2, SJ-3, SJ-4, and SJ-16) were isolated from matsufushi extract (Figure 4). SJ-2, a phenol stilbene, and the mixture of four compounds (Mix4; SJ-2, SJ-3, SJ-4, and SJ-16), but not each of the other separate compounds, inhibited the catecholamine secretion (Figure 5) and $^{45}\text{Ca}^{2+}$ influx [27] induced by ACh (0.3 mM). In *Xenopus* oocytes

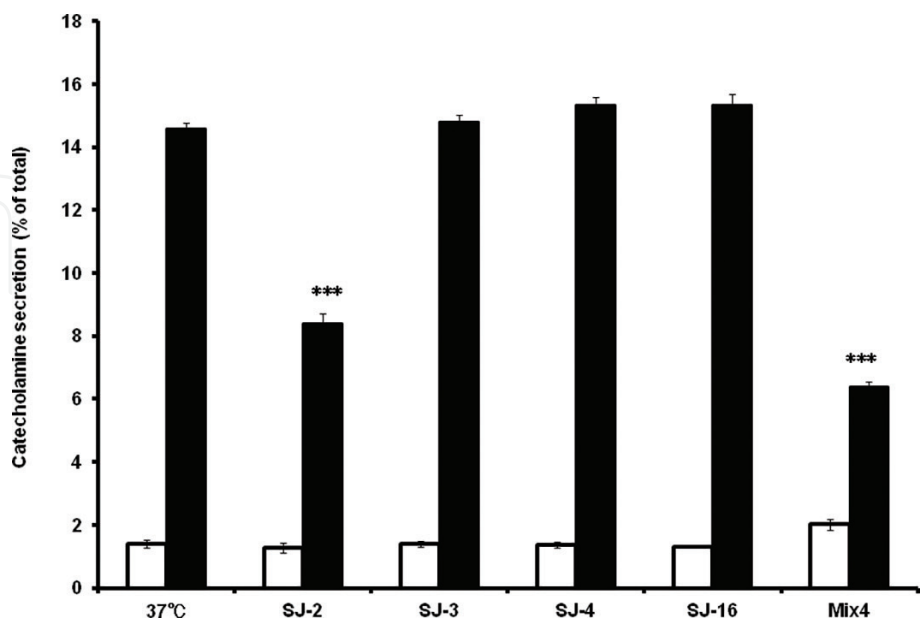


Figure 5.
Effects of SJ-2, SJ-3, SJ-4, SJ-16, and their mixture (Mix4) on catecholamine secretion induced by ACh in cultured bovine adrenal medullary cells. The cells (10^6 /well) were incubated with or without SJ-2 (10 μM), SJ-3 (10 μM), SJ-4 (10 μM), SJ-16 (10 μM), and their mixture (Mix4) (10 μM) for 10 min at 37°C. Catecholamine secretion is expressed as a percentage of the total catecholamines in the cells. Data are means $\pm$ SEM from three separate experiments carried out in triplicate. *** $P < 0.001$ vs. ACh alone. Rha: rhamnose (cited from [28]).

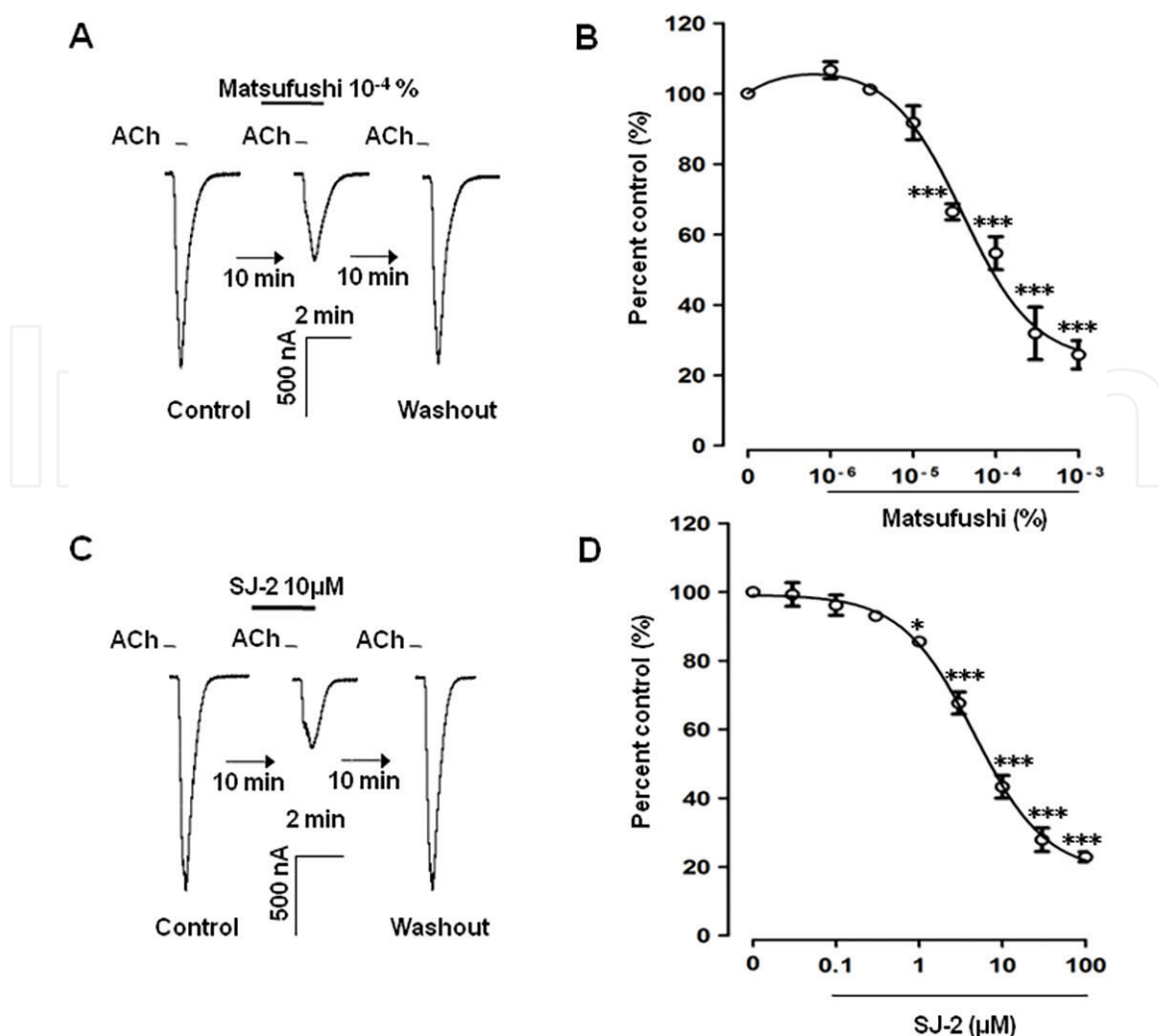


Figure 6.

Effects of ethanol extract of matsufushi and SJ-2 on inward currents induced by ACh in *Xenopus* oocytes expressing rat $\alpha 3\beta 4$ nAChRs. Representative traces from a single *Xenopus* oocyte are shown. The currents of matsufushi-treated (A) and SJ-2-treated (C) oocytes were recorded for 10 min after recording of the control currents, and the washout currents were obtained for 10 min after matsufushi extract and SJ-2 treatment. Matsufushi extract (0.0001%) and SJ-2 (10 μ M) suppressed the currents induced by the EC₅₀ (0.2 mM) of ACh, and the inhibitory effects were reversible. Concentration-response curve for the inhibitory effects of matsufushi extract (B) and SJ-2 (D) on ACh-induced currents. The peak current amplitude in the presence of matsufushi extract and SJ-2 was normalized to that of the control, and the effects are expressed as percentages of the control. Data are presented as means $\pm$ SEM from four separate experiments carried out in triplicate. $P < 0.05$ and $***P < 0.001$ vs. the control (cited from [28]).

expressed with $\alpha 3\beta 4$ nAChRs, matsufushi extract and SJ-2 reversibly inhibited ACh (0.2 mM)-induced Na⁺ currents (Figure 6A, C). Matsufushi extract (0.00003–0.001%) (Figure 6B) and SJ-2 (1–100 μ M) (Figure 6D) significantly suppressed the Na⁺ current in a concentration-dependent manner [27]. In addition, matsufushi and SJ-2 suppressed the ACh (0.3 mM)-induced ¹⁴C-catecholamine synthesis from ¹⁴C-tyrosine and tyrosine hydroxylase activity [27]. These results suggest that matsufushi extract inhibits ACh-induced catecholamine synthesis and secretion mainly due to SJ-2 via the suppression of Na⁺ influx mediated through nAChR-ion channels [27].

4. Effects of the extract of sweet tea on catecholamine secretion and synthesis in adrenal medullary cells

Sweet tea is the processed leaves of *Hydrangea macrophylla* var. *thunbergii* Makino (*hydrangeae dulcis folium*), which is listed in the Japanese Pharmacopeia XV and used as a sweetening agent for diabetic patients. It also has antimicrobial and anti-allergic

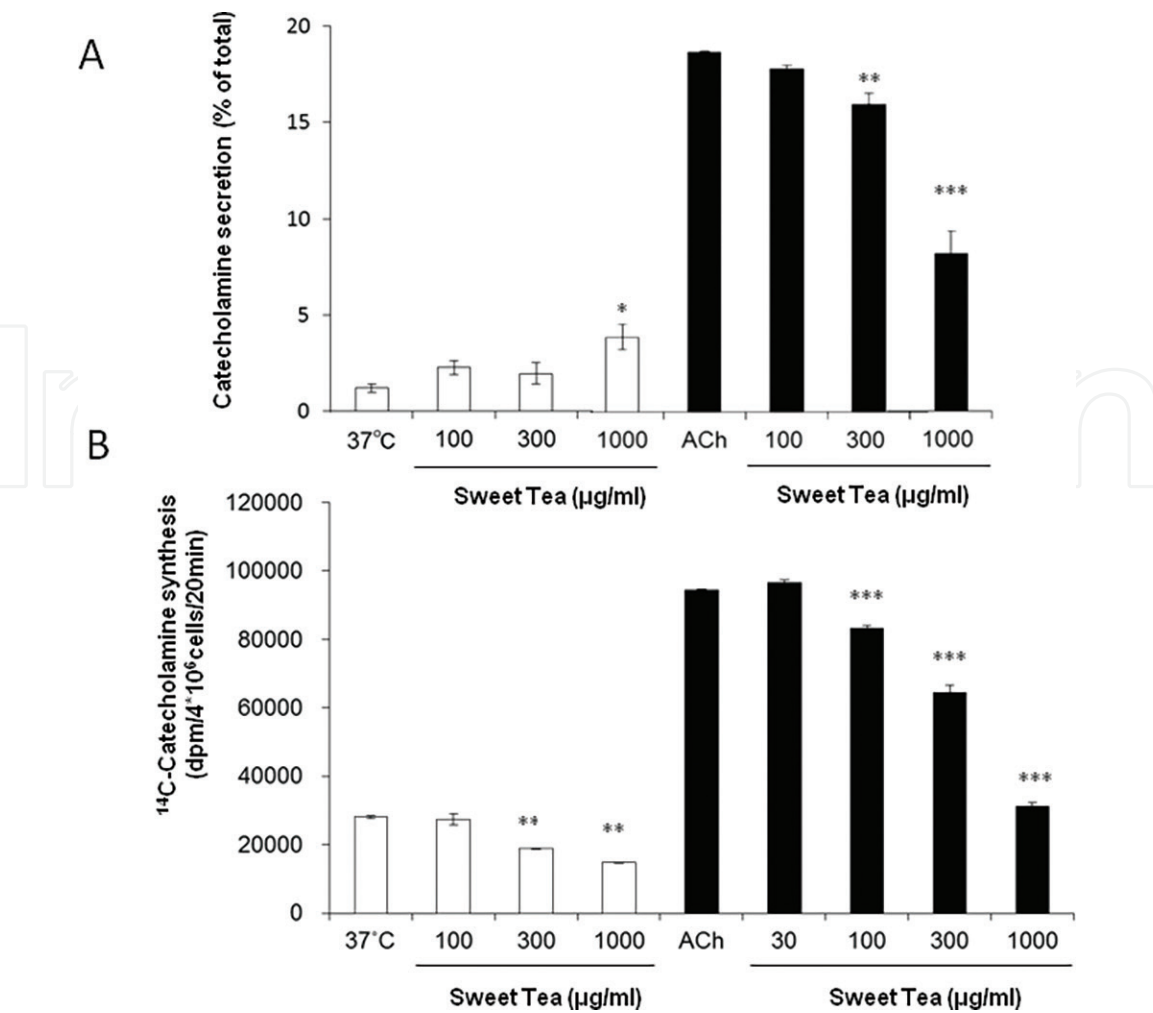


Figure 7. Effects of sweet tea on ¹⁴C-catecholamine synthesis from [¹⁴C]tyrosine (A) and tyrosine hydroxylase activity (B) in the cells. (A) Cells (4 × 10⁶/dish) were incubated with L-[U-¹⁴C] tyrosine (20 µM, 1 µCi) in the presence or absence of sweet tea (100–1000 µg/ml) and with or without 300-µM ACh at 37°C for 20 min. The ¹⁴C-catecholamines formed were measured. (B) Cells (10⁶/well) were incubated with L-[1-¹⁴C] tyrosine (18 µM, 0.2 µCi) in the presence or absence of sweet tea (100–1000 µg/ml) and with or without 300 µM ACh at 37°C for 10 min, and tyrosine hydroxylase activity was measured. Data are means ± SEM from three separate experiments carried out in triplicate. P < 0.05, compared with 37°C (control), and **P < 0.01 and ***P < 0.001 vs. ACh alone in (A), and **P < 0.01 vs. 37°C (control) and ***P < 0.001 vs. ACh alone in (B).

effects [30, 31]. Sweet tea is used as a healthy tea in Japan. There is, however, little evidence regarding sweet tea's effects on the sympathetic nervous system activity.

We investigated the effects of the extract of sweet tea on adrenal medullary cell function. A dry powder of sweet tea prepared from fermented leaves of *hydrangeae dulcis folium* was solubilized at 5.0 mg/ml and extracted at 90°C for 60 min. The extracted solution of sweet tea was used after centrifugation and filtration. The extract of sweet tea (1.0 mg/ml) slightly increased the basal secretion of catecholamines (Figure 7A), whereas it suppressed the catecholamine secretion induced by ACh (0.3 mM) in a concentration-dependent manner (300–1000 µg/ml) (Figure 7A). In addition, the extract of sweet tea (300- or 100–1000 µg/ml) inhibited basal and ACh (0.3 mM)-induced ¹⁴C-catecholamine synthesis from ¹⁴C-tyrosine, respectively (Figure 7B). Sweet tea at concentrations of 3 mg/ml is usually used for drinking.

5. The insight of pharmacological potential of herbs in the catecholamine signaling induced by ACh in adrenal medulla

Adrenal medullary cells are derived from the embryonic neural crest and share many physiological and pharmacological properties with postganglionic sympathetic

neurons [2]. The stimulation of AChRs in these cells increases the synthesis of catecholamines and causes the secretion of catecholamines into the systemic circulation [2, 11, 14]. In adrenal medullary cells, the Na^+ influx induced by ACh via nAChR-ion channels is a prerequisite for Ca^{2+} influx via the activation of voltage-dependent Ca^{2+} channels and the subsequent catecholamine secretion and synthesis; in contrast, high K^+ directly gates voltage-dependent Ca^{2+} channels to increase $^{45}\text{Ca}^{2+}$ influx [2, 11] (**Figure 1**).

As we noted, ikarisoside A and matsufushi (or SJ-2) inhibited the catecholamine secretion induced by ACh, but not the secretion induced by 56 mM K^+ [10, 27]. In addition, ikarisoside A [10] and matsufushi [27] or SJ-2 [27] suppressed the Na^+ current induced by ACh in *Xenopus* oocytes expressing $\alpha 3\beta 4$ nAChRs. These results suggest that the herbs and their components used as described herein inhibit the ACh-induced secretion and synthesis of catecholamines via a suppression of Na^+ influx mediated through nAChRs in adrenal medullary cells [10, 27].

It is well known that catecholamines have important roles in the regulation of normal function in the central and peripheral sympathetic nervous systems as a neurotransmitter but also in the adrenal medulla as an endocrine hormone [10]. Strong and prolonged stress causes massive amounts of catecholamine release, which can lead to cardiovascular diseases (such as hypertension, coronary heart disease, heart failure, and atherosclerosis), and such stress also suppresses the immune system to induce some cancers [2, 9, 10]. Indeed, chronic heart failure is associated with the activation of the sympathetic nervous system as manifested by an increased circulating level of noradrenaline and increased regional activity of the sympathetic nervous system [2, 32]. It was reported that the stress hormone adrenaline stimulates $\beta 2$ -adrenoceptors to activate the Gs-protein-dependent protein kinase A and the β -arrestin-1-mediated signaling pathway, which, in turn, suppresses p53 levels and triggers DNA damage [2, 33]. On the basis of these previous and present results, it appears that the herbs and their components such as ikarisoside A, matsufushi

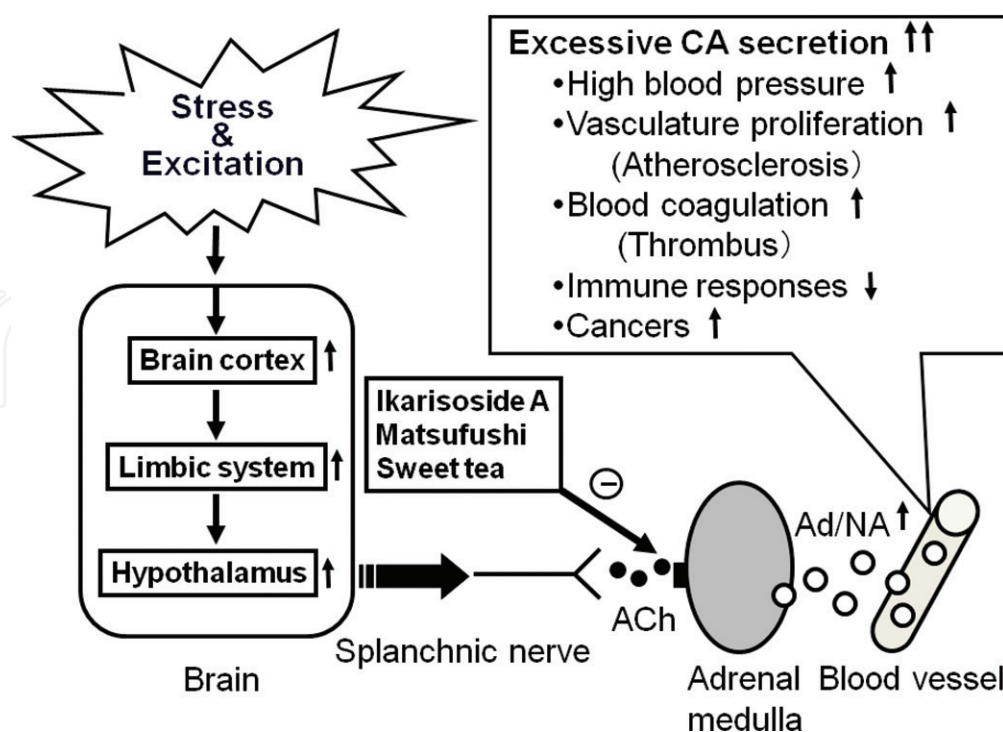


Figure 8.

Inhibitory mechanism of plant herbs (ikarisoside A, matsufushi, and sweet tea) on stress or excitation-induced excessive catecholamine secretion. Prolonged and strong stress or excitation stimulates the brain cortex, limbic system, and hypothalamus which evoke acetylcholine release from the splanchnic sympathetic nerves. Released acetylcholine induces a massive secretion of catecholamines from the adrenal medulla which may cause various deleterious symptoms or diseases such as high blood pressure (hypertension), vasculature proliferation (atherosclerosis), blood coagulation (thrombus), immune suppression, and cancers ([2] modified).

(or SJ-2), and sweet tea suppress the induction of a hyperactive catecholamine system induced by strong stress or emotional excitation (**Figure 8**).

6. Future perspective

Although the in vitro effects of the herbs and herb components described herein have been well clarified using cultured bovine adrenal medullary cells and *Xenopus* oocytes, the in vivo results are not yet clear. To confirm the pharmacological effects of these herbs on the catecholamine system, further in vivo studies of the effects of the administration of herbs to animals or humans are needed [2, 27]. We observed a disturbance of the autonomic nervous balance in women with climacteric symptoms measured by a power spectral analysis of heart rate variability [34]. Using this assay method, we will examine the effect of herbs on the autonomic nervous activity under some stress conditions.

7. Concluding remarks

We have reviewed the evidence that herbs and their components such as ikariso-side A, matsufushi (or SJ-2), and sweet tea inhibit the catecholamine synthesis and secretion induced by ACh in cultured bovine adrenal medullary cells and summarized them in **Table 1**. These findings may provide new insights into the pharmacological potentials of herbs on the hyperactive catecholamine system induced by stress.

Herb	Epimedium	Pine nodule	Sweet tea
Supplement	+	+	+
Compound	Ikarisoside A	SJ-2	ND
ACh-induced Na ⁺ current	↓	↓	ND
ACh-induced Ca ²⁺ influx	↓	↓	ND
Catecholamine secretion			
Basal	→	→	↑
ACh-induced	↓	↓	↓
Catecholamine synthesis			
Basal	→	→	↓
ACh-induced	↓	↓	↓

Supplement +, available in Japan; ND, not determined.

Table 1.
Summary of the effects of herbs and their components on catecholamine secretion and synthesis.

Conflict of interest

The authors have no conflict of interest to declare.

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Abbreviations

ACh	acetylcholine
nAChR	nicotinic acetylcholine receptor

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References

- [1] Lu MF, Xiao ZT, Zhang HY. Where do health benefits of flavonoids come from? Insights from flavonoid targets and their evolutionary history. *Biochemical and Biophysical Research Communications*. 2013;**434**(4):701-704
- [2] Yanagihara N, Zhang H, Toyohira Y, Takahashi K, Ueno S, Tsutsui M, et al. New insights into the pharmacological potential of plant flavonoids in the catecholamine system. *Journal of Pharmacological Sciences*. 2014;**124**:123-128
- [3] Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: A review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*. 2001;**74**(4):418-425
- [4] Ren ZL, Zuo PP. Neural regeneration: Role of traditional Chinese medicine in neurological diseases treatment. *Journal of Pharmacological Sciences*. 2012;**120**(3):139-145
- [5] Falcone Ferreyra ML, Rius SP, Casati P. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science*. 2012;**3**:222
- [6] Gehm BD, McAndrews JM, Chien P-Y, Jameson JL. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;**94**:14138-14143
- [7] Wu JM, Wang ZR, Hsieh TC, Bruder JL, Zou JG, Huang YZ. Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine (Review). *International Journal of Molecular Medicine*. 2001;**8**:3-17
- [8] Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French Paradox for coronary heart disease. *Lancet*. 1992;**339**:1523-1526
- [9] Westfall TC, Westfall DP. Neurotransmission: The autonomic and somatic motor nervous systems. In: Brunton LL, Chabner B, Knollman B, editors. *Goodman & Gilman's the Pharmacological Basis of Therapeutics*. 12th ed. New York: McGraw-Hill; 2011. pp. 171-218
- [10] Li X, Toyohira Y, Horisita T, Satoh N, Takahashi K, Zhang H, et al. Ikarisoside A inhibits acetylcholine-induced catecholamine secretion and synthesis by suppressing nicotinic acetylcholine receptor-ion channels in cultured bovine adrenal medullary cells. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2015;**388**:1259-1269
- [11] Wada A, Takara H, Izumi F, Kobayashi H, Yanagihara N. Influx of ²²Na through acetylcholine receptor-associated Na channels: relationship between ²²Na influx, ⁴⁵Ca influx and secretion of catecholamines in cultured bovine adrenal medulla cells. *Neuroscience*. 1985;**15**(1):283-292
- [12] Yanagihara N, Tank AW, Weiner N. Relationship between activation and phosphorylation of tyrosine hydroxylase by 56 mM K⁺ in PC12 cells in culture. *Molecular Pharmacology*. 1984;**26**:141-147
- [13] Yanagihara N, Tank AW, Langan TA, Weiner N. Enhanced phosphorylation of tyrosine hydroxylase at more than one site is induced by 56 mM K⁺ in rat pheochromocytoma PC12 cells in culture. *Journal of Neurochemistry*. 1986;**46**:562-568
- [14] Yanagihara N, Wada A, Izumi F. Effects of α_2 -adrenergic agonists on carbachol-stimulated catecholamine

synthesis in cultured bovine adrenal medullary cells. *Biochemical Pharmacology*. 1987;**36**:3823-3828

[15] Tachikawa E, Tank AW, Yanagihara N, Mosimann W, Weiner N. Phosphorylation of tyrosine hydroxylase on at least three sites in rat pheochromocytoma PC12 cells treated with 56 mM K⁺: determination of the sites on tyrosine hydroxylase phosphorylated by cyclic AMP-dependent and calcium/calmodulin-dependent protein kinases. *Molecular Pharmacology*. 1986;**30**:476-485

[16] Yoshimura R, Yanagihara N, Hara K, Nakamura J, Toyohira Y, Ueno S, et al. Dual phases of functional changes in norepinephrine transporter in cultured bovine adrenal medullary cells by long-term treatment with clozapine. *Journal of Neurochemistry*. 2001;**77**:1018-1026

[17] Kajiwarra K, Yanagita T, Nakashima Y, Wada A, Izumi F, Yanagihara N. Differential effects of short and prolonged exposure to carvedilol on voltage-dependent Na⁺ channels in cultured bovine adrenal medullary cells. *The Journal of Pharmacology and Experimental Therapeutics*. 2002;**302**:212-218

[18] Obara G, Toyohira Y, Inagaki H, Takahashi K, Horishita T, Kawasaki T, et al. Pentazocine inhibits norepinephrine transporter function by reducing its surface expression in bovine adrenal medullary cells. *Journal of Pharmacological Sciences*. 2013;**121**(2):138-147

[19] Liu M, Yanagihara N, Toyohira Y, Tsutsui M, Ueno S, Shinohara Y. Dual effects of daidzein, a soy isoflavone, on catecholamine synthesis and secretion in cultured bovine adrenal medullary cells. *Endocrinologie*. 2007;**148**:5348-5354

[20] Toyohira Y, Ueno S, Tsutsui M, Itho H, Sakai N, Saito N, et al. Stimulatory

effects of the soy phytoestrogen genistein on noradrenaline transporter and serotonin transporter activity. *Molecular Nutrition & Food Research*. 2010;**54**:516-524

[21] Zhang H, Toyohira Y, Ueno S, Shinohara Y, Itoh H, Furuno Y, et al. Dual effects of nobiletin, a citrus polymethoxy flavone, on catecholamine secretion in cultured bovine adrenal medullary cells. *Journal of Neurochemistry*. 2010;**114**:1030-1038

[22] Shinohara Y, Toyohira Y, Ueno S, Liu M, Tsutsui M, Yanagihara N. Effects of resveratrol, a grape polyphenol, on catecholamine secretion and synthesis in cultured bovine adrenal medullary cells. *Biochemical Pharmacology*. 2007;**74**:1608-1618

[23] Dou J, Liu Z, Liu S. Structure identification of a prenylflavonol glycoside from *Epimedium koreanum* by electrospray ionization tandem mass spectrometry. *Analytical Sciences*. 2006;**22**:449-452

[24] Choi HJ, Eun JS, Park YR, Kim DK, Li R, Moon WS, et al. Ikariside A inhibits inducible nitric oxide synthase in lipopolysaccharide-stimulated RAW 264.7 cells via p38 kinase and nuclear factor- κ B signaling pathways. *European Journal of Pharmacology*. 2008;**601**:171-178

[25] Choi HJ, Park YR, Nepal M, Choi BY, Cho NP, Choi SH, et al. Inhibition of osteoclastogenic differentiation by Ikariside A in RAW 264.7 cells via JNK and NF- κ B signaling pathways. *European Journal of Pharmacology*. 2010;**636**:28-35

[26] Li C, Li Q, Mei Q, Lu T. Pharmacological effects and pharmacokinetic properties of icariin, the major bioactive component in *Herba Epimedii*. *Life Sciences*. 2015;**126**:57-68

- [27] Li X, Horisita T, Toyohira Y, Shao H, Bai J, Bo H, et al. Inhibitory effects of pine nodule extract and its component, SJ-2, on acetylcholine-induced catecholamine secretion and synthesis in bovine adrenal medullary cells. *Journal of Pharmacological Sciences*. 2017;**133**:268-275
- [28] Bermudez J, Burgess MF, Cassidy F, Clarke GD. Activity of the oxidation of products of oleum terebinthinae "Landes" on guinea pig airway smooth muscle in vivo and in vitro. *Arzneimittel-Forschung*. 1987;**37**:1258-1262
- [29] Chen W, Liu Y, Li M, Mao J, Zhang L, Huang R. Anti-tumor effect of α -pinene on human hepatoma cell lines through inducing G2/M cell cycle arrest. *Journal of Pharmacological Sciences*. 2015;**127**:332-338
- [30] Yoshikawa M, Harada E, Naitoh Y, Inoue K, Matsuda H, Shimoda H, et al. Development of bioactive functions in *hydrangeae dulcis folium*. III. On the antiallergic and antimicrobial principles of *hydrangeae dulcis folium*. (1). Thunberginols A, B, and F. *Chemical & Pharmaceutical Bulletin (Tokyo)*. 1994;**42**:2225-2230
- [31] Wang Q, Matsuda H, Matsuhira K, Nakamura S, Yuan D, Yoshikawa M. Inhibitory effects of thunberginols A, B, and F on degranulations and releases of TNF- α and IL-4 in RBL-2H3 cells. *Chemical & Pharmaceutical Bulletin (Tokyo)*. 2007;**30**:388-392
- [32] Freedman NJ, Lefkowitz RJ. Anti- β_1 -adrenergic receptor antibodies and heart failure: causation, not just correlation. *The Journal of Clinical Investigation*. 2004;**113**:1379-1382
- [33] Hara MR, Kovacs JJ, Whalen EJ, Rajagopal S, Strachan RT, Grant W, et al. A stress response pathway regulates DNA damage through β_2 -adrenoreceptors and β -arrestin-1. *Nature*. 2011;**477**(7364):349-353
- [34] Yanagihara N, Seki M, Nakano M, Hachisuga T, Goto Y. An inverse correlation between cardiac vagal activity and the simplified menopause index in Japanese menopausal climacteric women. *Menopause*. 2014;**21**:669-672