Research Article
J. Ginseng Res. Vol. 34, No. 4, 314-320 (2010)
DOI:10.5142/jgr.2010.34.4.314

JGR Journal of Ginseng Research

Beneficial Role of Ginseng Saponin on Hemodynamic Functions of Porcine Blood Vessel

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The previous reports have showed that ginseng saponins, which are the active ingredients of *Panax* ginseng, cause the relaxation of artery that are contracted due to a various of hormones or potassium (K^+). Recently, we also showed that ginsenosides differentially regulate channel activity. The purpose of this study was to examine whether ginseng saponins affect contraction induced by K^+, serotonin (5-HT), or acetylcholine (Ach) in porcine coronary vessel. Treatment with concentrations of ginseng saponins caused a relaxation of 25 mM KCl-induced porcine coronary artery contraction. Also, ginseng saponin induced a significant dose-dependent relaxation of 3 μM 5-HT-induced porcine coronary artery with the endothelium. In the porcine artery with the endothelium, ginseng saponins induced a relaxation by 3 μM 5-HT in a concentration-dependent pattern. Ginseng saponins induced relaxation of both 25 mM KCl- and 3 μM 5-HT-induced coronary artery contraction in the absence and presence of the endothelium. In contrast, treatment with 100 μg/mL ginseng saponin did not induce relaxation in coronary artery contraction induced by Ach (0.01 μM to 30 μM) in the presence of the endothelium, but did cause significant relaxation of coronary artery contractions by Ach (0.01 μM to 30 μM) in the absence of the endothelium. These findings indicate that ginseng saponin (>100 μg/mL) significantly inhibits porcine coronary artery contractions caused by K^+, 5-HT, and Ach. Therefore, in this study, we demonstrated that ginseng saponin may show beneficial roles on abnormal coronary contraction.

Keywords: Ginseng saponin, Porcine blood vessel, Contraction, Vasodilation, Hemodynamic

INTRODUCTION

Coronary artery diseases is defined to arterial smooth muscle injury that include hardening and decrease of elasticity. This disease come about three patterns: plaques containing lipoid in the arterial walls, monckeberg medical calcification, and thickness because of hyperplasia, fibrosis, and narrowing. However, the exact mechanisms

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Received 23 Jul. 2010, **Revised** 27 Aug. 2010, **Accepted** 27 Aug. 2010

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are unknown.

Coronary disease is a primary cause of mortality in the world, and many psychological factors are involved in an increased risk [1,2]. Meantime, we understand that the pathophysiology of coronary arteries has undergone many remarkable changes. Coronary disease is the most predominant cause of death in Western nations [3]. Those who survive cardiac infarction have an about threefold increased risk of left heart dysfunction [4]. The likelihood of surviving cardiac ischemia is almost doubled in some nations [5].

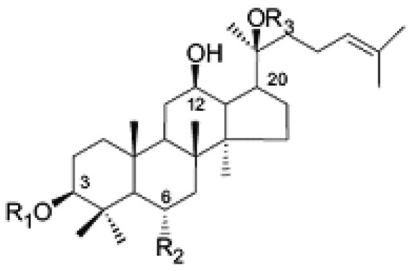
Ginseng saponin (GS), a widely recognized herbal substances, has been reported to have a wide range of therapeutic, and an increasing amount of studies has showed the evidence for the beneficial effects of GS in heart diseases. Injection of ginsenosides decreases blood pressure in both hypertension [6,7]. The antihypertensive effects may be due to inhibit vascular contraction. Also, ginsenosides relax rabbit pulmonary arteries [8] and rabbit and rat aortas contracted with phenylephrine [7] in a concentraction-dependent manner. Those effects require the presence of endothelium and are related by an increased production of endothelium-originated nitric oxide [7]. Ginseng is a relatively safe herbal for hypertension in humans [9]. Also it was shown that ginseng decreases vascular dysfunction found in hypertension [10]. Ginseng also results in many cardiac effects [11–18], but the mechanisms remain unclear. Also, even now, no evidence has been presented to indicate that GS shows inhibition on porcine coronary artery contraction.

In this study, we showed the inhibitory role of GS on contraction induced by either high K^+ [19], 5-HT [19], or acetylcholine [20] in porcine coronary arteries with or without endothelium. We found that GS exhibits inhibitory effects on porcine artery contraction caused by 25 mM KCl, 3 mM 5-HT, or 0.01 μM to 30 μM acetylcholine. Furthermore, this inhibitory role might be dependent or independent on the presence of the endothelium.

MATERIALS AND METHODS

Materials

Fig. 1 show the chemical structure and constituents of GS, which was kindly provided by the Korea Ginseng Corporation (Daejeon, Korea). Other analytical grade chemicals (KCl, acetylcholine, and serotonin) were purchased from Sigma (St. Louis, MO, USA). GS was dissolved in a physiological salt solution (PSS), which was centrifuged at 15,000 rpm for 10 min. The supernatant was transferred to another tube and filtered through a 0.2-mm syringe filter to remove any suspended solids.



Ginsenosides	R_1	R_2	R_3
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc
Rb_2	-Glc_2-Glc	-H	-Glu_6-Ara(pyr)
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)
Rd	-Glc_2-Glc	-H	-Glc
Re	-H	-O-Glc_2-Rha	-Glc
Rf	-H	-O-Glc_2-Glc	-H
Rg_1	-H	-O-Glc	-Glc
Rg_2	-H	-O-Glc_2-Rha	-H
Rg_3	-Glc_2-Glc	-H	-H

Fig. 1. Structures of nine representative ginsenosides. The structures differ at three side chains attached the common steroid ring. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates. Glc, glucopyranoside; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside.

Preparation of coronary artery strips and measurement of isometric contraction

The effects of GS on porcine coronary artery contraction induced by high K^+, 5-HT, and acetylcholine were evaluated in an organ bath chamber. Porcine hearts were obtained from an abattoir and transferred to laboratory [21]. The left circumflex coronary (LCC) arteries were dissected and placed in PSS containing 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, 0.016 mM CaEDTA, and 11.1 mM glucose (control solution). LCC arteries were cleaned of connective tissue and then cut into many ring that were 2 mm to 3 mm in width. Each specimen was divided into intact and endothelium-denuded samples. Five of the ten specimens were used with the endothelium intact; the endothelium was denuded in the remaining four samples. The endothelium was removed by gently rubbing the inner surface of the vessel with a cotton thread moistened with PSS. Endothelium removal was confirmed by stabilization of the specimen with 25 mM KCl. The prepared LCC artery rings were horizontally suspended between two stainless-steel stirrups in organ chambers filled with 3 ml of control solution at 37.8°C (pH 7.4) and bubbled with 95% O_2 and 5% CO_2. One of the stirrups was anchored to the organ chamber and the other was connected to a transducer coupler

(Grass Technologies, Warwick, RI, USA) connected to a recording system (ADInstruments, Sydney, Australia). The LCC arteries rings were progressively stretched to the optimal tension (2 g) prior to the addition of 25 mM KCl, 3 mM 5-HT, or 0.01 μM to 30 μM acetylcholine.

Measurement of isometric tension induced by high K^+, 5-HT, or acetylcholine

After the plateau of the contraction elicited by 25 mM KCl was obtained, the aortic rings were rinsed three times in warm PSS at 37.8°C. After a 30-min resting period in PSS, the aortic rings were exposed to 25 mM KCl a second time. After a stable plateau of vasoconstriction in the presence of 25 mM KCl was reached, the rings were stabilized using PSS. This response was repeated three times. Next, we tested drug effects. Specifically, following preincubation for 1 min with 1 to 300 μg/mL GS, the rings were again contracted using 25 mM KCl to test contraction inhibition of the coronary artery. A concentration curve was obtained based on coronary artery contraction in solutions containing 25 mM KCl and different dosages of GS. The inhibitory effects of GS were expressed as a percentage of the maximal response to 25 mM KCl. In all cases, each experiment was repeated five to six times. We also evaluated the inhibitory effects of different doses of GS on contractile responses induced by 3 μM 5-HT in the porcine coronary artery. Once the plateau of contraction elicited by 3 μM 5-HT was obtained, coronary artery rings were rinsed three times for 30 min with PSS. After a resting period of 30 min, the rings were exposed again to 3 μM 5-HT. After contraction with 3 μM 5-HT, the rings were stabilized using warm PSS. The rings were then preincubated for 1 min with 1 to 300 μg/mL GS in the presence or absence of the endothelium and 3 μM 5-HT-induced contraction was examined. Thus, after preincubation of GS in a dose-dependent manner, contractile responses to 3 μM 5-HT were obtained. The amplitude of contraction induced by 3 μM 5-HT was measured for each concentration. The inhibitory effects of 3 μM GS on 5-HT-induced contraction were expressed as a percentage of the maximal response to 3 μM 5-HT in each aorta. In all cases, each experiment was repeated five to six times. The inhibitory effects of GS on contractile responses to acetylcholine were also investigated in porcine coronary arteries with and without endothelium. After a stable plateau of 25 mM KCl-induced vasoconstriction was reached, the rings were stabilized using PSS for 30 min. This response was repeated three times. The rings were then preincubated for 1 min with 100 μg/mL GS and contracted using Ach in a dose-dependent manner (0.01 μM to 30 μM) to test the inhibitory effects GS on porcine coronary artery contraction in the presence or absence of the endothelium. Acetylcholine (0.01 μM to 30 μM) was added to the bath in a cumulative fashion. The response to each concentration was allowed to reach a plateau before the addition of the next concentration of Ach. The amplitude of contraction induced by Ach was measured for each concentration. The inhibitory effects of 100 μg/mL GS on Ach-induced were expressed as a percentage of the maximal response to 25 mM KCl in each aorta. In all cases, each experiment was repeated five to six times.

Data analysis

All numeric values are represented as the means±SEM. Tests for statistical significance were performed on the data set using an unpaired Student's *t*-test. A *p*-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effects of GS on high K^+-induced contraction

Ginsenosides are known to have differential roles on both voltage-dependent and ligand-gated ion channels. To further investigate, we first examined the inhibitory effects of GS on contraction induced by high K^+. In the presence of 25 mM KCl, the addition of GS (1 to 300 μg/mL) produced a concentration-dependent relaxation in endothelium-attached and -denuded porcine coronary arteries (Fig. 2A, B). The inhibitory effects of GS on coronary arteries with endothelium were 2.5±1.83, 4.7±3.1, 6.3±5.34, 12.7±8.87, 43.9±8.58, and 48.5±6.82 % at doses of 1, 3, 10, 30, 100, and 300 μg/mL, respectively (Fig. 2A). Also, the inhibitory effects of GS on endothelial-denuded coronary arteries were 3.7±0.99, 1.5±1.78, 5.8±2.04, 13.9±7.61, 35.9±5.83, and 38.6±6.72% at doses of 1, 3, 10, 30, 100, and 300 μM, respectively (Fig. 2B). Thus, the inhibitory effects of GS on high K^+-induced contraction was not dependent on an intact endothelium. IC_{50} values of GS were 45.88±10.76 and 39.55±9.47 μg/mL on coronary arteries in the presence and absence of the endothelium, respectively.

Inhibitory effect of GS on 5-HT-induced contraction

As the next step, we studied the effects of GS on coronary artery contraction induced by 3 μM 5-HT. As shown in Fig. 3A and 3B, GS displayed more potent dose-dependent inhibition in the presence of the endothelium than in its absence. Inhibition by GS on 5-HT-induced

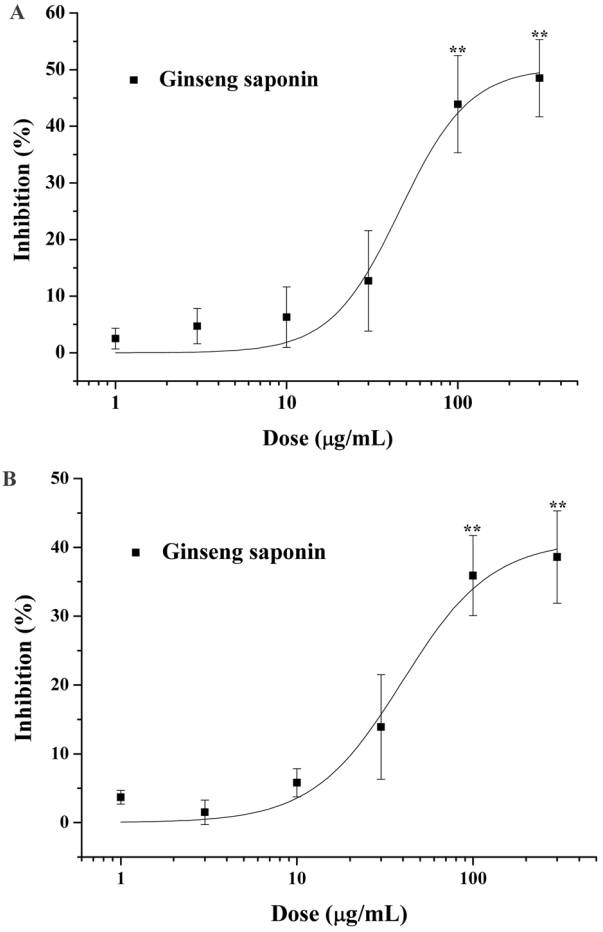


Fig. 2. The inhibitory effects of ginseng saponin at doses of 1, 3, 10, 30, 100, and 300 μg/mL in coronary arteries with and without endothelium that were constricted with 25 mM KCl. Relaxation effects evoked by ginseng saponin on coronary arteries in the presence (A) or absence (B) of the endothelium are also shown. Constriction induced by 25 mM KCl was inhibited by ginseng saponin in a concentration-dependent manner (>100 μg/mL). All experiments were performed in the presence of physiological salt solution, following protocols described in the Materials and Methods. Data are expressed as means±SEM (*n*=5–6/dose). **Significant inhibitory effects of ginseng saponin on contraction with 25 mM KCl (p<0.01).

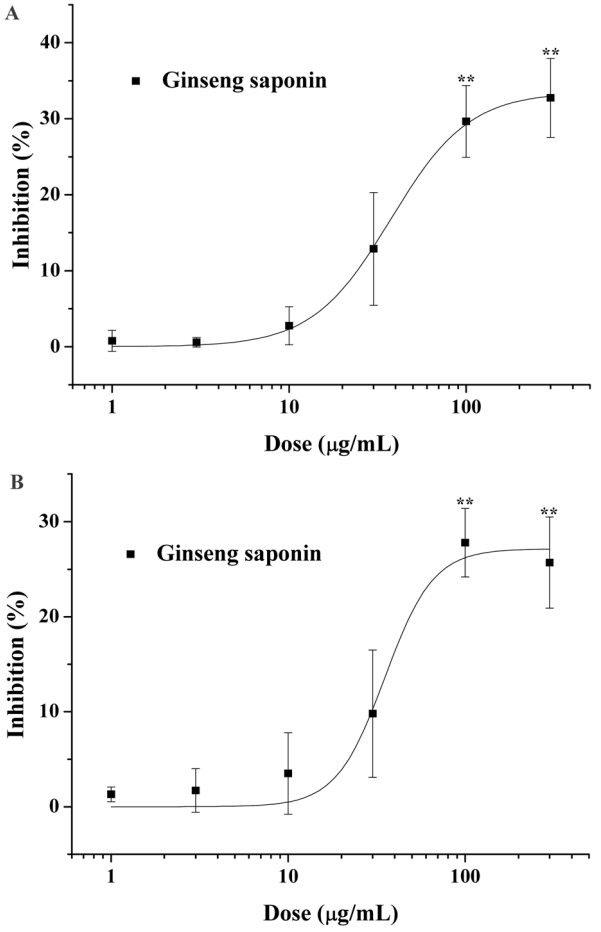


Fig. 3. The inhibitory effects of ginseng saponin at doses of 1, 3, 10, 30, 100, and 300 μg/mL in coronary artery with and without endothelium that were constricted with 3 μM 5-HT. Relaxation effects evoked by ginseng saponin on coronary arteries in the presence (A) or absence (B) of the endothelium are also shown. Constriction induced by 3 μM 5-HT was inhibited by ginseng saponin in a concentration-dependent manner (>100 μg/mL). All experiments were performed in the presence of physiological salt solution, following protocols described in the Materials and Methods. Data are expressed as means±SEM (*n*=5–6/dose). **Significant inhibitory effects of ginseng saponin on with 3 μM 5-HT (p<0.01).

coronary artery contraction was 0.78±1.37, 0.58±0.63, 2.76±2.51, 12.87±7.43, 29.65±4.72, and 32.75±5.18% at doses of 1, 3, 10, 30, 100, and 300 μM, respectively, in the presence of the endothelium. The IC_{50} of GS was 37.48±1.90 μg/mL in the presence of the endothelium (Fig. 3A). Additionally, in the absence of the endothelium, the inhibitory effects of GS on 5-HT-induced coronary artery contraction were studied. Inhibition of 5-HT-induced coronary artery contraction by GS was 1.3±0.78, 1.7±2.3, 3.5±4.3, 9.8±6.7, 27.8±3.6, and 25.7±4.8% at doses of 1, 3, 10, 30, 100, and 300 μg/mL, respectively (Fig. 3B). The IC_{50} of GS-induced inhibition was 35.10±7.12 μM on endothelium-denuded coronary arteries (Fig. 3B).

Inhibition of ginseng saponin on Ach-induced contraction

Since GS inhibited coronary artery contraction induced by high K^+ and 5-HT as shown in Figs. 2A, 2B, 3A, and 3B, we next examined whether GS could also inhibit Ach-induced coronary artery contraction. A 100 μg/mL volume of GS was determined to be the appropriate dose to show an inhibitory effect (Figs. 2A, 2B, 3A, and 3B). In contrast to the inhibition on KCl- or 5-HT-induced coronary artery contraction, GS (100 μg/mL) had no effect on acetylcholine-induced coronary ar-

tery contraction in the presence of the endothelium (Fig. 4A). In contrast, GS (100 μg/mL) inhibited Ach-induced porcine coronary artery contraction in the absence of the endothelium (Fig. 4B). In the control group (no GS treatment), Ach-induced coronary artery contraction was 1.37±1.58, 1.55±0.99, 2.52±1.78, 15.34±4.65, 59.85±5.27, 79.67±3.65, 82.45±4.71, and 85.87±5.73% at doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM, respectively, in the presence of the endothelium (Fig. 4A, ■). The EC_{50} of GS was 0.63±0.02 μM. Contraction with GS treatment was 2.04±0.86, 2.91±1.87, 2.97±1.98, 8.54±3.95, 53.72±4.67, 75.43±5.38, 77.65±4.54, and 80.92±4.7 % at doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 μM, respectively in the presence of the endothelium (Fig. 4A, ●). The EC_{50} was 0.73±0.04 μM. In the control group (no GS treatment), Ach-induced coronary artery contraction was 2.78±1.35, 2.56±2.83, 1.63±3.16, 5.83±2.75, 55.67±6.32, 58.71±5.98, 63.75±5.01, and 64.98±6.77% at doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM, respectively, in the absence of the endothelium (Fig. 4B, ■). The EC_{50} of GS was 0.56±0.06 μM. Contraction with GS treatment (100 μg/mL) was 1.69±0.99, 0.78±1.85, 0.98±2.65, 3.26±2.89, 35.45±5.43, 38.76±6.12, 40.32±4.57, and 43.65±4.38 % at doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM, respectively, in the presence of the endothelium (Fig. 4B, ●). The EC_{50} was 0.59±0.06 μM. Thus, a significant difference was observed in the inhibitory potency of GS on Ach-induced coronary artery contraction as compared to controls (Fig. 4B, $^{**}p<0.01$).

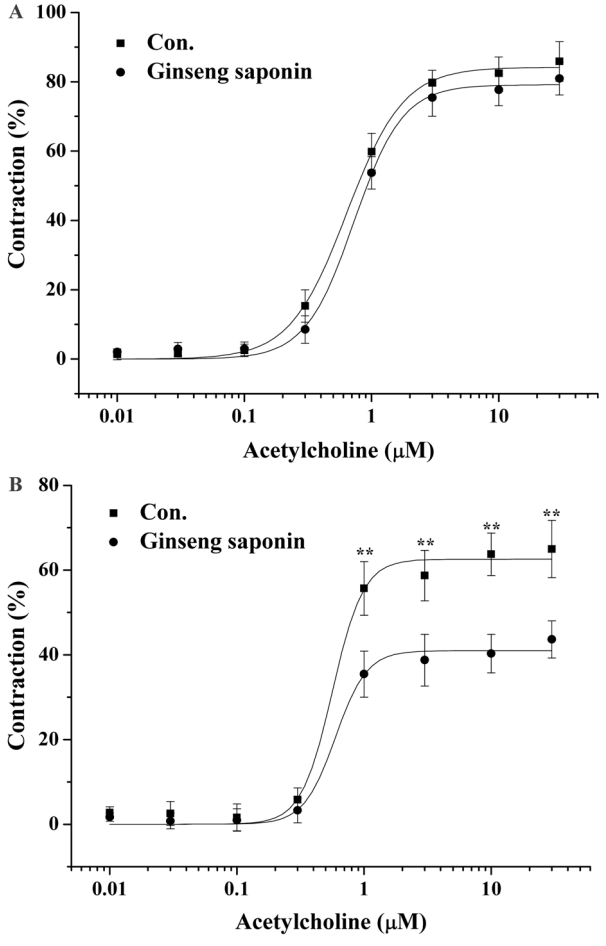


Fig. 4. The inhibitory effects of 100-μg/mL ginseng saponin incubated for 1 min with and without endothelium on arteries constricted with acetylcholine at doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM. Contraction evoked by acetylcholine is shown in the presence (A, ■) or absence (B, ■) of the endothelium (control). Constriction induced by acetylcholine was inhibited by preincubation of 100-μg/mL ginseng saponin in the absence of the endothelium (B, ●). However, the inhibition of contraction was not observed in the presence of the endothelium (A, ●). All experiments were performed in the presence of PSS, following protocols described in the Materials and Methods. Data are expressed as means±SEM (n=5–6/dose). **Significant inhibitory effects of ginseng saponin as compared to the control group (p<0.01).

Although ginseng have gained increased popularity worldwide for many beneficial effects, the underlying mechanisms remain unclear. Information regarding the effects of ginseng on heart diseases is even more controversial. Therefore, we studies the effects of GS on porcine coronary artery contraction induced by various substances in the absence or presence of the endothelium.

In the present study, we demonstrated that GS inhibited high K^+- and 5-HT-induced porcine coronary artery contraction. GS had no effect on acetylcholine-induced porcine coronary artery contraction with the endothelium, but inhibited acetylcholine-induced contraction in a dose-dependent manner without the endothelium. Finally, GS was more effective in the inhibition of high K^+- and 5-HT-induced porcine coronary artery contractions. These results indicate that GS has effects on high K^+- or 5-HT-induced porcine coronary artery contractions and that the endothelium may not play a role in GS-induced artery relaxation. In a previous study, ginsenosides were able to directly inhibit vascular muscle in the rat aorta [7]. Although GS caused inhibition in K^+- and 5-HT-induced porcine artery contraction without the endothelium, GS had no effect on Ach-induced coronary artery contraction with the endothelium. Therefore, these results shows that endothelium-dependent relaxation might exist in the porcine coronary artery. The effects indicated that the effects on coronary arteries with endothelium may relate a NO-dependent mechanism, as demonstrated by Sumner [22], Martin et al. [23], and Wallis and Martin [24]. The presence or absence of endothelium can influence contraction in the arteries [25-28]. Therefore, these data suggest that the inhibitory

effects is present in the porcine coronary artery and that products of the endothelium-secreted relaxant substance may involve to regulate 5-HT receptors, as shown by Yamano et al. [29], Hinton et al. [30], and Nieto et al. [31].

In summary, we found that GS inhibited high KCl-, 5-HT-, and Ach-induced porcine coronary artery contractions in a both dependent and independent of the endothelium. The findings suggest that GS can be utilized as the medicine of coronary diseases.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0074590).

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