North American ginseng (P. quinquefolius) suppresses β adrenergic-dependent signalling, hypertrophy and cardiac dysfunction

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North American ginseng (P. quinquefolius) suppresses β adrenergic-dependent signalling, hypertrophy and cardiac dysfunction

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**Abstract:** There is increasing evidence for a beneficial effect of ginseng on cardiac pathology. Here we determined whether North American ginseng can modulate the deleterious effects of the β-adrenoceptor agonist isoproterenol on cardiac hypertrophy and function using *in vitro* and *in vivo* approaches. Isoproterenol was administered for 2 weeks at either 25 mg/kg/day or 50 mg/kg/day (ISO25 or ISO50) via a subcutaneously implanted osmotic mini-pump to either control rats or those receiving ginseng (0.9 g/L in the drinking water *ad libitum*). Isoproterenol produced time- and dose-dependent left ventricular dysfunction although these effects were attenuated by ginseng. Improved cardiac functions were associated with reduced heart weights as well as prevention in the upregulation of the hypertrophy-related fetal gene expression. Lung weights were similarly attenuated suggesting reduced pulmonary congestion. In *in vitro* studies, ginseng (10 µg/ml) completely suppressed the hypertrophic response to 1 µM isoproterenol in terms of myocyte surface area as well as reduction in the upregulation of fetal gene expression. These effects were associated with attenuation in both protein kinase A and cAMP response element-binding protein phosphorylation. Ginseng attenuates adverse cardiac adrenergic responses and may therefore be an effective therapy to reduce hypertrophy and heart failure associated with excessive catecholamine production.

*Key words:* ginseng, isoproterenol, heart failure, cardiomyocyte hypertrophy, β-adrenergic signaling, PKA, CREB
Introduction

Heart failure (HF) is a progressive severe clinical syndrome associated with high morbidity and mortality (Ruetten et al. 2005). It is also associated with sympathetic nervous system hyperactivity resulting in enhanced circulating catecholamine levels likely contributing to HF progression (Hall et al. 2014). Indeed, β-adrenergic receptor blockers have been demonstrated to significantly reduce mortality and morbidity in heart failure patients (Foody et al. 2002).

Ginseng is a well-known traditional medicine which has been used in Asian societies for thousands of years and which has emerged as one of the most frequently-utilized alternative medicines in western countries (Karmazyn et al. 2011). Ginseng has been reported to exert numerous clinical benefits including those on the cardiovascular system (Karmazyn et al. 2011). With respect to cardiac pathology ginseng has been shown to attenuate hypertrophy and heart failure following coronary artery ligation as well as to protect the ischemic and reperfused myocardium (Guo et al. 2011; Li et al. 2014; Moey et al. 2012). However, little is known concerning the effect of ginseng on catecholamine-dependent cardiac responses. The present study was carried out to address this issue by determining the effect of North American ginseng (Panax quinquefolius) on the myocardial response to two weeks administration of isoproterenol to rats. In addition, we further determined the effect of ginseng on the response of cultured ventricular myocytes to 24 hours of isoproterenol treatment. Our results demonstrate that ginseng effectively mitigates isoproterenol-induced cardiac hypertrophy as well as left ventricular dysfunction. Evidence from both sets of studies using cultured cardiomyocytes suggests that this occurs by preventing protein kinase A (PKA) and cAMP response element-binding protein (CREB) phosphorylation.

Materials and methods
**Ginseng extract**

Four-year-old North American ginseng roots collected from 5 different farms in Ontario, Canada were provided by the Ontario Ginseng Growers Association and shipped to Naturex (South Hackensack, NJ, USA) for ginsenosides extraction using a hydroalcoholic process. The ginseng extract was provided by the Ontario Ginseng Innovation and Research Consortium at the University of Western Ontario.

**Animals**

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the Canadian Council on Animal Care (Ottawa, ON, Canada). The studies have been approved by the Animal Care and Use Committee of the University of Western Ontario (Protocol # 2013-31). For *in vivo* studies male Sprague-Dawley rats weighing 180-220 g were purchased from Charles River Canada (St. Constant, Quebec, Canada) whereas for *in vitro* studies one to three day old Sprague-Dawley rats were bred in the Health Sciences Animal Care Facilities at the University of Western Ontario.

**In vivo studies**

Isoproterenol or vehicle was administered for 2 weeks via a subcutaneously implanted osmotic mini-pump (ALZET, Cupertino, California, USA, Model 2ML2; infusion rate of 5.0 µL/h) under inhalation anaesthesia with 2% isoflurane to either control animals or to animals receiving ginseng. Minipumps were prepared to provide an isoproterenol dose of either 25 mg/kg/day (ISO25) and 50 mg/kg/day (ISO50) whereas the ginseng was administered immediately after minipump implantation in the drinking water at a concentration of 0.9 g/L *ad libitum*. A total of 65 male Sprague-Dawley rats were randomly assigned into the following 6 groups: saline (*N*=10),
ISO25 (N=12), ISO50 (N=18), ISO25+ ginseng (N=7), ISO50+ ginseng (N=12), and, lastly ginseng alone (N=6). All animals were monitored regularly for body weight and mortality throughout the study.

**Echocardiography**

Echocardiography was performed at baseline as well as 7 and 14 days after osmotic minipump implantation using a Vevo 770 microimaging system equipped with a real-time scan head of 17.5 MHz (VisualSonics, Toronto, Ontario, Canada). Rats were anesthetized with 2% isoflurane and echocardiography was performed as described previously (Guo et al. 2011; Moey et al. 2012). Briefly, short-axis M-Mode echocardiography was performed to measure the following parameters: left ventricle internal diameter during diastole (LVID:d), left ventricle internal diameter during systole (LVID:s), left ventricle anterior wall thickness during diastole (LVAW:d), left ventricle anterior wall thickness during systole (LVAW:s), left ventricular posterior wall thickness during diastole (LVPW:d), left ventricular posterior wall thickness during systole (LVPW:s), ejection fraction (EF) and fractional shortening (FS). Doppler mode was used to determine peak early diastolic filling velocity (E wave), peak late diastolic filling velocity (A wave), and E/A ratios. All images were analyzed using the Vevo 770 Protocol-Based Measurements software.

**Non-invasive blood pressure measurements**

Blood pressure was measured during the echocardiography procedure using a CODA standard tail-cuff blood pressure system (Kent Scientific, Torrington, Connecticut, USA) as previously described (Skrbic et al. 2015). Blood pressure parameters were measured by placing the blood pressure cuff around the root of the tail. Systolic and diastolic blood pressure as well as heart rate were determined at baseline and 7, 14 days after isoproterenol infusion.
Gravimetric measurements

At the end of the 14 day follow-up period rats were anaesthetized with a ketamine (50 mg/kg) and xylazine (10 mg/kg) mixture (i.p.), and the heart and lung tissues were collected and weighed. The hearts were separated into the left ventricle free wall (LV) and the right ventricle free wall (RV), wet weights were obtained and tissues were immediately frozen on dry ice for further measurement of gene expressions and protein levels by real-time reverse transcription-polymerase chain reaction (RT-PCR) and western blotting, respectively as described below.

In vitro experimental protocol

Neonatal ventricular myocytes were isolated as described previously (Gan et al. 2013). Cells were grown in fetal bovine serum-containing medium for 24 h after isolation, then followed by serum-free medium for 24 h. To observe the effect of ginseng on isoproterenol-induced cardiac hypertrophy, cardiomyocytes were pretreated with ginseng (10 µg/mL) for 1 h before isoproterenol (1 µmol/L) administration for 24 h.

Determination of cell surface area

Cardiomyocytes images were captured using an Infinity 1 camera (Lumenera Corp, Ottawa, Ontario, Canada) connected to a Leica DMIL inverted microscope (Leica, Wetzlar, Germany). The surface area of 50 myocytes was measured using SigmaScan Pro5 software (Systat, Richmond, California, USA) to provide an N value of one.

RNA isolation and expressions

Total RNA was extracted from either left or right ventricular tissues or cardiomyocytes using Trizol (Life Technologies, Burlington, Ontario, Canada) according to the manufacturer’s instructions and quantified by measuring the absorbance at 260 nm. Three µg of total RNA was
used for reverse transcription to cDNA using M-MLV reverse transcriptase according to the manufacturer’s protocols. The expression levels of atrial natriuretic peptide (ANP), beta myosin heavy chain (β-MHC), alpha skeletal actin (α-SKA), or 18S ribosomal RNA (18S rRNA as a loading internal control) genes were determined in 10 µL reaction volume using EvaGreen qPCR Mastermix (Applied Biological Materials, Richmond, British Columbia, Canada). Fluorescence was measured and quantified using a DNA Engine Opticon 2 System (Bio-Rad Laboratories, Mississauga, Ontario, Canada). PCR conditions to amplify all 3 genes were 10 seconds at 94°C followed by annealing at 58°C for 20 seconds for ANP and β-MHC, 58°C for 30 seconds for α-SKA and 56°C for 20 seconds for 18S rRNA followed by elongation at 72°C for 30 seconds. All genes were amplified for 44 cycles. Primer sequences for genes of interest are as follows: ANP, forward 5’-CTGCTAGACCACCTGGAGGA-3’, reverse 5’-AAGCTTGTGCAGCCTAGTCC-3’; β-MHC, forward 5’-GCACTGGCCAAGTCAGTGTA-3’, reverse 5’-CGAACATGTGGTGGTTGAAG-3’; α-SKA, forward 5’-CACGGCATTATCACCAACTG-3’, reverse 5’-CCGGAGGCATAGAGAGACAG-3’ and 18S forward 5’-GTAACCCGTTGAACCCCATT-3’, reverse 5’-CCATCCAATCGGTAGTAGCG-3’.

**Western blotting**

The tissue homogenate from LV or RV and the whole cardiomyocyte lysates were subjected to western blotting for determination of protein levels of p-PKAα/β/ϒ (Thr 198) and PKAα (A-2) (Santa Cruz Biotechnology Inc., Dallas, Texas, USA). In addition, the cytosolic and nuclear extracts of cardiomyocytes were subjected to western blotting for determination of protein levels of p-CREB-1 (Ser 133) and CREB-1 (D-12) (Santa Cruz). Western blots were performed as described previously (Gan et al. 2013). All primary antibodies were used at a 1:500 dilution.
whereas goat anti-rabbit, goat anti-mouse, donkey anti-goat, goat anti-mouse secondary antibodies were used at a 1:5000 dilution (LI-COR Biosciences, Lincoln, Nebraska, USA). All membranes were scanned using an Odyssey Clx Infrared Imaging System (Li-COR) and the images were analyzed for density using FluorChem 8000 software (Alpha Innotech, San Leandro, California, USA).

**Immunofluorescence**

PKA translocation and nuclear expression of phosphorylated CREB were assessed as described previously with slight modifications (Gan et al. 2013). Cardiomyocytes were grown on collagen precoated glass coverslips in culture medium. After treatments, cells were fixed in cold 1:4 acetone-methanol at 4°C for 30 min, permeabilized with 0.1% Triton X-100 in PBS for 5 min and then blocked with 1% BSA in PBS for 1 h. Cells were incubated with primary antibodies p-PKAα/β/ϒ (Thr 198, 1:50 dilution) and p-CREB-1 (Ser 133, 1:50 dilution) overnight at 4°C followed by incubation with Alexa Fluor 594 goat anti-rabbit IgG (1:250 dilution) and Alexa Fluor 594 Donkey anti-goat IgG (1:250 dilution) for 1 h in darkness, respectively. Cells were mounted on the glass slide using a fluorescent mounting medium (Dako, Burlington, Ontario, Canada). Immunofluorescence was assessed with a Zeiss Axio Observer D1 fluorescence microscope (Zeiss, Gottingen, Germany) and quantified using SigmaScan Pro5 software (Systat).

**cAMP assay**

The cAMP concentrations of both myocytes and LV tissue were measured by a cyclic AMP enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan, USA) described previously (Kou et al. 2012). For myocytes, after treatments, cells were washed, and lysed and incubated in 100 µL of HCl (0.1 M, 20 min) at room temperature. Cells were scraped and centrifuged (1000 x
g, 10 min), and the supernatant was assayed for intracellular cAMP levels. For tissue, the frozen LV was weighed and added 5 volumes of 5% trichloroacetic acid in water, and homogenized and centrifuged (1500 x g, 10 min) to remove the precipitate. The supernatant was extracted the trichloroacetic acid using water-saturated ether repeatedly. After the residual ether was removed, the LV samples as well as myocytes samples were assayed for cAMP concentrations according to the protocols provided by the manufacturers. The cAMP concentrations in myocytes and LV tissue were normalized to the amount of protein (pmol/mg protein).

**Statistical analysis**

All are presented as means ± SEM. Data were analyzed using a one-way ANOVA followed by a Student-Newman-Keuls post hoc test to determine group differences. Survival rates were using log-rank (Mantel-Cox) test. $P < 0.05$ was considered significant.
Results

Animal body weights, blood pressures and heart rates

As shown in Table 1A and 1B we observed no differences in animal body weights during the two week treatment period. Animals treated with isoproterenol demonstrated a sustained significant increase in both systolic and diastolic blood pressures as early as one week after initiating treatment. These increases in blood pressure were unaffected by ginseng at the one week period but were significantly attenuated at the two week period. Heart rates increased in both ISO groups although these increases were partially but not completely attenuated by ginseng (Table 1B). Heart rates were generally stable throughout the 2 week period in sham animals not receiving isoproterenol and completely unaffected by ginseng treatment (Table 1A).

Heart and lung weights following in vivo isoproterenol treatment

We determined the degree of cardiac hypertrophy two weeks after isoproterenol administration. As summarized in Fig. 1 total heart weights increased significantly with both isoproterenol doses although this was attenuated by ginseng, however statistically significant inhibition in total heart weights was seen only in the ISO50 group. These changes were associated with markedly elevated lung weights, an effect also significantly blunted by ginseng in the ISO50 group (Fig. 1).

Having shown that ginseng prevents the isoproterenol-induced increases in total heart weights we further assessed hypertrophic responses by carrying out gravimetric analyses and determining molecular markers of hypertrophy in both left and right ventricles after two week treatment with both isoproterenol doses. These data are summarized in Fig. 2 and show identical hypertrophy with both the ISO25 and ISO50 groups that was evident in both left and right ventricles and as demonstrated by tissue weights as well as expression levels of ANP, β-MHC and α-SKA. Based
on assessment of these parameters, hypertrophic responses to isoproterenol by both the left and right ventricles were significantly diminished by ginseng treatment.

**Left ventricular function assessed by serial echocardiography and mortality**

The effect of ginseng on isoproterenol induced cardiac dysfunction was assessed by serial echocardiography at a weekly basis. These data are summarized in Table 2A and 2B. Isoproterenol treatment resulted in myocardial remodeling as evidenced by reduced left ventricular chamber size during both diastole and systole as well as increased in both anterior and posterior wall thickening although these effects were generally more pronounced in the ISO50 group. Ginseng significantly attenuated these responses which was particularly evident at the two week time point. Representative M-mode and Doppler echocardiography images are shown in Fig. 3. Left ventricular remodeling was also associated with progressive reduction in left ventricular function assessed by determination of ejection fraction and fractional shortening with generally a more rapid and pronounced effect seen with the ISO50 group. Ginseng significantly and markedly attenuated the left ventricular dysfunction seen with both isoproterenol doses. In addition, both isoproterenol doses produced a significant increase in the E/A ratios although this effect was normalized by ginseng at the end of the two week treatment period.

Isoproterenol infusion produced substantial mortality which was more pronounced in the ISO50 group and evident as early as one day after commencing isoproterenol infusion (Fig. 4). Ginseng completely prevented mortality in the ISO25 group whereas mortality was reduced by 43.7% in the ISO50 group receiving ginseng (Fig. 4). No mortality was seen in those animals not receiving isoproterenol. There were no statistical significant differences between any of the treatment groups except in the ISO50 group not treated with ginseng where a significant
incidence of mortality was seen compared to control animals (Fig. 4).

**β adrenergic signaling in vivo and in vitro**

Having shown clear evidence for a beneficial effect of ginseng on cardiac function we next determined whether these effects of ginseng are associated with reduced activation of adrenergic signaling at the end of the two week treatment period. These results are summarized in Fig. 5 and show that both isoproterenol doses elevated cAMP levels (Fig. 5A) and increased activation of PKA at both isoproterenol doses (Fig. 5B and 5C). Similar effects were seen with respect to the transcriptional factor CREB as determined by the degree of phosphorylation (Fig. 5D and 5E). Ginseng completely suppressed PKA and CREB activation (Fig. 5B-5E) although surprisingly cAMP levels were not significantly affected (Fig. 5A).

Assessment of potential mechanisms was further pursued in myocytes exposed to isoproterenol for 24 hours. As shown in Fig. 6A-6E, ginseng produced a marked and significant attenuation of the isoproterenol-induced hypertrophic response as determined by cell size measurements (Fig. 6A and 6B) as well as expression of three molecular markers of hypertrophy (Fig. 6C-6E) thus mimicking the antihypertrophic effect of ginseng seen in rats subjected to isoproterenol infusion.

As shown in Figs. 7 and 8, the antihypertrophic effect of ginseng on cardiomyocytes was also associated with abrogation of isoproterenol-induced activation of adrenergic signaling. In this regard the elevation in cAMP levels by 24 hour isoproterenol treatment was completely suppressed by ginseng (Fig. 7A). PKA activation as determined by phosphorylation status occurred within 5 min following isoproterenol addition and persisted for most of the 24 hour treatment period, with the exception of values seen at 12 hours (Fig. 7B). We therefore next assessed the effect of ginseng at the 30 min and 24 hour time point. As shown in Fig. 7C, ginseng
completely suppressed isoproterenol-induced PKA phosphorylation at both 30 min and 24 hours after isoproterenol administration. Moreover, this effect was associated with a near-complete inhibition of PKA translocation into nuclei at both time points as determined by immunofluorescence (Fig. 7D and 7E).

Isoproterenol administration produced two distinct effects on CREB activity. First, as shown in Fig. 8A and 8B isoproterenol produced a time-dependent translocation of CREB into nuclei which occurred as early as six hours after its addition. In addition, a significant increase in nuclear P-CREB was observed 24 hours after isoproterenol administration whereas no effect was seen on cytosolic CREB with the latter demonstrating substantially lower abundance than that observed in nuclei (Fig. 8C). Both the stimulation in nuclear CREB phosphorylation as well as CREB nuclear translocation were significantly reduced to near-control levels by ginseng.
Discussion

There is currently substantial interest in the use of alternative medications for the treatment of many cardiovascular disorders although clinical efficacy of this approach has yet to be proven primarily due the paucity of well-controlled large scale clinical trials (Rabito and Kaye 2013; Yeh et al. 2006). Among these alternative medicines is the herbal product ginseng which has been used as a therapeutic agent in Asian societies for thousands of years. Ginseng is a highly complex herb containing hundreds of bioactive compounds possessing a myriad of biological effects (Attele et al. 1999). We and others have shown that ginseng can attenuate hypertrophy as well as heart failure using a number of experimental models (Guo et al. 2011; Li et al. 2014; Moey et al. 2012). However, it is not known whether ginseng can specifically affect adrenergic-dependent responses particularly as this pertains to the development of catecholamine-induced cardiac hypertrophy. Accordingly, in the present study we used relatively low doses of isoproterenol to produce heart failure-like responses (Carll et al. 2011). This model of chronic infusion of isoproterenol has been widely used to mimic the sustained β-adrenoceptor stimulation and elevated endogenous catecholamines during the development of heart failure (Okumura et al. 2009; Saadane et al. 1999). Consistent with previous studies (Okumura et al. 2009; Saadane et al. 1999), our data show that infusion of isoproterenol at either of two doses to rats resulted in cardiac hypertrophy and heart failure as evidenced by increased heart weights, seen in both left and right ventricles as well as increased expression of three distinct molecular markers of hypertrophy. Moreover, a similar hypertrophic response was observed in cultured myocytes following administration of isoproterenol for 24 hours thus paralleling results obtained with isoproterenol infusion in vivo.

The major finding of our study is that ginseng effectively inhibited all indices of hypertrophy
which was translated into improved cardiovascular performance including the inhibition of left ventricular systolic dysfunction as seen with improvements in both ejection fractions and fractional shortening. These effects were also associated with reduced mortality in those animals treated with ginseng, indeed with a complete protection seen in the ISO25 group. The cause of the mortality seen in isoproterenol-treated animals is uncertain but this is unlikely due to pump failure due to the relatively modest reductions in ejection fractions and the fact that a large number of deaths occurred within the first few days of starting isoproterenol infusion, especially in the ISO50 group. Although animals were not chronically monitored during the two week treatment period it is reasonable to propose that these deaths were likely related to arrhythmias as previously reported in isoproterenol-treated animals, an effect possibly due to the development of both early delayed afterdepolarization (Tweedie et al. 2000) which, if they reach threshold, can result in triggered lethal arrhythmias. Indeed, electrical remodeling is a major contributor to arrhythmogenesis in the hypertrophied myocardium (Tomaselli and Marban 1999). Although ginseng has been shown to modify the behavior of various cardiac ion channels (Bai et al. 2003), whether it can affect arrhythmogenesis has not been adequately explored. The possibility that ginseng can reduce mortality through an antiarrhythmic mechanisms may be of importance since arrhythmias are well established as substantial contributors to mortality in patients with heart failure (Kjekshus 1990).

Serial echocardiographic analyses revealed marked systolic and diastolic dysfunctions which were attenuated by ginseng. The latter included preservations of ejection fractions particularly evident in the ISO50 group as well as normalization of E/A ratios. These effects were likely mediated by reduced myocardial remodeling in isoproterenol treated rats, especially in terms of attenuating hypertrophy. Indeed, ginseng reduced indices of cardiac hypertrophy as determined
by gravimetric analyses and molecular markers of the hypertrophic response. Moreover, the isoproterenol-induced decreased left ventricle internal diameters during diastole and systole, particularly in the ISO50 group, as well as the increased anterior and posterior wall thicknesses were generally reduced in ginseng treated animals.

Our results also show that pulmonary edema was substantially reduced in isoproterenol-treated rats receiving ginseng. Pulmonary edema and congestion are complex pathophysiologic processes resulting from heart failure. As pulmonary edema can be a consequence of left ventricular dysfunction (Azzam et al. 2001; Chen et al. 2012; Soltysinska et al. 2011), it is reasonable to assume that the beneficial effect of ginseng reflects improved left ventricular performance seen with ginseng treatment. However, isoproterenol, and indeed other catecholamines, can directly produce pulmonary tissue remodeling when infused into rats (Rassler et al. 2012) and it is therefore conceivable that some of the effect of ginseng may have been due to direct inhibition of pulmonary responses to isoproterenol, independent of cardiac effects.

To understand potential mechanisms by which ginseng attenuates the hypertrophic effect of isoproterenol we focused primarily on the β adrenergic pathway and, more specifically on the transcriptional factor CREB which has been implicated in cardiomyocyte hypertrophy as well as heart failure (Ozgen et al. 2008). Our results show that ginseng effectively suppresses isoproterenol-induced stimulation of β adrenergic signaling as shown by a reduction in cAMP levels and attenuation of PKA and CREB activation. Surprisingly we did not observe a significant inhibition by ginseng of the isoproterenol-induced elevation in cAMP levels despite a significant blunting of both PKA and CREB activation, yet cAMP levels were depressed by ginseng in isoproterenol-treated myocytes. A precise reason for this apparent discrepancy is
uncertain but it may suggest that the ability of ginseng to reduce cAMP levels in vivo may have occurred earlier during the 14 day isoproterenol treatment period, a possibility supported by effective inhibition of PKA and CREB activation in this model. Moreover, many of the effects of excessive catecholamines are likely due to enhanced oxidative stress likely unrelated to β-adrenergic receptor signaling (Adameova et al. 2009), although the exact contribution of this process in our model is uncertain.

In conclusion, our in vivo and in vitro results of this present study demonstrate that North American ginseng attenuates beta adrenergic activation-induced cardiac hypertrophy as well as heart failure by preventing PKA activation and CREB phosphorylation. Ginseng may therefore be an effective therapy to reduce hypertrophy and heart failure associated with excessive catecholamine production. Moreover, as emerging evidence has demonstrated the potential benefits of ginseng and its constituent ginsenosides in cardiovascular diseases, this study may also provide support for the potential use of ginseng as adjunctive therapy with standard medications for the treatment of heart failure.
Acknowledgement

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Disclosures

The authors have no conflicts to disclose.
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and Nkx-2.5 in adrenergic-induced cardiac hypertrophy and during regression in adult mice.


Figure legends

**Fig. 1.** Ginseng decreased isoproterenol induced increases in heart weight (A) and lung weight (B). (No treatment (saline): N=10; ISO25: N=9; ISO25+GS: N=7; ISO50: N=10; ISO50+GS: N=9; GS: N=6). Data are shown as means±SEM. *P<0.05 vs. saline; #P<0.05 vs. respective ISO group. ISO, isoproterenol; GS, ginseng; BW, body weight.

**Fig. 2.** Ginseng attenuated isoproterenol induced cardiac hypertrophic responses including ventricle weight and gene expression of ANP, β-MHC, and α-SKA in both left and right ventricles. No treatment (saline): N=8; ISO25: N=7; ISO25+GS: N=6; ISO50: N=8; ISO50+GS: N=8; GS: N=6). Data are shown as means±SEM. *P<0.05 vs. saline; #P<0.05 vs. respective ISO group. ISO, isoproterenol; GS, ginseng; LV, left ventricle; RV, right ventricle; ANP, atrial natriuretic peptide; β-MHC, β-myosin heavy chain; α-SKA, α-skeletal actin.

**Fig. 3.** Representative M-mode (A) and Doppler (B) echocardiography images for animals at 0 week, 1 week and 2 weeks after isoproterenol treatment in the absence or presence of ginseng administration. ISO, isoproterenol; GS, ginseng.

**Fig. 4.** Survival curves for animals during the two week isoproterenol treatment period in the absence or presence of ginseng administration. No mortality was seen in those animals not receiving isoproterenol (Saline: N=10; ISO25: N=12; ISO25+GS: N=7; ISO50: N=18; ISO50+GS: N=12; GS: N=6). *P<0.05 from control animals.

**Fig. 5.** Representative western blots and quantified data showing the effect of ginseng
on cAMP levels (A), PKA (B-C) and CREB (D-E) activation in left ventricle of hearts from rats treated for 14 days with isoproterenol ($N=6$ for all data except for Panel A where $N=5$). Data are shown as means±SEM. *$P<0.05$ vs. saline; #$P<0.05$ vs. ISO group. ISO, isoproterenol; GS, ginseng; PKAα cat, protein kinase Aα catalytic subunit; CREB 1, cAMP response element-binding protein 1; cAMP, cyclic adenosine monophosphate.

**Fig. 6.** Ginseng suppressed isoproterenol induced cardiomyocytes hypertrophy. (A) Representative micrographs of cardiomyocytes subjected to different treatments of isoproterenol and ginseng, respectively. Ginseng attenuated isoproterenol induced significant increase in cell surface area (B) as well as gene expression of ANP (C), β-MHC (D) and α-SKA (E), respectively ($N=6-8$). Data are shown as means±SEM. *$P<0.05$ vs. Control; #$P<0.05$ vs. ISO group. ISO, isoproterenol; GS, ginseng; ANP, atrial natriuretic peptide; β-MHC, β-myosin heavy chain; α-SKA, α-skeletal actin.

**Fig. 7.** Ginseng significantly suppressed isoproterenol induced cardiomyocyte hypertrophy through β-adrenergic signaling pathway. (A) Ginseng reversed isoproterenol induced significant elevation in cAMP levels in cardiomyocytes ($N=6$). (B) Representative western blots and quantified data showing that exposure of cardiomyocytes to isoproterenol for 5 min, 15 min, 30 min, 1 h, 12 h, and 24 h results in PKA phosphorylation, respectively ($N=6$). (C) Ginseng attenuated isoproterenol induced PKA phosphorylation in cardiomyocytes at both 30 min and 24 h time point, respectively ($N=6$). Representative immunofluorescent images (D) and quantified data of nuclear: cytosolic fraction ratio (E) indicating that ginseng inhibited PKA translocation into nuclei in cardiomyocytes treated with isoproterenol 30 min and 24 h,
respectively (N=6). Data are shown as means±SEM. *P<0.05 vs. Control; #P<0.05 vs. ISO group. ISO, isoproterenol; GS, ginseng; PKAα cat, protein kinase Aα catalytic subunit.

**Fig. 8.** Ginseng significantly attenuated isoproterenol induced CREB nuclear translocation and phosphorylation in cardiomyocytes. Representative immunofluorescent images (A) and quantified data of nuclear: cytosolic fraction ratio (B) show that exposure of cardiomyocytes to isoproterenol for 6 h and 24 h resulted in significant CREB nuclear translocation but not after 1 h (N=6). Ginseng significantly reduced these effect at 24 h time point. (C) Ginseng significantly inhibited CREB nuclear phosphorylation at 24 h time point after isoproterenol addition (N=6). Data are shown as means±SEM. *P<0.05 vs. control; #P<0.05 vs. ISO group. ISO, isoproterenol; GS, ginseng; CREB, cAMP-responsive element (CRE)-binding protein.
Table 1A. Body weights, blood pressures and heart rates of rats during the two week treatment period

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<td>86.7±6.0</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>358±13 349±11</td>
<td>353±19 353±14</td>
</tr>
<tr>
<td></td>
<td>357±17</td>
<td>346±14</td>
</tr>
</tbody>
</table>

Note: Data are shown as means ± SEM, *P<0.05 vs. 0 week data in the same group, respectively,
(Sham (saline only): N=10; Ginseng alone: N=6). SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.
Table 1B. Body weights, blood pressures and heart rates of rats during the two week treatment period

<table>
<thead>
<tr>
<th></th>
<th>ISO25</th>
<th>ISO25+ginseng</th>
<th>ISO50</th>
<th>ISO50+ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 week</td>
<td>1 week</td>
<td>2 weeks</td>
<td>0 week</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>208±9</td>
<td>267±13*</td>
<td>310±10*</td>
<td>202±11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>108.9±6.9</td>
<td>130.4±5.1</td>
<td>137.3±6.8*</td>
<td>106.9±5.3</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86.0±5.1</td>
<td>105.0±4.3*</td>
<td>106.8±4.5*</td>
<td>83.4±3.8</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>358±18</td>
<td>444±24*</td>
<td>455±13*</td>
<td>367±13</td>
</tr>
</tbody>
</table>

Note: Data are shown as means ± SEM. *P<0.05 vs. 0 week data in the same group, respectively, #P<0.05 vs. ISO25 or ISO50 group, respectively (ISO25: N=9; ISO25+ginseng: N=7; ISO50: N=10; ISO50+ginseng: N=9). ISO, isoproterenol; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.
Table 2A. Echocardiographic parameters in ISO induced rats in the absence or presence of ginseng administration

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ginseng alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 week</td>
<td>1 week</td>
</tr>
<tr>
<td>EF (%)</td>
<td>81.34±2.05</td>
<td>80.28±2.34</td>
</tr>
<tr>
<td>FS (%)</td>
<td>50.58±2.14</td>
<td>52.54±2.35</td>
</tr>
<tr>
<td>LVID:d (mm)</td>
<td>6.45±0.15*</td>
<td>7.29±0.14</td>
</tr>
<tr>
<td>LVID:s (mm)</td>
<td>3.62±0.13</td>
<td>3.87±0.19</td>
</tr>
<tr>
<td>LVAW:d (mm)</td>
<td>1.60±0.10</td>
<td>1.66±0.10</td>
</tr>
<tr>
<td>LVAW:s (mm)</td>
<td>2.36±0.17</td>
<td>2.46±0.10</td>
</tr>
<tr>
<td>LVPW:d (mm)</td>
<td>1.67±0.14</td>
<td>1.81±0.10</td>
</tr>
<tr>
<td>LVPW:s (mm)</td>
<td>2.42±0.16</td>
<td>2.69±0.18</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.27±0.10</td>
<td>1.31±0.11</td>
</tr>
</tbody>
</table>

**Note:** Data are shown as means±SEM, *P<0.05 vs. 0 week data in the same group, respectively, (Sham (saline): N=8; Ginseng alone: N=6). There were no significant differences between sham and ginseng alone groups at 0 time, 1 week, and 2 weeks, respectively.

LVID:d, left ventricle internal diameter during diastole; LVID:s, left ventricle internal diameter during systole; LVAW:d, left ventricle anterior wall during diastole; LVAW:s, left ventricle anterior wall during systole; LVPW:d, left ventricular posterior wall thickness in diastole; LVPW:s, left ventricular posterior wall thickness in systole; EF%, ejection fraction%; FS%, fractional shortening%. 
### Table 2B. Echocardiographic parameters in ISO induced rats in the absence or presence of ginseng administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ISO25</th>
<th>ISO25+ginseng</th>
<th>ISO50</th>
<th>ISO50+ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 week</td>
<td>1 week</td>
<td>2 weeks</td>
<td>0 week</td>
</tr>
<tr>
<td>EF (%)</td>
<td>79.23±2.38</td>
<td>69.48±2.78*</td>
<td>64.74±2.10*</td>
<td>78.32±2.39</td>
</tr>
<tr>
<td>FS (%)</td>
<td>48.98±2.32</td>
<td>41.81±2.44</td>
<td>39.18±1.82*</td>
<td>48.48±2.38</td>
</tr>
<tr>
<td>LVID:d (mm)</td>
<td>6.44±0.17</td>
<td>7.25±0.10*</td>
<td>7.12±0.12*</td>
<td>6.41±0.12</td>
</tr>
<tr>
<td>LVID:s (mm)</td>
<td>3.71±0.15</td>
<td>3.64±0.13</td>
<td>3.45±0.11</td>
<td>3.67±0.20</td>
</tr>
<tr>
<td>LVW:d (mm)</td>
<td>1.63±0.13</td>
<td>1.88±0.11</td>
<td>1.99±0.09</td>
<td>1.60±0.11</td>
</tr>
<tr>
<td>LVW:s (mm)</td>
<td>2.44±0.14</td>
<td>2.89±0.10*</td>
<td>3.11±0.10*</td>
<td>2.38±0.15</td>
</tr>
<tr>
<td>LVPW:d (mm)</td>
<td>1.69±0.23</td>
<td>2.25±0.11</td>
<td>2.57±0.14*</td>
<td>1.67±0.12</td>
</tr>
<tr>
<td>LVPW:s (mm)</td>
<td>2.43±0.27</td>
<td>3.11±0.10*</td>
<td>3.18±0.11</td>
<td>2.41±0.16</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.28±0.10</td>
<td>1.62±0.10</td>
<td>1.73±0.10*</td>
<td>1.29±0.10</td>
</tr>
</tbody>
</table>

**Note:** Data are shown as means±SEM, *P<0.05 vs. 0 week data in the same group, respectively, †P<0.05 vs. ISO25 or ISO50 group, respectively (ISO25: N=7; ISO25+ginseng: N=6; ISO50: N=8; ISO50+ginseng: N=8). ISO, isoproterenol; LVID:d, left ventricle internal diameter during diastole; LVID:s, left ventricle internal diameter during systole; LVW:d, left ventricle anterior wall during diastole; LVW:s, left ventricle anterior wall during systole; LVPW:d, left ventricular posterior wall thickness in diastole; LVPW:s, left ventricular posterior wall thickness in systole; EF%, ejection fraction%; FS%, fractional shortening%.
Figure 2

LV

RV

ANP/18SmRNA (fold change)

β-MHC/18SmRNA (fold change)

α-SKA/18SmRNA (fold change)

LVW/BW (g/kg)

RVW/BW (g/kg)

ISO25

ISO50

GS -

+ ISO25

+ ISO50

* p < 0.05

# p < 0.01

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Canadian Journal of Physiology and Pharmacology
Figure 3

A

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ISO25</th>
<th>ISO25+GS</th>
<th>ISO50</th>
<th>ISO50+GS</th>
<th>GS</th>
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<tbody>
<tr>
<td>0 week</td>
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<td>2 weeks</td>
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B

<table>
<thead>
<tr>
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<th>ISO50</th>
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<td>2 weeks</td>
<td></td>
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</tbody>
</table>
Figure 4

A: Survival (%) over Days for different treatment groups:
- Saline
- ISO25
- ISO25+ginseng
- Ginseng

B: Survival (%) over Days for different treatment groups:
- Saline
- ISO50
- ISO50+ginseng
- Ginseng

* indicates significant difference
Figure 5

A

**cAMP** (pmol/mg protein)

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<tr>
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B

**P-PKα cat**

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C

**P-PKα cat**

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D

**P-CREB-1**

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E

**P-CREB-1**

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</table>
Figure 6

A. Control

ISO

ISO+GS

GS

B. Cell surface area

Control

ISO

ISO+GS

GS

C. ANP/18SmRNA

Control

ISO

ISO+GS

GS

D. β-MHC/18SmRNA

Control

ISO

ISO+GS

GS

E. α-SKA/18SmRNA

Control

ISO

ISO+GS

GS

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Figure 7

A

CAMP (pmol/mg protein) vs. treatment conditions:
- Control
- ISO
- ISO+GS

B

Graph showing fold change in P-PKα cat/PKAα cat for different time points:
- Control
- 5 min
- 15 min
- 30 min
- 1 h
- 12 h
- 24 h

C

Graph showing fold change in P-PKα cat/PKAα cat for different time points:
- ISO
- GS

D

Immunofluorescence images of nuclear/cytoplasmic ratio for:
- Control
- ISO
- ISO+GS
- GS

30 min
24 h

E

Graph showing fold change in nuclear/cytoplasmic P-PKα cat for:
- ISO
- GS

30 min
24 h
Figure 8

A

Control | ISO | ISO+GS | GS

1 h

6 h

24 h

B

Nuclear:cytosolic P-CREB-1 (fold change)

ISO GS

1 h 6 h 24 h

0.0 0.5 1.0 1.5 2.0

* #

C

P-CREB-1 CREB-1

P-CREB-1/CREB-1 (fold change)

ISO GS

nuclear cytosolic

* #

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