

Original Research Article

Protective effect of ginsenoside Rd against isoproterenol-induced myocardial infarction in Wistar rats

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Abstract

Purpose: To investigate the protective effect of ginsenoside Rd (GRd) against isoproterenol (ISO)-induced myocardial infarction in a rat model.

Methods: Forty healthy male rats were equally divided into four groups (10/group). Rats in the control group received only saline, whereas rats in GRd group were intraperitoneally (i.p.) injected GRd at a dose of 50 mg/kg body weight (b.wt.) for 14 days. The other two groups were ISO-treated rats. One group (MI model) received ISO at a dose of 80 mg/kg b.wt i.p. for 2 days, while the other group (GRd + ISO group) was pretreated with GRd at a dose of 50 mg/kg for 14 days prior i.p. administration of the same dose of ISO.

Results: Treatment with GRd (for 14 days) prior to ISO exposure resulted in significant increase in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as well as significant decreases in heart-to-body weight ratio, infarct size, inflammatory markers {tumour necrosis factor alpha (TNF- α), nuclear factor kappa B (NF- κ B), interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6)}, relative to ISO-treated rats ($p > 0.05$). In addition, prior exposure to ISO led to significant elevation in malondialdehyde (MDA), cardiac troponin T (cTnT), creatine kinase (CPK), lactate dehydrogenase (LDH) and apoptotic markers (caspase-3 and caspase-9). However, pre-treatment with GRd reversed the ISO-induced histopathological changes (necrosis/oedema and altered cardiac fibres) in cardiac tissue.

Conclusion: These results demonstrate that GRd ameliorates ISO-induced cardiotoxicity in rats via upregulation of the activities of antioxidants, and suppression of inflammatory and apoptotic biomarkers. Thus, GRd has cardio-protective properties.

Keywords: Ginsenoside Rd, Myocardial infarction, Apoptosis, Inflammation, Antioxidants

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INTRODUCTION

Myocardial infarction (MI) is a key contributor to mortality and morbidity among all types of cardiovascular diseases (CVDs). Indeed, it has been estimated that MI alone will claim

approximately 23.3 million lives by the year 2030 [1]. The National Centre for Cardiovascular Diseases in China has reported that CVD-linked mortality has risen to 40 % in China due to modified lifestyle and rapid socioeconomic growth [2]. A prolonged obstruction of blood

supply (especially coronary artery) to the myocardial tissue (i.e. hypoxia) results in apoptosis of myocytes, and eventually contributes to MI. However, the precise mechanism (pathophysiology) that underlies MI is still not clear. Nonetheless, few studies have demonstrated that events such as hypoxia, necrosis, mitochondrial dysfunction (altered energetics), apoptosis, oxidative stress, and inflammation are the major contributors to MI [3,4].

Isoproterenol (ISO) is a β -adrenergic agonist (synthetic catecholamine) and has been reported to cause oxidative stress in myocytes/myocardium due to excessive production of free radicals and increased lipid peroxidation. These result in the development of infarct (due to increased coronary vasoconstriction) and eventually end up in MI [5]. The experimental rat model of ISO-induced MI is considered the best MI model for accessing cardiac dysfunction in human MI. This is due to the fact that the pathological and morphological changes in ISO-induced MI in rats resemble those of human MI [6].

Lately, many epidemiological studies have indicated that consumption of plant-derived products (polyphenols) is inversely associated with the prevalence of CVDs especially MI owing to its antioxidant, anti-inflammatory and anti-apoptotic properties [7,8].

Ginsenoside Rd (GRd) is the biologically active principle in *Panax ginseng Radix* or Chinese ginseng, a popular Chinese herb prescribed for various ailments of the cerebrovascular and cardiovascular system [9]. It is a steroid saponin with various pharmacological (antioxidant, anti-inflammatory, anti-apoptotic and neuroprotective) properties [10,11]. Previous studies have demonstrated that GRd exerts protective effect against myocardial ischemic/reperfusion injury via modulation of Akt/GSK and Nrf2/HO-1 signalling pathways in various animal models [12,13]. Moreover, the cardioprotective properties of *Panax ginseng* against ISO-induced cardiotoxicity in rat model has been reported [14]. The present study was designed to investigate the cardioprotective property of GRd pre-treatment against ISO-induced MI in rats.

EXPERIMENTAL

Drug and reagents/chemicals

Isoproterenol hydrochloride, hematoxylin and eosin (H and E); triphenyl tetrazolium chloride (TTC), formaldehyde, isopropanol and xylene

were purchased from Sigma-Aldrich (MO, USA). Ginsenoside Rd (GRd, 98 % pure) was product of Tai-He Biopharmaceutical Co. Ltd, Guangzhou (China). All other chemicals used in this study were of analytical or HPLC grade.

Experimental rats

Forty healthy male Wistar strain rats (n = 40) weighing 210 - 220 g were procured from a local laboratory animal center in Wenshan City, China. They were housed in the animal house of People's Hospital of Wenshan Zhuang and Miao Autonomous Prefecture. The rats were housed in a ventilated steel cage and maintained at 22 – 23 °C with 50 – 55 % humidity under 12-h daylight/12-h dark cycle. The protocol used in this study was reviewed and approved by the Institutional Review board of People's Hospital of Wenshan Zhuang and Miao Autonomous Prefecture (approval no. PHWZM-2015/145a). The study was carried out as per the guidelines of National Research Council (NRC) for the use and care of laboratory animals [15].

Animal grouping and MI induction

Myocardial infarction (MI) was induced by intraperitoneal (*i.p.*) injection of ISO at a dose of 80 mg/kg for two days (15th and 16th day). Forty healthy male rats were equally assigned to four groups. Control group rats received only saline, whereas rats in the GRd group received only GRd (50 mg/kg *i.p.*) for 14 days. Rats in the MI model were injected with ISO (80 mg/kg, *i.p.*) for 2 days on the 15th and 16th days. The rats in the GRd + ISO treatment groups were pre-treated with GRd (50 mg/kg, *i.p.*) for 14 days before ISO injection for induction of MI.

Sample preparation

On the 17th day (end of the treatment), all rats were weighed using standard animal weighing scale (East High Measurement Co., Ltd, Nanjing, China). Then, the rats were anesthetized with pentobarbital sodium (40 mg/kg; *i.p.*), and sacrificed by cervical dislocation. Blood sample was collected in non-heparinized tubes and allowed to clot, and serum samples were separated by centrifuging at 3000 × g for 15 min at 4 °C. The serum samples were kept at -80 °C until analysis. Heart tissues were excised immediately, rinsed in ice-cold saline, blotted and weighed. Portions the heart tissues were homogenized in phosphate-buffered saline (PBS; pH 7.4) using potter-Elvehjem type homogenizer. The homogenate was centrifuged at 12000 × g for 15 min at 4 °C, and the supernatant portion was used for biochemical assays. The remaining

cardiac tissues were fixed in 10 % formalin for histopathological studies.

Biochemical assays

Measurement of myocardial infarct size

Triphenyl tetrazolium chloride (TTC) staining was used to measure the myocardial infarct size according to the method described elsewhere [12], with slight modification. The left ventricle was transversely cut (base apex axis) into slices 2 - 3 mm thick. The ventricular slices were incubated in 1 % TTC in PBS for 20 min at 37 °C, and then fixed with 10 % formaldehyde. Viable ischemic tissue slices were stained red (non-infarcted), whereas non-ischemic tissue slice appeared white or pale grey (infarcted). The image of each slice was captured using LEICA digital camera, and the percentage of infarct size was analyzed with Image J Software (ver 2.8) from National Institutes of Health (MD, USA).

Assay of cardiac antioxidants and lipid peroxidation products

The activities of cardiac endogenous antioxidant enzymes GPx, CAT, and SOD as well as the levels of cardiac MDA were measured using commercial kits (Shanghai Yantuo Biotechnology Ltd., Shanghai, China) in accordance with the manufacturer's protocol.

Assessment of cardiac capacity/function

The activities of serum creatine kinase-MB (isoform) and lactate dehydrogenase (LDH) were assayed using commercial kits from Biosino Biotechnology and Science Inc., Beijing, China, in line with the manufacturers' protocol. Serum cTnT levels were assessed using commercial Elecsys troponin-T stat kit (Roche Diagnostics Ltd., Risch-Rotkreuz, Switzerland).

Evaluation of cardiac inflammatory markers

Nuclear factor kappa B p65 subunit (NF- κ B p65) in the nuclear fraction was extracted from cardiac tissue homogenate using nuclear and cytoplasmic extraction kit from Guge Biotechnology, Wuhan, China, and was measured using NF κ B p65 ELISA kit (Invitrogen, Thermo Fisher Scientific Inc., MA, USA). The levels of IL-1 β , TNF- α , and IL-6 in cardiac tissue homogenate were determined with rat-specific Quantikine ELISA kits (R and D system, Minneapolis, MN, USA), based on suppliers instructions.

Determination of cardiac apoptotic markers

The activities of rat-specific caspase-3 and caspase-9 in the cardiac tissue homogenate (supernatant) were determined using commercial ELISA kits (Beyotime Int. Biotech, Jiangsu, China) in line with the kit protocol.

Histopathological examination

Cardiac tissues were fixed in 10 % formalin and dehydrated sequentially by first treating with xylene, followed by isopropanol. The tissue samples were embedded in liquid paraffin wax to form tissue blocks. Then, the blocks were cut into 5- μ m slices using a microtome (Leica RM, Wetzler, Germany), and the slices were attached to microscopic slides. Cardiac tissue slides from different groups were stained overnight with hematoxylin and eosin (H & E) at 4 °C, and thereafter examined under a light microscope (Nikon, Tokyo, Japan) for pathological changes. The slides were read by a pathologist naïve to the treatments, and photomicrographs were captured.

Statistical analysis

Data are expressed as mean \pm standard deviation (SD) for 10 animals in each group. Statistical differences between groups were analyzed with one-way analysis of variance (ANOVA), followed by Tukey's multi-comparison test. All statistical analyses were done with SPSS software, ver 22 (IBM Inc, NY, USA). Values of $p < 0.05$ were taken as significant.

RESULTS

Effect of GRd on heart weight, body weight, and heart weight-to-body weight ratio

Table 1 shows the impact of GRd on body weight, heart weight and heart-to-body weight ratio in the experimental rats. No significant changes were observed in body weight in all the experimental groups. When compared with rats in the control group, heart weight and heart-to-body weight ratio were significantly increased in the ISO-exposed rats ($p < 0.05$). However, rats pre-treated with GRd for 14 days had significant reductions in heart weight and heart-to-body weight ratio, relative to ISO-injected rats.

Effect of GRd on myocardial infarct size

Figure 1 depicts the effect of GRd on myocardial infarct size after TTC staining. When compared with rats in the control group, infarct size was significantly increased (32.5 %) in the ISO

(model) rats ($p < 0.01$); most of the heart tissue was unstained. However, treatment with GRd led to significant decrease in infarct size (14 %) ($p < 0.01$), with highly stained heart tissue slices (red), when compared with ISO-treated rats.

Effect of GRd on activities of some cardiac antioxidants and lipid peroxidation products

The effect of GRd on the activities of various cardiac antioxidants and lipid peroxidation products in experimental rats was investigated. As shown in Table 2 the activities of cardiac antioxidants (GPx, CAT and SOD) were significantly decreased ($p < 0.01$), with a marked increase ($p < 0.01$) in the level of MDA (lipid peroxidation product) in the ISO-treated rats. However, pre-treatment with GRd for 14 days prior to ISO exposure produced significant increases ($p < 0.05$, $p < 0.01$) in the activities of cardiac antioxidants GPx, CAT, and SOD, and significant decreases in the level of MDA, relative to ISO-injected rats ($p < 0.05$).

Effect of GRd on various cardiac markers in serum and cardiac tissue

Table 3 shows the effect of GRd on various cardiac markers in experimental rats. The mean levels of serum (LDH, CK-MB, cTnT) diagnostic markers of ISO-injected rats were significantly increased ($p < 0.01$) than control. However, pre-treatment with GRd significantly reversed ($p < 0.05$, $p < 0.01$) these ISO-induced elevations in cardiac markers to near normal levels, relative to ISO rats.

Table 1: Effect of GRd on heart weight, body weight and heart-to-body weight ratio

Parameter	Control	GRd	ISO	GRd+ISO
Body weight (g)	246.34 ± 8.50	245.72 ± 4.80	239.05 ± 7.00 ^a	245.40 ± 6.48 ^b
Heart weight (g)	0.570 ± 0.08	0.550 ± 0.07	0.790 ± 0.09 ^{a*}	0.687 ± 0.07 ^{b*}
Heart-to-body weight ratio (%)	0.231 ± 0.01	0.223 ± 0.02	0.330 ± 0.04 ^{a*}	0.279 ± 0.02 ^{b*}

Data are expressed as mean ± standard deviation (SD, n = 10). * $p < 0.05$; # $p < 0.01$: "a" represents comparison between ISO and control; "b" represents comparison between GRd + ISO group and ISO group

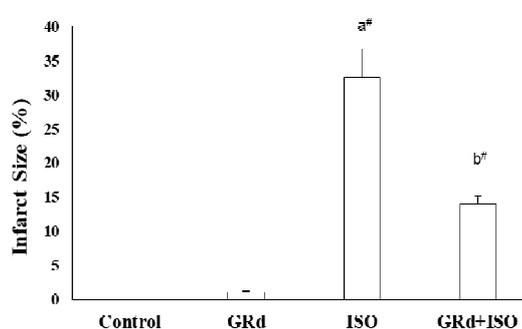


Figure 1: Effect of GRd on myocardial infarct size after TTC staining in experimental rats. Data are expressed as mean ± standard deviation (SD) for 10 animals in each group. P value (* $p < 0.05$; # $p < 0.01$): "a" represent the comparison between ISO vs. Control, where "b" represents the comparison between GRd + ISO vs. ISO group. GRd: Ginsenoside Rd; ISO: isoproterenol

Table 2: Effect of GRd on cardiac antioxidants and lipid peroxidation products in experimental rats

Parameter	Control	GRd	ISO	GRd+ISO
GPx ($\mu\text{g}/\text{mg pro}$)	9.87 ± 0.12	10.02 ± 0.11	6.89 ± 0.08 ^{a#}	8.44 ± 0.10 ^{b*}
CAT (U/mg pro)	14.24 ± 2.10	15.66 ± 1.50	7.80 ± 0.88 ^{a#}	12.38 ± 1.47 ^{b#}
SOD (U/mg pro)	6.72 ± 0.80	7.01 ± 0.85	3.97 ± 0.50 ^{a#}	5.45 ± 0.75 ^{b#}
MDA (nmols/mg pro)	0.52 ± 0.06	0.51 ± 0.04	0.88 ± 0.11 ^{a#}	0.66 ± 0.10 ^{b*}

Data are expressed as mean ± standard deviation (SD) for 10 animals in each group. * $p < 0.05$; # $p < 0.01$: "a" represents comparison between ISO and control, while "b" represents comparison between GRd + ISO group and ISO group. One unit of SOD Unit (U) was defined as the amount of enzyme that causes 50 % inhibition (IC_{50}) of reduction of tetrazolium salt. One unit of CAT (U) was defined as the amount of enzyme utilized to reduce 50 % of H_2O_2 . Pro = Protein

Table 3: Effect of GRd on serum cardiac markers in experimental rats

Parameter	Control	GRd	ISO	GRd+ISO
cTn T (ng/mL)	0.50 ± 0.05	0.48 ± 0.06	1.82 ± 0.14 ^{a#}	0.79 ± 0.10 ^{b*}
LDH (IU/L)	85.18 ± 6.19	88.32 ± 7.15	149.90 ± 12.25 ^{a#}	107.44 ± 14.87 ^{b*}
CK-MB (IU/L)	67.56 ± 7.23	73.91 ± 6.15	160.38 ± 14.10 ^{a#}	102.25 ± 15.20 ^{b#}

Data are expressed as mean ± standard deviation (SD) for 10 animals in each group; * $p < 0.05$; # $p < 0.01$: "a" represents comparison between ISO and control; "b" represents comparison between GRd + ISO group and ISO group

Table 4: Effect of GRd on cardiac inflammatory markers

Parameter	Control	GRd	ISO	GRd+ISO
NF-κB p65 (pg/mg pro)	82.94 ± 10.98	81.34 ± 12.10	194.77 ± 21.25 ^{a#}	120.81 ± 15.84 ^{b#}
TNF-α (ng/mg pro)	114.71 ± 18.34	115.30 ± 19.30	217.18 ± 22.00 ^{a#}	149.99 ± 20.27 ^{b#}
IL-1β (ng/mg pro)	60.45 ± 6.90	62.24 ± 8.49	152.70 ± 19.45 ^{a#}	86.45 ± 11.27 ^{b#}
IL-6 (pg/mg pro)	75.56 ± 10.12	78.36 ± 11.95	182.68 ± 21.00 ^{a#}	108.83 ± 20.30 ^{b#}

Data are expressed as mean ± standard deviation (SD) for 10 animals in each group; $p < 0.05$; $^{\#}p < 0.01$. "a" represents comparison between ISO and control; "b" represents comparison between GRd + ISO group and ISO group. (Pro = Protein)

Effect of GRd on cardiac inflammatory markers

As indicated in Table 4, the levels of different cardiac inflammatory markers i.e. NF-κB p65 (nuclear fraction), and IL-1β, TNF-α and IL-6 (cytosolic fraction pro-inflammatory cytokines) were significantly increased in ISO-injected rats ($p < 0.01$). Significant decreases in the levels of these inflammatory markers (NF-κB p65, IL-1β, TNF-α, IL-6) were observed in GRd-administered rats, when compared to the ISO-treated rats ($p < 0.01$).

Effect of GRd on cardiac apoptotic markers

The effect of GRd on the activities of various cardiac apoptotic markers in the experimental rats is illustrated in Figure 2. Significant increases were seen in the activities of apoptotic markers (caspase-3 and caspase-9) in ISO-injected rats ($p < 0.01$). Interestingly, the activities of these apoptotic markers were significantly decreased by pre-treatment with GRd for 14 days, when compared with ISO-injected rats ($p < 0.01$).

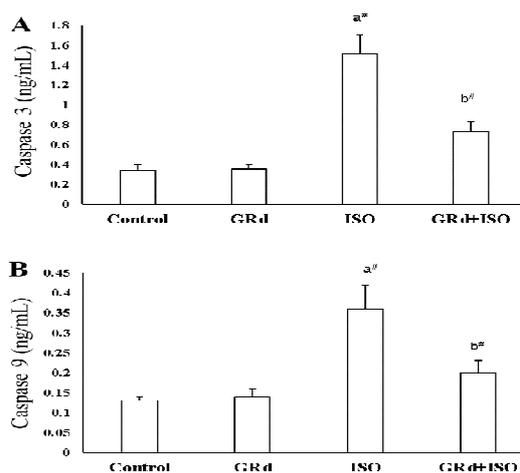


Figure 2: Effect of GRd on the activities of caspase-3 (A) and caspase-9 (B) in experimental rats. Data are expressed as mean ± standard deviation (SD) for 10 animals in each group. $^{\#}p < 0.05$; $^{\#}p < 0.01$. "a" represents comparison between ISO and control, while "b" represents comparison between GRd + ISO group and ISO group

Effect of GRd on histopathological changes

Figure 3 shows the effect of GRd on histopathological changes in cardiac tissue of ISO-treated rats, as revealed by H & E staining. Representative photomicrographs of control cardiac tissue presented regular myofibrillar structure without any pathological conditions such as oedema or inflammation (Figure 3A). Similarly, photomicrographs of rats treated with GRd alone revealed the presence of normal cardiac architecture (myofibrillar structure, Figure 3B), whereas, the ISO-treated rats showed evidence of myofibrillar degeneration (disruption) characterized by elevated neutrophil granulocyte infiltration (inflammation), necrosis and interstitial oedema (Figure 3C). Rats pre-treated with GRd for 14 days prior to ISO exposure had lower degree of myofibrillar degeneration (disruption), fewer neutrophil granulocyte infiltration and reduced interstitial oedema, which reveal the cardio-protective property of GRd (Figure 3D).

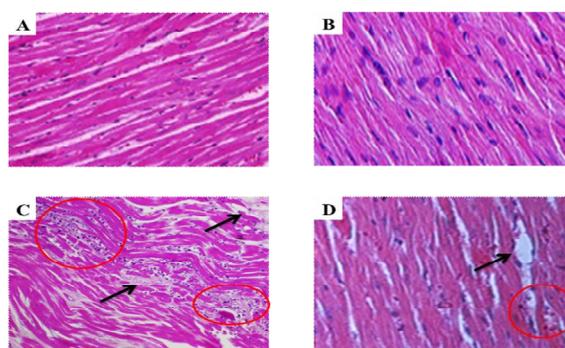


Figure 3: Effect of GRd on histopathological changes in cardiac tissue due to ISO treatment (H & E). Control cardiac tissue presented normal myofibrillar structure (Figure 3 A). Similarly, cardiac tissue of rats treated with GRd alone revealed presence of normal cardiac architecture (myofibrillar structure) (Figure 3 B). However, ISO-treated rats showed myofibrillar degeneration (disruption) characterized by elevated neutrophil granulocyte infiltration (indicated by a circle), necrosis and interstitial oedema (indicated by arrow) (Figure 3C). Rats pre-treated with GRd for 28 days prior to ISO treatment showed lesser myofibrillar degeneration (disruption), with fewer neutrophil granulocyte infiltration and less interstitial oedema (indicated by arrow mark), relative to the ISO group (Figure 3 D)

DISCUSSION

The results of the present study revealed that GRd exerts potent cardio-protective action against ISO-induced MI in rats by decreasing heart weight, lipid peroxidation and cardiac inflammatory markers, as well as enhancing antioxidant enzymes and reversing histopathological lesions. Evidence suggests that auto-oxidation of catecholamines/quinones (after ISO treatment) leads to excessive free radical generation which results in peroxidation of cardiomyocyte membrane lipids, changes in membrane permeability, cardiomyocyte necrosis and MI [16,17]. Heart-to-body weight ratio and heart weight were significantly increased in ISO-treated rats due to increased membrane permeability (enhanced oxidative stress), leading to increased movement of water contents (oedema) and increased heart weight [18]. However, 14 days of pre-treatment with GRd produced significant reductions in heart weight and heart-to-body weight ratio, relative to the ISO-injected rats. The effect produced by GRd is most likely due to its antioxidant and membrane stability-enhancing properties.

The MI rats had higher infarct size, whereas, rats treated with GRd for 14 days had decreased infarct size, relative to the ISO-induced rats. Previous studies showed that treatment with GRd considerably reduced infarct size and attenuated myocardial ischemic injury [13]. This is in agreement with the results obtained in the present study which show that pre-treatment with GRd exerts potent cardio-protective property. Numerous studies have revealed that oxidative stress (imbalance in pro- and antioxidants) as evidenced by significant elevation in MDA, and decreased SOD, CAT, and GPx occur during myocardial ischemic condition [19]. The activities of cardiac antioxidants (GPx, CAT, and SOD) were significantly decreased, with concomitant increase in MDA in rats treated with ISO. However, GRd treatment reversed the decreases in the activities of these cardiac antioxidants, while decreasing MDA. These results are in agreement with those reported in a previous study which also demonstrated that treatment with GRd considerably attenuated free radical generation by increasing the levels of SOD, and GSH [11]. Furthermore, it has been reported that treatment with GRd markedly upregulated the expression of nuclear factor-related factor 2 (Nrf2) and enhanced the production of SOD and glutathione [13].

The serum levels of LDH, CK-MB and cTnT were significantly increased in the ISO-treated rats due to elevated lipid peroxidation (membrane

damage), which resulted in the leakage of these cardiac markers from cardiomyocytes into the serum. The amount of this marker enzyme in the blood is directly proportional to the number of damaged, necrotic cardiomyocytes [18]. Pre-treatment with GRd restored the levels of cardiac markers to near normalcy by maintaining the membrane integrity due to enhanced free radical scavenging activity. Previous studies have shown that treatment with GRd markedly lowered the production of various cardiac markers due to free radical and anti-lipid peroxidation property, resulting in increased population of cardiomyocytes [13].

Inflammation markers (cytokines) are crucial prognostic factors in cardiovascular patients, and are useful for early diagnosis of ischemia and myocardial lesions [20]. Nuclear factor kappa B (NF- κ B) plays a central role (act as a transcriptional factor) in regulating various inflammatory factors especially pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6), and it is indirectly involved in the recruitment of various immune cells. Normally, NF- κ B is anchored in the cytoplasm (inactive form), but inflammatory stimuli (LPS or ISO) can trigger its activation, leading to translocation of activated NF- κ B p65 subunit into the nucleus where it binds to the promoter region of pro-inflammatory cytokine genes and upregulates the expression of these pro-inflammatory cytokines [21]. Rats injected with ISO had pronounced increases in cardiac NF- κ B p65 (nuclear fraction), and IL-1 β , TNF- α and IL-6 (cytosolic fraction-proinflammatory cytokines). The ISO treatment (inflammatory stimuli) results in oxidative stress in cardiomyocytes, which in turn initiates an inflammatory response [6]. Thus, the levels of these inflammatory markers were considerably increased in ISO-treated rats. However, pre-treatment with GRd reversed the ISO-induced increases in the levels of these inflammatory markers. These results are consistent with the results of Cong and Chen [11].

Although several caspases are involved in apoptosis, caspase-9 (initiator-caspase) and caspase-3 (executioner-caspase) are highly expressed during hypoxia (ischemic) and oxidative stress conditions [22] especially in MI [23,24]. Thus, the levels of these major cardiac apoptotic markers were determined in the present study. Studies have indicated that oxidative stress due to ISO leads to apoptosis in cardiomyocytes and eventually results in ischemic injury and myocardial infarction [25]. The activities of caspase-3 and caspase-9 were significantly increased in ISO-treated rats due to activation of the β -adrenergic receptor which led

to apoptosis in cardiomyocytes. However, GRd pre-treatment led to significant decreases in the activities of these caspases. Studies have indicated that treatment with GRd significantly inhibited the activation of caspase-3 and caspase-9, thereby arresting the apoptosis response [12].

Histopathological analysis were carried out to assess pathophysiological changes in the experimental rat groups. Photomicrographs of cardiac tissues from rats in control and GRd-alone groups showed normal myofibrillar structures without oedema or inflammation. However, cardiac tissue from ISO-treated rats showed myofibrillar degeneration characterized by elevated infiltration of neutrophil granulocytes (inflammation), necrosis and interstitial oedema. Rats pre-treated with GRd for 14 days prior to ISO exposure showed lower degree of myofibrillar degeneration (disruption), with fewer infiltration of neutrophil granulocyte and reduced interstitial oedema. This indicates the cardio-protective property of GRd through protection of cardiac fibers from necrosis and apoptosis. Treatment with Korean ginseng rich in ginsenoside showed better prominent cardiac fibres with cross striation after ISO-induced cardiotoxicity [14].

Study limitations

The major limitation of the present study is that hemodynamic parameters and lipid profiles were not investigated.

CONCLUSION

The findings of the present study demonstrate that GRd exerts cardio-protective effects against ISO-induced MI through enhancement of the activities of various antioxidants, significant reduction in infarct size, reduction of the levels of cardiac inflammatory biomarkers, and reduction of apoptotic markers. However, further studies are required to confirm the cardioprotective properties of GRd as well as the mechanism underlying the process.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this study

Authors' contributions

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaojun Sun, Chunli Li, and Jing Lu involved in conception and designing of this study. Xiaojun Sun, and Chunli Li conducted this study. Chunli Li, and Jing Lu interpreted and carried statistical analysis. Xiaojun Sun, and Jing Lu both drafted this manuscript.

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