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The cardio and renoprotective role of ginseng against epinephrine-induced myocardial infarction in rats: Involvement of angiotensin II type 1 receptor/protein kinase C

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ABSTRACT

The expression of angiotensin II type 1 receptor (AT1 receptor)/protein kinase C (PKC) in heart tissues has a vital role in myocardial infarction (MI). The current work aimed to clarify the renal complication enhanced by MI following epinephrine injection via AT1 receptor/ PKC expression; in addition, the impact of ginseng extract on epinephrine-induced MI and its renal complication was assessed. Adult male albino Wistar rats were pretreated orally with ginseng extract (200 & 400 mg/kg/day) for 14 days, then two successive doses of epinephrine injection (100 mg/kg, i.p.). Epinephrine evoked electrocardiographic (ECG) and renal changes accompanied with a significant increase in heart and kidney contents of malodialdehyde (MDA), nitric oxide (NO), protein kinase C (PKC), heart contents of nuclear factor-kabba B (NF-κB) and angiotensin 1receptor (AT1R), as well as a decrease in heart and kidney reduced glutathione (GSH) and nuclear factor-erythroid-related factor 2 (Nrf2) contents. In histopathological investigations epinephrine exhibited deleterious heart changes in the form of acute MI with the presence of necrosis of cardiomyocytes with iNOS expression and produced glomerulus and renal tubules degeneration. Pretreatment of rats with ginseng extract in both doses significantly corrected epinephrine-induced heart and renal changes. The current work revealed that epinephrine-induced MI associated with aggravated renal complication and ginseng extract has cardio and reno protective role against this as it reduces infarct size, preserves cardiac and renal tissues and functions through activating Nrf2 and down-regulating NF-κB, PKC, AT1R and iNOS.

1. Introduction

Myocardial infarction (MI) is a cardiac remodeling in which there is a consequence of molecular, cellular, tissue, and organ levels maladaptation to cardiac stress and oxygen derived free radicals genesis in the heart leading to necrosis and apoptosis of cardiomyocytes [1]. MI produces 3 million case worldwide [2] and according to the World Health Organization (WHO), it is one of the major diseases in 2020 [3]. New therapies are needed to treat MI because current treatments have only a limited impact on survival and annual cost.

Cardiac stress, the sustained and abnormal neurohormonal stress, is a key pathophysiological process leading to post-MI heart failure. One of the neurohormons involved in cardiac stress is epinephrine that is responsible for "fight or flight" mechanism [4]. Circulating epinephrine level is increased in the early phase of MI as it is released from adrenal medulla and post-ganglionic synapses due to sympathetic discharge. It plays an important role in the generation of the highly cytotoxic free radicals associated with MI morbidity; this is due to its auto-oxidation, with a subsequent depletion of the natural antioxidant defense mechanisms, as reduced glutathione (GSH), catalase, and superoxide dismutase [5]. Nuclear factor-erythroid-2 (NF-E2)-related factor 2 (Nrf2) is a vital key player in epinephrine-induced MI. It is a sensitive markers in cardiovascular diseases pathogenesis [6]. It can also prolong survival and modulate innate and adaptive immune responses involved in these diseases. Nrf2 controls the antioxidant genes expression and detoxifying enzymes (cytoprotective phase II), including quinone oxido-reductase-1 and heme-oxygenase-1 [7].

The angiotensin II type 1 receptor (AT1 receptor) is one of the renin angiotensin aldosterone system. It is expressed in the cardiovascular system and activates different types of protein kinases as protein kinase

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C (PKC) that generates ROS and produces hypertrophy, hyperplasia and renal damage. The expression of PKC in heart tissues revealed an important role in MI. PKC isozyme is a regulator controlling a signal transduction pathways of biological responses such as gene expression, cell proliferation and differentiation [8]. Wang et al. demonstrated the increased cardiac activity of PKC in heart failure of cardiomyopathic hamsters [9]. Moreover, PKC elevation was observed in pressure-overloaded cardiac hypertrophy in rats [10]. Furthermore, overexpression of cardiac PKC was provoked in transgenic mice with cardiac hypertrophy and diminished ventricular function [11].

Interestingly, heart-kidney crosstalk has been reported; hypertension and atherosclerosis can lead to chronic kidney disease through tolerance of oxidative stress and inflammation [12], also, the main reasons of death in renal failure patients are cardiovascular diseases, mainly heart failure, that account for 50 % of all deaths in renal failure population [13]. Ronco et al. redefined this heart-kidney crosstalk as cardiorenal syndrome (CRS) [14]. Administration of epinephrine produces renal blood flow decrement via stimulation of renal receptors, resulting in a change of renal functions [7].

Phytotherapy has been used in the treatment or prevention of many disorders for thousands of years. Ginseng is an important phototherapeutic agent; it possesses protective effects against many vital organs disorders, such as liver, brain, kidney, and heart diseases, as well as a significant anticarcinogenic effect [15]. The purpose of this study was to investigate the possible progression of renal damage following epinephrine-induced MI in rats. In addition, we aimed to explore the role of ginseng extract as cardio and renoprotective against this MI model, and to find its role in regulation of Nrf2, NF-κB, PKC, AT1R and iNOS expressions that are affected by epinephrine.

2. Materials and methods

2.1. Animals

Adult male Wister albino rats weighing 120–140 g were purchased from the animal house colony of the National Research Centre (NRC, Cairo, Egypt), and were kept in the animal house under conventional laboratory conditions. The animals were kept in a quiet place and were allowed free access to water and standard food pellets throughout the period of investigation. All research procedures and the care of the animals were in compliance with the Instructional Animal Care and Use Committee (IACUC) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). This study had a certificate number 15,164 before starting the experiment from the ethics committee of NRC (Cairo, Egypt).

2.2. Plant materials

Panax ginseng roots were collected from Agriculture Research Centre, Ministry of Agricultural Research Centre, Egypt.

2.3. Drugs, chemicals and kits

Epinephrine was purchased from Sigma–Aldrich (MO, USA). All other chemicals used were of analytical grade.

Creatinine, blood urea nitrogen (BUN), nitric oxide (NO), malodialdehyde (MDA) and reduced glutathione (GSH) standard kits were purchased from Biodiagnostic (Cairo, Egypt).

Nuclear factor-erythroid-related factor 2 (Nrf2) and nuclear factor-kabba B (NF-κB) enzyme linked immunoassay (ELISA) kits were obtained from Glory Science Co., Ltd, USA. Protein kinase C (PKC), and angiotensin 1receptor (AT1R) ELISA kits were obtained from Elabscience and Life Span BioScience, Inc., China.

2.4. Preparation of plant extract

Panax ginseng roots (100 g) were grinded and extracted with 70 % aqueous ethanol (1000 mL) in a 2000 mL conical flask and kept on an orbital shaker (Stuart, England) at 160 rpm at room temperature for 24 h. Then, the extract was centrifuged (Sigma 3–18 ks Centrifuge, Germany) at 5000 rpm for 20 min at 25 °C to separate cell debris from the supernatant. The extraction step was repeated twice. The extract was filtered using Whatman NO.1 filter paper. The filterate was dried in rotavap under vaccum of 120 millibar in water bath at 40 °C. The distillate was added on the marc, recollected again and re-evaporated in the rotavap. Then, the extract was freeze dried (lyophilized) using a freeze-drier (CHRIST (ALPHA 1-4 LSC plus), Germany) to eliminate water traces. The extracts were then placed in glass vials and stored frozen at −20 °C till being used [16]. The contents of ginsenoside was measured using high-performance liquid chromatography and size exclusion chromatography [17].

2.5. Experimental design

MI was induced in rats according to Zaafan et al. [18] using an intrapretonial (i.p.) injection of epinephrine (100 mg/kg) for two successive days. Rats were randomly allocated into six groups (n = 8). The normal control group received saline orally (p.o.) for 14 days then two successive saline injection. Two groups received ginseng extract (200 or 400 mg/kg; p.o), respectively, for 14 days and served as control groups. Forth group received two successive doses of epinephrine injection (100 mg/kg; i.p). The remaining two groups received ginseng extract (200 or 400 mg/kg; p.o), respectively, for 14 days [19] then, received epinephrine injection in the last day of ginseng extract administration.

After 24 h of epinephrine injection, animals were anesthetized with urethane (1.5 g/kg; i.p.) for electrocardiogram monitoring (ECG; Powerlab/8sp, Australia), then, blood samples were taken from the abdominal aorta and used for determination of creatinine and BUN levels [20]. Rats were then sacrificed by decapitation [21] and the hearts and kidneys were rapidly isolated, washed with ice-cold saline. A weighed part of each heart and kidney (0.5 g) were homogenized using a homogenizer (Medical instruments, MPW-120, Poland), in 2 mL of ice -cooled phosphate buffer (pH 7.4) to prepare 20 % (w/v) homogenates [22]. The supernatant were used for the estimation of heart and kidney NO, MDA, GSH, Nrf2 and PKC as well as heart NF-κB and AT1R contents. The other half of the heart and the other kidney were preserved in 10 % formalin for histological examination and immunohistochemical investigation of iNOS

2.6. Estimation of kidney function and oxidative stress biomarkers

Serum creatinine and BUN levels were determined using Biodiagnostic kits. Heart and kidney NO was estimated using standard Biodiagnostic kits. This kit provides an accurate method for measurement of endogenous nitrite concentration as indicator of NO production. MDA and GSH contents were estimated using Biodiagnostic kits.

2.7. Estimation of Nrf2 and PKC heart and kidney contents as well as NF-κB and AT1R heart contents

The contents of heart and kidney Nrf2 and PKC as well as heart NF-κB and AT1R were estimated by enzyme linked immunoassay (ELISA) kits. We followed the manufacturer's instructions of Glory Science Co., Ltd, USA and Elabscience and Life Span BioScience, Inc., China for calculating the results. Standards and samples were pipetted into wells with immobilized antibodies specific for rat Nrf2, PKC, NF-κB and AT1R, then were incubated 30 min at 37°C. After incubation and washing, horseradish peroxidase-conjugated streptavidin of Nrf2, PKC, NF-κB and AT1R was pipetted into the wells and incubated 30 min at 37°C, which were washed once again. TMB (chromogen; tetramethylbenzidine)

substrate solution was added to the wells and incubated 15 min at 37°C; color developed proportionally to the amount of Nrf2, PKC, NF-κB and AT1R bound. Color development was discontinued (Stop Solution) and after 10 min color intensity was measured at 450 nm.

2.8. Histological examination

The parts of the heart and kidney that were fixed in 10 % formalin solution were then dehydrated in ascending grades of alcohol and embedded in paraffin. Four sections/group, at four micron thickness, were taken and stained with hematoxylin and eosin (H&E). The sections were evaluated under light microscope (200×) for necrosis, nuclear pyknosis, and macrophage activity. Severity of changes was graded using a scale of no change (0), mild changes (1), moderate changes (2) and severe changes (3).

2.9. Immunohistochemical detection of iNOS

Immunohistochemical staining of anti-iNOS antibodies was performed by streptoavidin–biotin. Four sections/group, at four micron thickness, were deparaffinized and incubated with fresh 0.3 % hydrogen peroxide in methanol for 30 min at room temperature. The specimens were then incubated with anti-iNOS antibody as a primer antibody at a 1:100 dilution. The specimens were counterstained with H&E.

Immunohistochemical staining was scored in a semiquantitative manner to determine differences between the control group and the experimental groups. Weak (-) mild (+), moderate (++), and strong (+++), signals was observed and recorded. This analysis was performed in at least 10 areas from the brain, in 4 sections from each animal at x400 magnification.

2.10. Statistical analysis

All the values are presented as means ± standard error of the means (SE). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's HSD test for multiple comparisons. Graphpad Prism software, version 5 (Inc., USA) was used to carry out these statistical tests. The difference was considered significant when $p < 0.05$.

3. Results

3.1. Determination of total ginsenoside content

The results of the current study revealed that ginseng root extract has 26.11 % total ginsenoside content of dry weight of the extract.

3.2. Effects of pretreatment with ginseng extract on ECG pattern of epinephrine-injected rats

Normal HR, QTc and RR intervals were detected in control groups received ginseng extract (200 mg and 400 mg/kg), while, epinephrine injection showed a significant decrease in HR and QTc intervals. In addition, it produced RR interval elevation as compared to normal group. Pretreatment of the animals with the low dose of ginseng extract significantly increased HR and QTc intervals as compared to epinephrine-treated rats; while, pretreatment of the animals with the high dose normalized all ECG parameters (Table 1).

3.3. Effects of pretreatment with ginseng extract on serum creatinine and BUN levels

The mean levels of creatinine and BUN in ginseng extract control groups were not changed when compared with the normal group, while, induction of MI by epinephrine significantly increased the serum creatinine and BUN levels by 155.70 % and 461.94 %, respectively, as compared to normal group. The pre-treatment with the low dose of ginseng extract exhibited a significant decrease in the epinephrine-induced elevation of serum creatinine and BUN levels by 27.72 % and 45.15 %, respectively. Also, the high dose of ginseng extract made a correction of epinephrine effects decreasing serum creatinine and BUN levels by 46.53 % and 59.50 %, respectively, as compared with epinephrine group (Table 2).

3.4. Effects of pretreatment with ginseng extract on the heart NO, MDA, and GSH contents

Both doses of ginseng extract administration have no effect on the normal contents of the heart NO, MDA, and GSH. Induction of MI by epinephrine significantly increased heart NO and MDA contents by 183.32 % and 356.81 %, respectively, and decreased heart GSH content by 25.90 % as compared with normal control group. Pretreatment with low dose of ginseng extract significantly decreased only heart MDA content by 66.32 %, while, pretreatment with the high dose of ginseng extract significantly decreased heart NO and MDA contents by 42.47 % and 75.13 %, respectively, in addition pretreatment of the animals with high dose of ginseng extract increased heart GSH content by 41.86 %, respectively, as compared with epinephrine group (Table 3).

3.5. Effects of pretreatment with ginseng extract on kidney NO, MDA and GSH and contents

The kidney contents of NO, MDA and GSH in ginseng extract control groups were not changed as compared with the normal group. Injection of epinephrine significantly increased kidney NO and MDA contents by 51.88 % and 74.81 %, respectively, and decreased kidney GSH content by 13.00 % as compared with normal control group. Pretreatment with the low dose of ginseng extract did not change kidney NO and MDA contents, while, the high dose of ginseng extract significantly decreased kidney NO and MDA contents by 15.51 % and 23.48 %, respectively, in addition pretreatment of the animals with both doses of ginseng extract increased kidney GSH content by 8.29 % and 14.75 %, respectively, as compared with epinephrine group (Table 4).

3.6. Effects of pretreatment with ginseng extract on heart and kidney Nrf2 and heart NF-κB contents

Normal heart and kidney Nrf2 and heart NF-κB contents were

Table 1
Effects of pretreatment with ginseng extract on electrocardiogram (ECG) pattern.

	Normal control	Ginseng extract (200 mg/kg)	Ginseng extract (400 mg/kg)	Epinephrine control	Epinephrine + Ginseng extract (200 mg/kg)	Epinephrine + Ginseng extract (400 mg/kg)
Heart Rate (bpm)	322.10 ± 2.49	318.10 ± 3.72[b]	320.10 ± 2.45[b]	108.64 ± 12.90[a]	271.14 ± 8.36[a,b]	315.42 ± 2.59[b]
RR interval (s)	0.25 ± 0.00	0.26 ± 0.01[b]	0.26 ± 0.01[b]	0.45 ± 0.01[a]	0.44 ± 0.00[a]	0.28 ± 0.03[b]
QTc interval	0.12 ± 0.00	0.15 ± 0.01[b]	0.15 ± 0.01[b]	0.07 ± 0.01[a]	0.10 ± 0.01[a,b]	0.13 ± 0.00[b]

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons.
[a] Significantly different from normal control (Saline) at $P < 0.05$.
[b] Significantly different from epinephrine control at $P < 0.05$.

Table 2
Effects of pretreatment with ginseng extract on serum creatinine and urea levels.

	Normal control	Ginseng extract (200 mg/kg)	Ginseng extract (400 mg/kg)	Epinephrine control	Epinephrine + Ginseng extract (200 mg/kg)	Epinephrine + Ginseng extract (400 mg/kg)
Creatinine (mg/dl)	0.32 ± 0.01	0.34 ± 0.00[b]	0.33 ± 0.01[b]	0.81 ± 0.00[a]	0.58 ± 0.01[a,b]	0.43 ± 0.01[a,b]
Blood Urea Nitrogen (mg/dl)	26.80 ± 2.87	28.80 ± 2.33[b]	27.80 ± 2.42[b]	150.60 ± 1.66[a]	82.60 ± 1.29[a,b]	61.00 ± 1.87[a,b]

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons.
[a] Significantly different from normal control (Saline) at $P < 0.05$.
[b] Significantly different from epinephrine control at $P < 0.05$.

Table 3
Effects of pretreatment with ginseng extract on heart GSH, MDA and NO contents.

	Normal control	Ginseng extract (200 mg/kg)	Ginseng extract (400 mg/kg)	Epinephrine control	Epinephrine + Ginseng extract (200 mg/kg)	Epinephrine + Ginseng extract (400 mg/kg)
NO (nmol/mL)	20.60 ± 0.07	21.26 ± 0.38[b]	20.92 ± 0.30[b]	58.38 ± 1.57[a]	55.45 ± 1.25[a]	33.58 ± 0.35[a,b]
MDA (nmol/mL)	5.83 ± 0.60	5.71 ± 0.55[b]	5.60 ± 0.51[b]	26.62 ± 2.47[a]	8.97 ± 0.46[a,b]	6.62 ± 0. 14[b]
GSH (mg/dl)	10.40 ± 0.09	10.13 ± 0.21[b]	10.27 ± 0.11[b]	7.71 ± 0.05[a]	7.87 ± 0.06[a]	10.93 ± 0.11[b]

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons.
[a] Significantly different from normal control (Saline) at $P < 0.05$.
[b] Significantly different from epinephrine control at $P < 0.05$.

Table 4
Effects of pretreatment with ginseng extract on kidney GSH, MDA and NO contents.

	Normal control	Ginseng extract (200 mg/kg)	Ginseng extract (400 mg/kg)	Epinephrine control	Epinephrine + Ginseng extract (200 mg/kg)	Epinephrine + Ginseng extract (400 mg/kg)
NO (nmol/mL)	30.69 ± 0.09	31.49 ± 0.70[b]	30.78 ± 0.13[b]	46.60 ± 0.06[a]	45.89 ± 0.33[a]	39.38 ± 0.21[a,b]
MDA (nmol/mL)	4.43 ± 0.03	4.31 ± 0.12[b]	4.31 ± 0.14[b]	7.74 ± 0.06[a]	7.72 ± 0.06[a]	5.92 ± 0.05[a,b]
GSH (mg/dl)	8.31 ± 0.15	8.57 ± 0.23[b]	8.71 ± 0.50[b]	7.23 ± 0.02[a]	7.83 ± 0.22[a,b]	8.29 ± 0.05[b]

Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons.
[a] Significantly different from normal control (Saline) at $P < 0.05$.
[b] Significantly different from epinephrine control at $P < 0.05$.

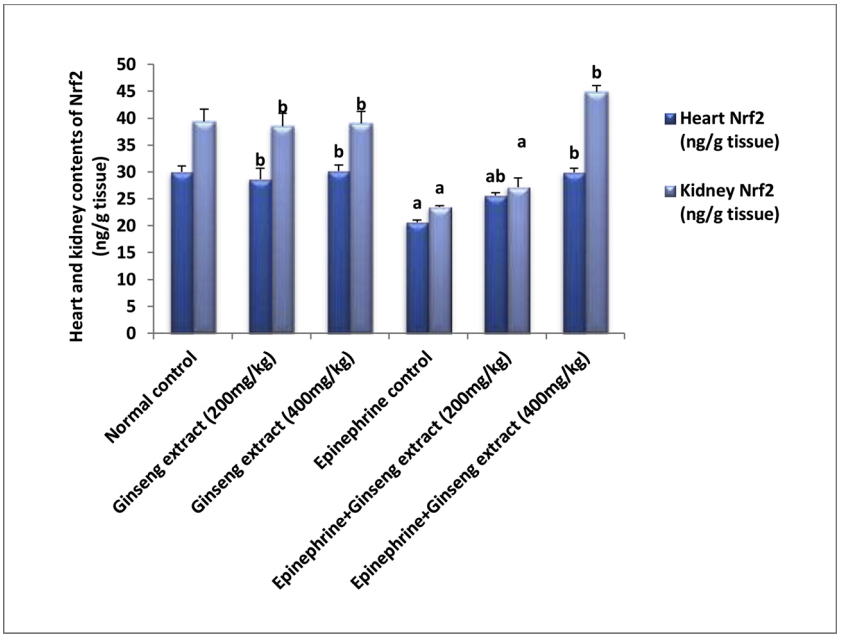

Fig. 1. Effects of pretreatment with ginseng extract on Nrf2 level.
Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons. [a] Significantly different from normal control (Saline) at $P < 0.05$. [b] Significantly different from epinephrine control at $P < 0.05$.

observed with both doses of ginseng extract administration. Heart and kidney Nrf2 contents were reduced in the epinephrine group by 31.11 % and 40.50 % respectively, on the other hand, higher heart Nrf2 contents was estimated in animals pre-treated with both doses of ginseng extract by 23.94 % and 44.92 % respectively and the higher kidney Nrf2 content was observed in animals pre-treated with the high dose of ginseng extract only by 91.49 % as compared to epinephrine group (Fig. 1). Epinephrine injection showed a significant elevation in heart NF-κB content by 4.92 % as compared to control rats. While, pretreatment with the both doses of ginseng extract suppressed the elevated heart NF-κB contents by 83.18 % and 83.49 %, respectively, as compared to epinephrine group (Fig. 2).

3.7. Effects of pretreatment with ginseng extract on heart and kidney PKC and heart AT1R contents

Normal heart and kidney PKC and heart AT1R contents were observed with both doses of ginseng extract administration. Epinephrine injection exhibited increase in contents of heart and kidney PKC by 167.29 % and 75.83 %, respectively, as compared to normal rats. On the other hand, pretreatment with the high dose of ginseng extract suppressed the elevated heart PKC contents by 32.74 %, while the kidney PKC content was decreased in pretreatment with both doses of the ginseng extract by 22.82 % and 50.14 %, respectively, as compared to epinephrine group (Fig. 3). Heart AT1R content was increased in the epinephrine group by 3.26 fold, as compared to normal control group, while lower heart AT1R content was observed in animals pretreated with both doses of ginseng extract by 73.94 % and 77.21 % respectively, as compared to epinephrine group (Fig. 4).

3.8. Effects of pretreatment with ginseng extract on histopathological study

Heart of normal control, ginseng extract (low dose) and ginseng extract (high dose) revealed normal cardiac muscle fibers with no evidence of necrosis or inflammatory reaction demonstrated in these groups (Fig. 5a–c, respectively). In contrast, characteristic and pathognomonic histopathological alterations were demonstrated in epinephrine control group in the form of acute myocardial infarction with presence of multifocal areas of coagulative necrosis of cardiomyocytes that appeared intensely eosinophilic with karypyknosis. The necrotized cardiomyocytes were intensely infiltrated with inflammatory cells mostly macrophages (Fig. 5d). Disintegration and dissolution of necrotic cardiomyocytes were also evident in some examined sections. Regressions of these histopathological lesions were recorded in epinephrine and ginseng extract (low dose) group in which the heart showed focal necrotic area infiltrated by macrophages (Fig. 5e). On the other hand, the most remarkable improvement was recorded in epinephrine and ginseng extract (high dose) group in which the heart appeared normal in most examined sections and necrosis was restricted to individual cardiomyocytes with mild leukocytic cell infiltration (Fig. 5f). No necrosis, pyknosis or macrophage activity were demonstrated in normal and control groups while scaled in the epinephrine group as 3, 2 & 3, respectively. On the other hand, necrosis, pyknosis and macrophage activity were reduced and scaled as 2, 1 & 2, respectively, in epinephrine and ginseng extract (low dose) group and 1, 0 & 0, respectively, in epinephrine and ginseng extract (high dose) group (Table 5).

Kidney tissues of the normal control and ginseng extract controls rats revealed normal renal glomeruli surrounded by urinary space and normal proximal, distal and convoluted tubules (Fig. 6A). In epinephrine-treated kidneys, there were degeneration changes in the glomerulus such as shrinkage and widening of urinary space. In addition, renal tubules revealed vacuolation, hydrophic degeneration of epithelium and pyknotic nuclei, interstitial inflammatory cells and intertubular hemorrhage were observed in this group (Fig. 6B). The rats of epinephrine and ginseng extract (low dose) treatment group displayed a improvement in architecture of kidney. Some glomeruli showed mild dilatation of Bowman's space with mildde generated tubules and pyknosis nuclei (Fig. 6C). Group of epinephrine and ginseng extract (high dose) showed almost normal architecture of the kidney with the exception of only few degenerated tubulesand pyknosis nuclei (Fig. 6D).

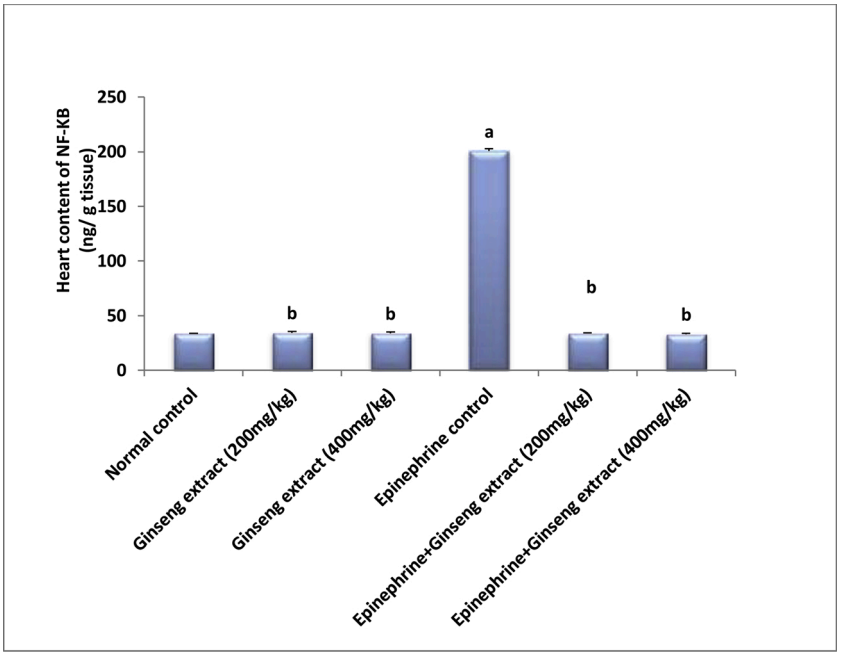

Fig. 2. Effects of pretreatment with ginseng extract on NF-κB level.
Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons. [a] Significantly different from normal control (Saline) at $P < 0.05$. [b] Significantly different from epinephrine control at $P < 0.05$.

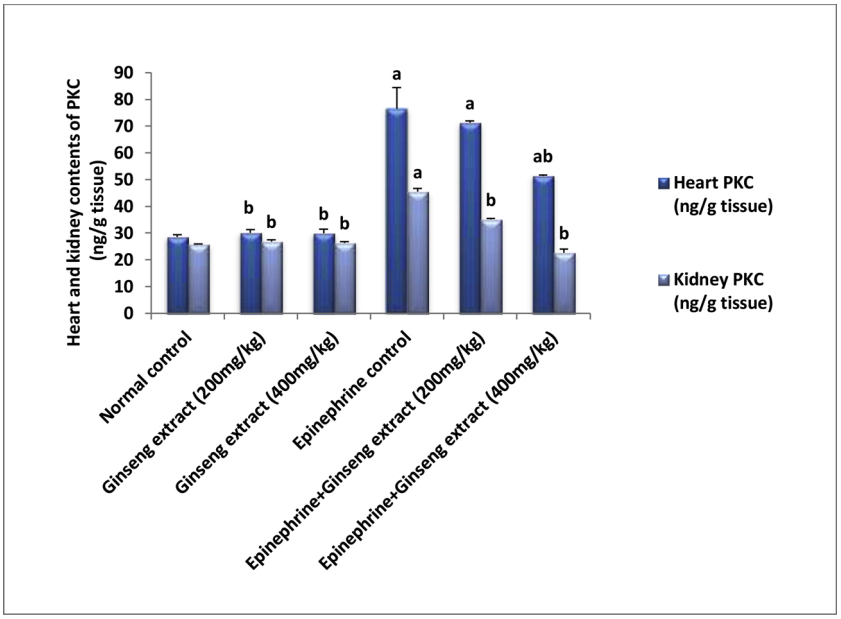

Fig. 3. Effects of pretreatment with ginseng extract on PKC level.
Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons. [a] Significantly different from normal control (Saline) at $P < 0.05$. [b] Significantly different from epinephrine control at $P < 0.05$.

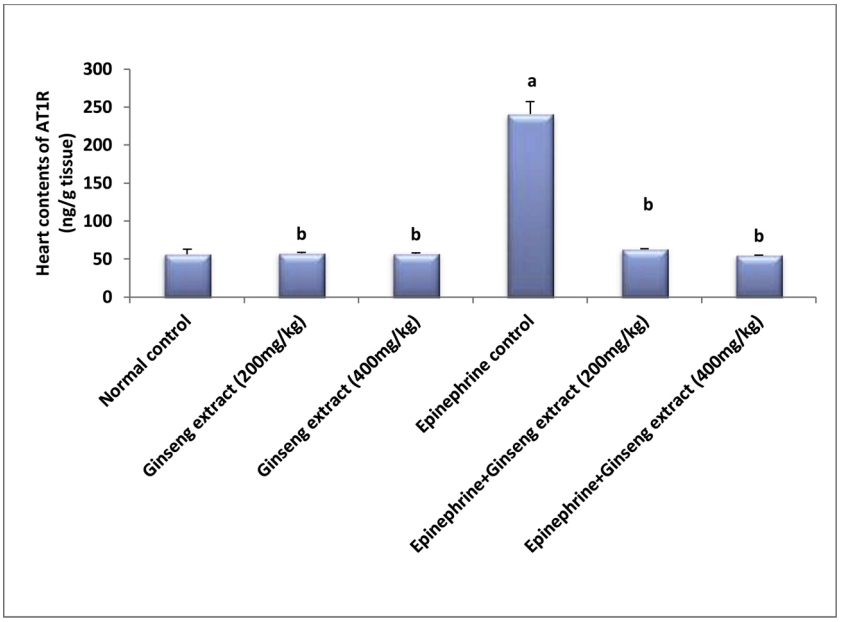

Fig. 4. Effects of pretreatment with ginseng extract on AT1R level.
Data were expressed as mean ± SE. Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons. [a] Significantly different from normal control (Saline) at $P < 0.05$. [b] Significantly different from epinephrine control at $P < 0.05$.

3.9. Immunohistochemical results

No cytoplasmic heart iNOS immunoreactivity was detected in the cardiac myocytes from normal control and ginseng extract controls rats (Fig. 7A–C). On the other hand, epinephrine injection revealed strong immunoreactivity of iNOS was localized in cardiomyocytes with some immunostaining noted within the inflammatory cells (Fig. 7D). Co-administration of epinephrine and ginseng extract (low dose) resulted in moderate expression of iNOS immunostaining in cardiomyocytes (Fig. 7E), However, treatment with epinephrine and ginseng extract (high dose) resulted in mild iNOS expression in cardiomyocytes (Fig. 7F). Semiquantitative comparison of the intensity of iNOS immunoreactivity in the heart tissue of control and the experimental groups was shown in Table 6.

Kidney iNOS expression in the normal control and ginseng extract controls rats no observed (Fig. 8A–C). In group treated with epinephrine revealed strong positive immune-reaction of iNOS in the form brown color was predominant in the glomerular and tubular region (Fig. 8D). The administration of epinephrine and ginseng extract (low dose) showing decreased expression of iNOS (Fig. 8E). In addition, epinephrine and ginseng extract (high dose) group received showed the recovery to near normal expression of iNOS but still not exact as control group

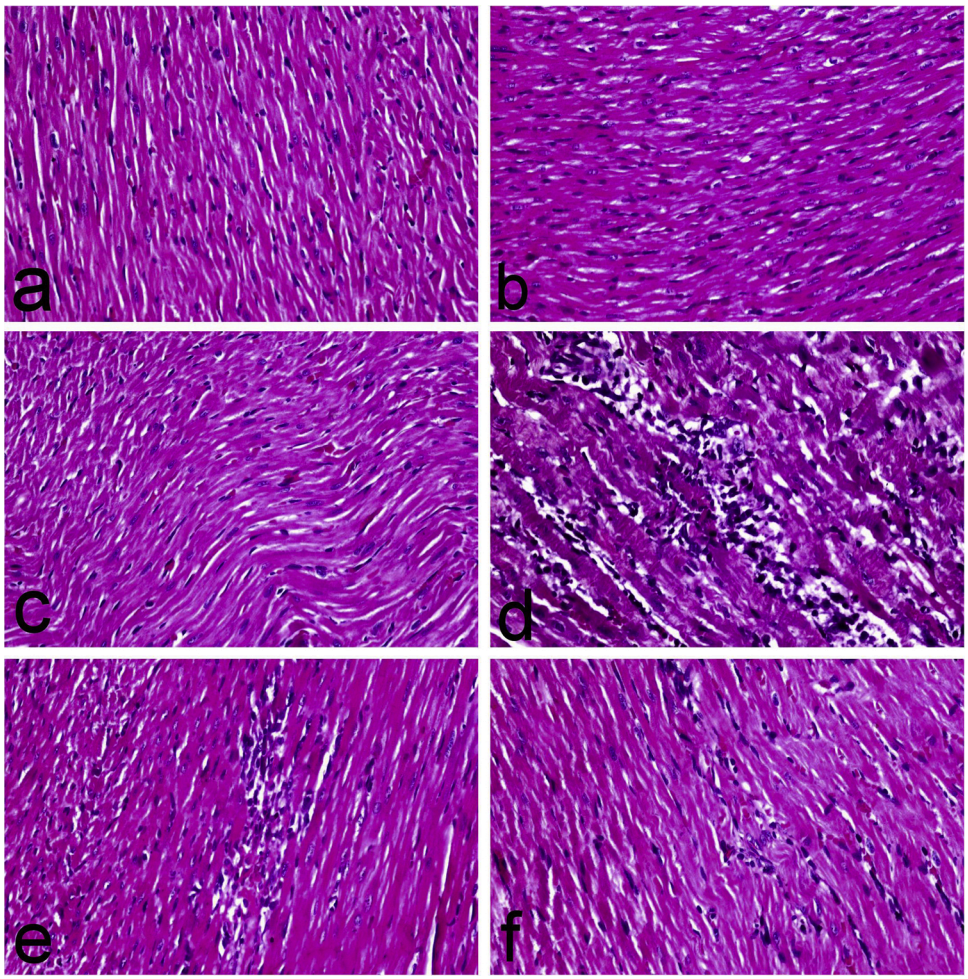

Fig. 5. Photomicrographs of heart.
(A) control rats showing normal cardiac muscle fibers,(B) Ginseng extract (200 mg/kg) treated rats showing normal cardiac muscle fibers, (C) Ginseng extract (400 mg/kg) treated rats showing normal cardiac muscle fibers, (D) Epinephrine control group showing coagulative necrosis of cardiomyocytes that are intensely infiltrated with macrophages, (E) Epinephrine + Ginseng extract (200 mg/kg) group showing focal necrotic area infiltrated by macrophages, and (F) Epinephrine + Ginseng extract (400 mg/kg) group showing little necrosis of individual cardiomyocyte with very mild leukocytic cell infiltration (H&E, 40X).

Table 5
Effect of ginseng extract on histopathological changes in the hearts of epinephrine-induced rats.

Groups	Normal control	Ginseng extract (200 mg/kg)	Ginseng extract (400 mg/kg)	Epinephrine control	Epinephrine + Ginseng extract (200 mg/kg)	Epinephrine + Ginseng extract (400 mg/kg)
Necrosis	0	0	0	3	2	1
Pyknosis	0	0	0	2	1	0
Macrophage Activity	0	0	0	3	2	0

Histopathological changes; 0 No change, 1 Mild, 2 Moderate, 3 Severe.

(Fig. 8F). Semiquantitative comparison of the intensity of iNOS immunoreactivity in the kidney tissue of control and the experimental groups was shown in Table 6.

4. Discussion

The current study explains the basic features of MI rat model induced by i.p. injection of epinephrine and its renal complication. In addition, this work explores the impact of ginseng extract on MI induced by epinephrine. Catecholamines (epinephrine, nor epinephrine and isoproterenol)- induced MI in rats are models for investigating the effects of various cardioprotective agents, and these models are mimics to those taking place in human MI [20]. In previous study, epinephrine administration elevated ST segment which represent cardiac ischemia [23] and caused ECG-changes as it caused RR interval elevation and HR and QTc interval reduction due to myocardial damage [24]. That result is in line with our results that showed significant changes in cardiovascular parameters following i.p injection of epinephrine comprised a reduction of HR and QTc interval with a prolongation of RR interval and arrhythmia duration.

One of the basic features of MI induced by i.p. injection of epinephrine is renal complication. Our results implied that the kidney's ability to function declined as evidenced by the elevation in the serum levels of creatinine and BUN, as well as the decreased GSH and elevated MDA and NO kidney contents in epinephrine-treated rats. We hypothesized, also, degeneration and atrophy of glomerulus, vacuolation of epithelium renal tubules and interstitial inflammatory cells in epinephrine group contributed to renal dysfunction. These data suggest renal dysfunction following cardiac impairment. Cronin et al. have suggested poor renal function via tubular degeneration and obstruction in norepinephrine-induced acute renal failure [25]. Conger et al. showed azotemia (increase in BUN) between the second and fourth days after norepinephrine infusion into the renal artery that induced acute renal failure. The pathogenetic mechanism of acute renal failure induced by

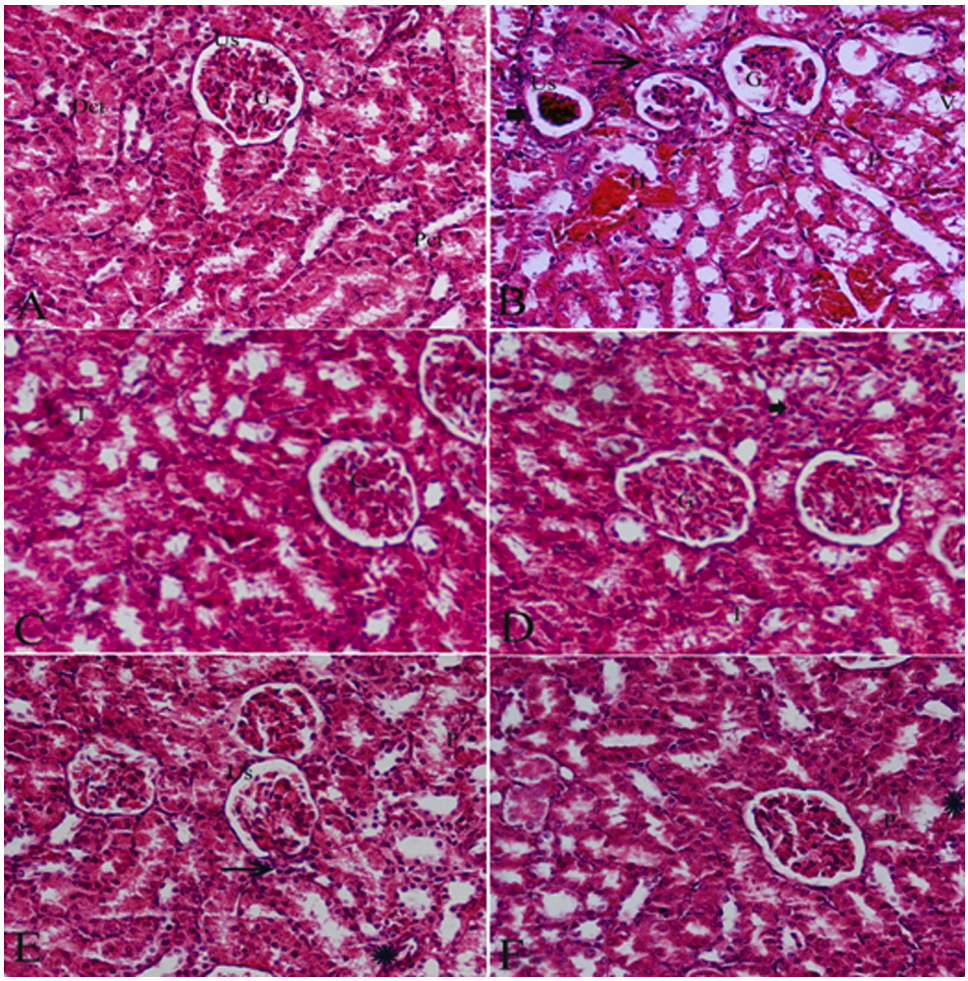

Fig. 6. Photomicrographs of renal kidney. A. control group rats showing normal glomeruli (G) with an intact urinary space (Us), proximal convoluted (Pct) and distal convoluted (Dct) tubules. B. Photomicrographs of renal kidney in epinephrine group showing degeneration of glomerulus (G), atrophy of glomerulus (arrowhead), widening of urinary space (Us), vacuolation of epithelium renal tubules (V), pyknotic nuclei (P), interstitial inflammatory cells (arrow) and intertubular hemorrhage (H). C. Photomicrographs of renal kidney in ginseng extract (low dose) group showing improvement in architecture, some glomeruli showed mild dilatation of Bowman's space with milddegenerated tubulesand pyknosis nuclei. D. Photomicrographs of renal cortex in ginseng extract (high dose) group showing almost normal architecture with the exception of only few degenerated tubulesand pyknosis nuclei **(**H&E, 40X**).**

norepinephrine has been attributed to ischemic insult that plays a role in the maintenance of renal dys-function [26]. Clinically, Van dokkum et al. showed that patients with cardiovascular events are vulnerable to more renal function loss [27].

The second feature of MI was impaired oxidant-antioxidant balance. In the previous study, epinephrine induced myocardial infarction in rats through oxidative stress [28]. Our results revealed that MI provoked by i.p. injection of epinephrine is diagnosed by a marked reduction of antioxidant level of heart GSH and elevation of heart MDA contents as compared to normal control. This elevation of heart MDA content indicated high peroxide generation levels inducing cardiac necrosis as shown in our histopathological study. Lipid peroxidation is a pathogenic key in MI and its complications and reflects the major stages of tissue damages [29]. NO was found in progression of the MI induced by isoprenaline (one of catecholamine). The increased activity of iNOS, synthesized cytotoxic NO, showed for periods of time, hours or days in MI. NO action can be linked to reactive species provoking DNA fragmentation or dysfunctional mitochondria [30]. Epinephrine injection, in current study, exhibited a significant increase in heart NO content compared to normal control group suggesting its role in MI induced by epinephrine. This result was paralleled with our histopathological study that illustrated distortion of the structure, cardiac myocytes necrosis, and atrophy of cardiac muscle associated with overexpression of iNOS. The critical antioxidant regulator of cardiovascular homeostasis is Nrf2 and its target genes via the suppression of oxidative stress, which is involved in cardiac injury in rat [31] preserving cardiac function and reducing infarct size [32]. In current study, downstream of Nrf2 was observed in the progression of epinephrine model of MI destroying cardiac structure and inducing necrotic changes as shown in our histopathology.

The third feature of MI, in this study, was inflammation involving PKC, isozyme controlling remodeling of the myocardium [33]. Our model of MI was characterized by a significant increase in PKC expression, in rat heart and kidneys; these results emphasized by histopathological study that showed inflammatory cells that involved in the pathogenesis of MI and its renal complication. PKC is activated by catecholamines [34]. *In vitro* study of Joyeux et al. explored that selective PKC inhibitor abolished MI [35]. In *vivo*, Wang et al. explained that MI-induced by occlusion of the left coronary artery elevated PKC expression [33]. In addition, there is a crosstalk between sympathetic nerve system and renin-angiotensin system as Ang II can provoke the sympathoexcitation and associated with epinephrine release through AT1R [36]. In this study, after MI, the increased level of AT1R in epinephrine-treated group is associated with increased level of PKC and NF-κB inducing the intrinsic apoptotic pathway in heart tissue as shown in histopathological section. Vivar et al. explained the expression of AT1R after MI in cultured cardiac fibroblasts with an early loss of mitochondrial membrane potential [37] and NF-κB has been involved in the upregulation of the AT1R [38] triggering iNOS expression, provoking apoptosis [39,40]. Moreover, mitochondrial ROS generation, after external stimuli, activates PKC expression, leading to the progression of apoptosis [41]. Ang II increased generation of mitochondrial ROS, via activating NAD(P)H oxidase, through AT1 receptors which in turn stimulates protein kinases activation that is highly related to cardiovascular diseases risk [42].

In previous study, ginseng ameliorated myocardial ischemia-

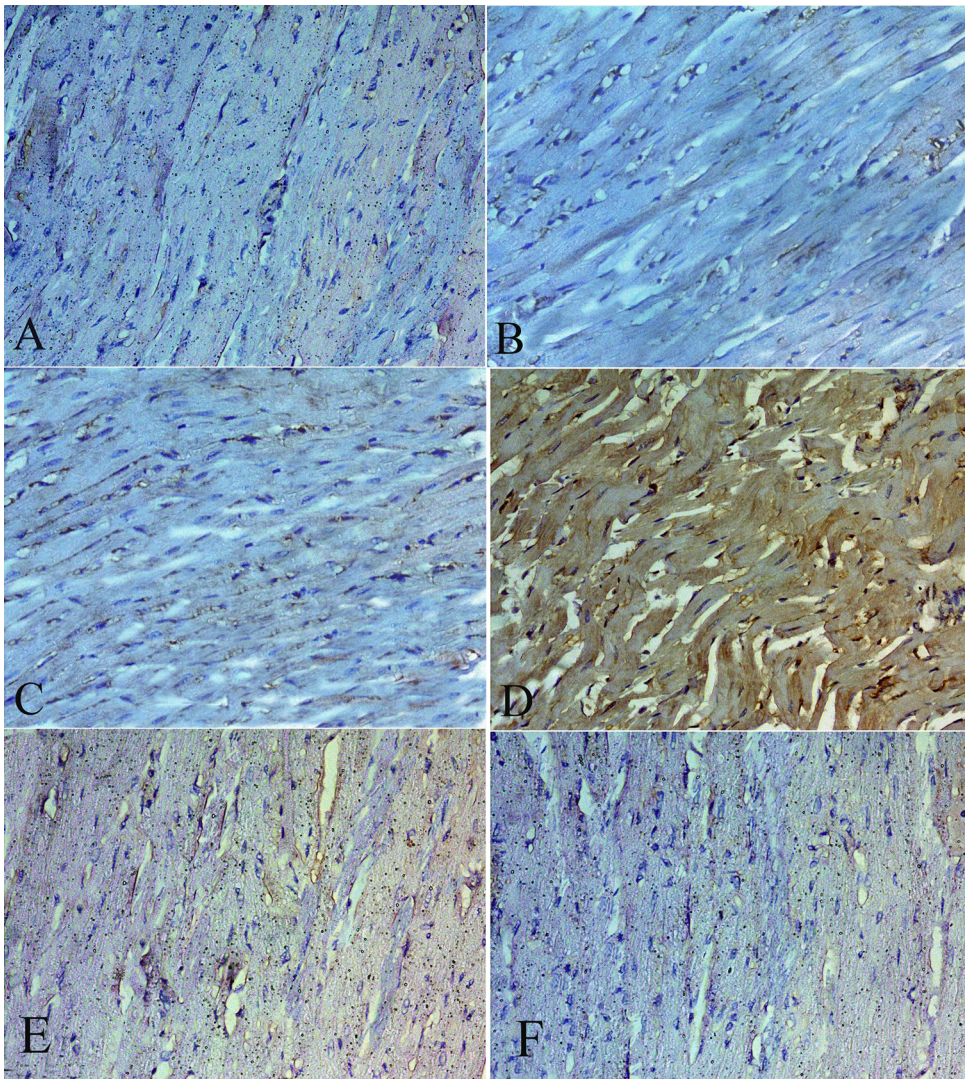

Fig. 7. iNOS -immunohistochemical staining of heart section of rats.
Negative iNOS expression was observed in the cardiac myocytes from control group (A). Ginseng extract low dose group showed negative immunoreactivity for iNOS immunoreactions (B). Ginseng extract high dose group showed negative immunoreactivity for iNOS immunoreactions (C). Strong positive immunereaction of iNOS was localized in cardiomyocytes with some immunostaining noted within the inflammatory cells in epinephrine group (D). Co-administration of epinephrine and ginseng extract (low dose) resulted in moderate expression of iNOS immunostaining in cardiomyocytes (E), However, treatment with epinephrine and ginseng extract (high dose) resulted in mild iNOS expression in cardiomyocytes (F).

Table 6
Semiquantitative comparison of the intensity of iNOS immunoreactivity in the heart and kidney tissue of control and the experimental groups.

Groups	Heart	Kidney
Control	–	–
Ginseng extract (200 mg/kg)	–	–
Ginseng extract (400 mg/kg)	–	–
Epinephrine	+++	+++
Epinephrine and ginseng extract (200 mg/kg)	++	++
Epinephrine and ginseng extract (400 mg/kg)	+	+

The intensity of the staining was recorded as weak (−), mild (+), moderate (++), and strong (+++).

reperfusion injury of isolated rat heart models due to the presence of saponins [43]. In this study, we have explored the antiarrhythmic and cardioprotective effect of ginseng extract and its potential renoprotective role on epinephrine induced MI in rats and revealed the possible mechanisms of protection. The results of this study indicated cardiac protection by prophylactic treatment of ginseng extract in two different doses (200 & 400 mg/kg) for two weeks before administration of epinephrine, as evidenced by a decreasing in HR and the QTc intervals, delaying the onset of arrhythmia and improving RR interval as compared to epinephrine group. These results supported by our histopathological study that exhibited that ginseng extract attenuated myocyte hypertrophy and myocardial inflammation induced by epinephrine.

Ginseng contains high concentrations of saponins such as ginsenosides which inhibit oxidative stress and inflammation alleviated the renal damage progression in type 1 diabetes [44]. The protective potential of ginseng extract was estimated on the renal damage linked with MI involving oxidative stress, inflammation and apoptosis, the pretreatment with both doses of ginseng extract exerted a renoprotective effect through decreasing serum creatinine and BUN levels as well as attenuation of glomerulus degeneration, vacuolation of epithelium renal tubules and interstitial inflammatory cells. Jung et al. explored that ginseng extract has a renoprotective effect in cisplatin nephrotoxicity that accompanied by the caspase-dependent anti-inflammatory pathway. Antioxidants are believed to have a potential health benefit against MI [45]. Ginseng, a dietary antioxidant, implicated in oxidative stress associated chronic diseases such as cardiovascular disease [46], and rescued renal cells from oxidative damage [47]. In this study, the pretreatment with both doses of Ginseng extract augment the overall cells capacity to detoxify harmful substances via enhancing GSH and counteracting MDA contents. Moreover, our results exhibited down regulation of heart and kidney inducible NO contents in ginseng extract treated groups through inhibition of iNOS expression as reported in our histopathological investigation as compared to normal values, while in another study, the authors have shown cardioprotective effects of ginseng extract through upregulation of eNOS activity in I/R injury

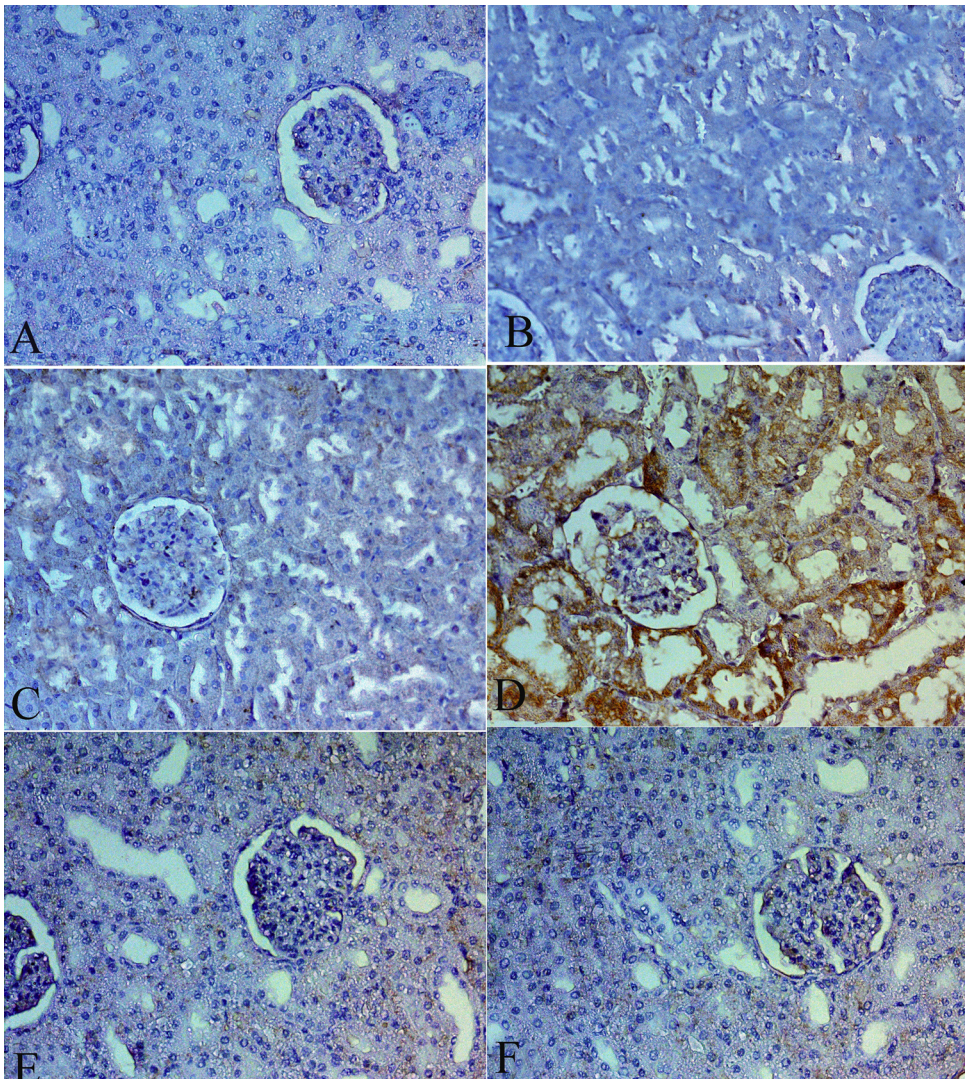

Fig. 8. iNOS -immunohistochemical staining of kidney section of rats.
Negative iNOS expression was observed in the control group (A). Ginseng extract low dose group showed negative immunoreactivity for iNOS immunoreactions (B). Ginseng extract high dose group showed negative immunoreactivity for iNOS immunoreactions (C). Strong positive immune-reaction of iNOS in the form brown color was predominant in the glomerular and tubular region of epinephrine group (D). Co-administration of low dose of ginseng extract with epinephrine showed decreased expression of iNOS (E). Ginseng extract high dose with epinephrine administration showed the recovery to near normal expression of iNOS but still not exact as control group (F).

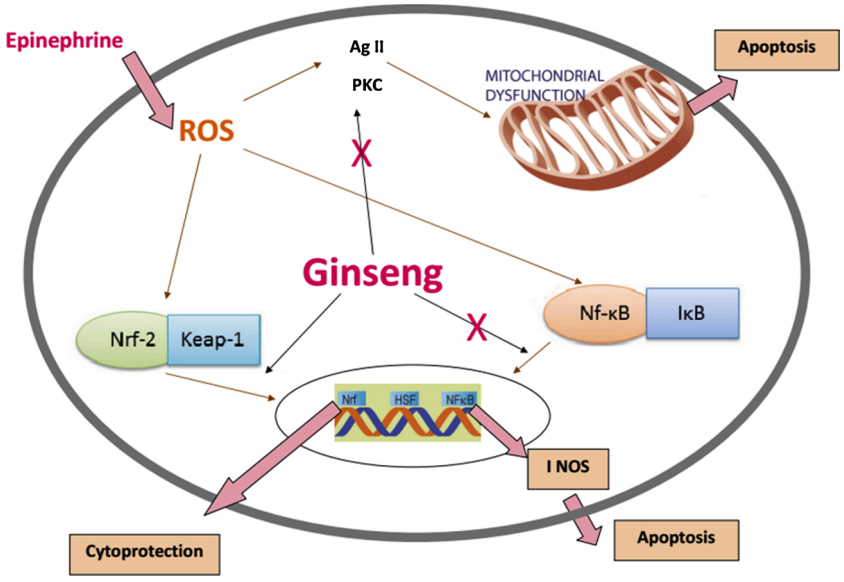

Fig. 9. Cardio and renoprotective role of ginseng extract against Epinephrine-Induced Myocardial Infarction via Nrf2 expression and NF-κB/PKC/AT1R down regulation. Nrf2: Nuclear factor-erythroid-related factor 2; NF-κB: nuclear factor-kabba B; PKC: Protein kinase C and AT1R: angiotensin 1receptor.

hearts [48]. Furthermore the influence of ginseng extract as Nrf2 expression inducers against kidney and cardiac damage has never been examined before, in this study, we have presented evidence that ginseng extract induced Nrf2 expression in heart and kidney that exhibited cell defense processes emphasizing its cardio renoprotective role (Fig. 9), Nrf2 is a critical regulator of cardiovascular homeostasis as it acts as oxidative stress suppressor. *In vitro* study, the Nrf2 signaling pathway revealed an important role in protecting cardiac cell injury [49] and preventing the heart from remodeling and cardiac dysfunction [50].

Ginseng extract in the two dose levels, in the current work, suppressed PKC activity and AT1 receptors in heart and kidney suggesting that ginseng extract blocked membrane-bound NAD(P)H oxidase ameliorating mitochondrial dysfunction and apoptosis (Fig. 9); so, we reported for the first time that PKC was involved in the antiarrhythmic effect of ginseng. In a different study, Ederhy et al. have put forward the beneficial effects of several molecular-targeted therapy drugs as PKC inhibitors in MI through regulating QTc interval [51]. In addition, Palaniyandi et al. show that PKC provoked heart failure associated with pro-inflammatory responses and fibrosis [52]. Ginseng extract also reduced NF-κB leading to down-regulating of AT1R and iNOS expressions reducing apoptotic pathway in heart and kidney tissues that produced by epinephrine (Fig. 9). In previous studies, ginseng extract abrogated the expression of cleaved caspase-3 which in turn reduced the percentage of apoptotic pig kidney (LLC-PK1) cells in cisplatin-induced nephrotoxicity [53] and modulated machrophage reducing myocardial infarct size and apoptotic cell death in myocardial ischemia/reperfusion injury [54].

5. Conclusion

Our results preclude the possibility that Nrf2/NF-κB imbalance and PKC/AT1R activation induced by epinephrine may be important events in the pathogenesis of MI and its renal complication. Therefore, therapeutic interventions monitoring ECG changes and having antioxidant activity are not the sole factors optimizing MI, also, Nrf2/NF-κB balance and PKC/AT1R down regulating may exert beneficial effects against it. In this respect, this experimental study have shown cardio and renoprotective role of ginseng extract that we exclude the possibility that ginseng extract abolished ECG changes, stimulated Nrf2 and inhibited NF-κB/PKC/AT1R, as well as corrected renal disorder in epinephrine model of MI. Therefore, ginseng extract may benefit human patients with MI and renal disorder as it considered as Nrf2 enhancer and NF-κB/PKC/AT1R inhibitor. The importance of ginseng extract in clinical entity will need further investigation in future.

CRediT authorship contribution statement

Abeer A.A. Salama: Conceptualization, Project administration, Funding acquisition, Methodology, Validation, Formal analysis, Writing – editing and reviewing. **Dina Mansour:** Methodology. **Rehab Hegazy:** Methodology, editing and reviewing.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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