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A Cardiomyopeptin Preparation Protects against Isoproterenol-Induced Chronic Heart Failure in Rats

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Abstract

The present study investigated the effect of an herbal extract-supplemented cardiomyopeptin preparation (HECP), in the rat model of chronic heart failure. HECP pre-/co-treatment at a daily dose of 0.072 to 0.124 g/kg for 30 days protected against isoproterenol (ISO)-induced chronic myocardial damage in rats in a dose-dependent manner, with the extent of protection, as assessed by plasma cardiac troponin I level, being up to 76%. The cardioprotection afforded by HECP was associated with the enhancement of myocardial mitochondrial antioxidant status, amelioration of plasma parameters on cardiac dysfunction and hypertrophy, as well as an increase in myocardial endothelial nitric oxide synthase activity. Myocardial apoptotic and anti-apoptotic parameters were suppressed and stimulated, respectively. The cardioprotection afforded by HECP was accompanied by an increase in exercise capacity, an indirect functional index of the myocardium, in ISO-challenged rats. In conclusion, HECP may offer a promising prospect in preventing chronic heart failure in human subjects.

Keywords

Cardiomyopeptin, Herbal Extract, Chronic Heart Failure, Isoproterenol

1. Introduction

Despite significant improvement in medical and surgical management, coronary heart disease remains one of the major causes of morbidity and mortality in industrialized countries, with a large portion of patients suffering from myocardial

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infarction and hence heart failure caused by ischemic heart disease [1]. In the case of chronic myocardial ischemia or psychological stress, the increase in sympathetic activity can lead to myocardial damage caused by the elevated level of epinephrine/norepinephrine, which undergoes autooxidation and produces reactive oxygen species in the myocardium [2]. The exploration for therapeutic agents aimed at reducing the extent of myocardial infarction and hence myocardial dysfunction has become an area of intensive research. In this regard, the prophylactic use of pharmacological agents may provide an effective means of reducing the extent of myocardial damage caused by chronic ischemic heart disease. Cardiomyopeptin, a small polypeptide, is extracted from the myocardium of young pigs/cows [3]. Recent studies showed that cardiomyopeptin protected against anoxia-ischemia injury in cultured cardiomyocytes [4] [5] and ischemia-reperfusion injury in rats [6]. Chinese medicine has been practiced for more than two thousand years, with a complete system set of theories and practices, as well as abundant sources of herbal drugs [7]. Experimental and clinical investigations have demonstrated that Chinese herbal drugs can effectively relieve myocardial ischemia and/or reperfusion by intervening on various signal pathways, thereby protecting against myocardial injury [8]. In the present study, an herbal extract-supplemented cardiomyopeptin preparation (HECP), consisting of cardiomyopeptin and a mixture of water extracts of Ginseng Radix, Polygonati Rhizoma and Bamboo leaf, was formulated to explore possible cardioprotection after oral administration. Long-term administration of isoproterenol (ISO), a potent β-adrenergic agonist, can cause myocardial damage like those observed in clinical myocardial infarction [9]. We, therefore, endeavored to investigate the cardioprotective effect of HECP using a rat model of ISO-induced chronic heart failure.

2. Materials and Methods

2.1. Reagents

Isoproterenol (ISO), glutathione (GSH), and oxidized glutathione (GSSG) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Formulation of Herbal Extracts-Supplemented Cardiomyopepetin Preparation (HECP)

Cardiomyopeptin was isolated from cattle heart tissue after homogenization, enzyme digestion, filtration, and concentration followed by lyophilization. The crude extract contained around 50% (w/w) cardiomyopeptin. Ginseng Radix, Polygonati Rhizoma and Phyllostachys Floret (bamboo leaf) were processed by water extraction; the dried extract contained 12% total saponins, 6.1% crude polysaccharides, and 12% crude polysaccharides/29% total flavonoids, respectively HECP was formulated as follows: every 100 g of HECP contained 45 g cardiomyopeptin; 10 g Ginseng total saponins; 10 g Polygonati crude polysaccharides;

2 g Phyllostachys crude polysaccharides/total flavonoids and 37 g maltitol.

2.3. Animal Care

Adult Sprague-Dawley (SD) male rats (8 to 10-week-old; 250 to 300 g) were maintained under a 12 h dark/light cycle at an ambient temperature of about 22˚C and allowed water and food *ad libitum* in the Laboratory Animal Facilities at the Hong Kong University of Science and Technology (HKUST). All experimental protocols were approved by the Committee of Research Practice, HKUST.

2.4. A Rat Model of ISO-Induced Chronic Heart Failure

Rats were randomly divided into groups and orally administered by gavage with daily doses (0.072 - 0.124 g/kg) of HECP or vehicle for 30 days. The medium dose of HECP was the human equivalent dose. To induce chronic heart failure, rats were subcutaneously injected with ISO at 0.1 mg/kg or an equal volume of the vehicle from day 16 to day 30 once at an interval of 24 hours for fifteen consecutive days.

2.5. Biochemical Assessments of ISO-Induced Chronic Heart Damage

Plasma cardiac troponin I (cTnI) level, a low molecular weight contractile protein, was measured by using Cloud-Clone (Katy, USA) ELISA kit. Plasma activities of creatine kinase-MB (CK-MB) and CK were measured with assay kits (EKF Diagnostics, Barleben, Germany). Myocardial mitochondrial antioxidant status was assessed by measuring glutathione redox status (GSH/GSSG) and the activity of manganese superoxide dismutase (Mn-SOD) [10]. The extent of mitochondrial lipid peroxidation was assessed by measuring the malondialdehyde (MDA) level in the mitochondrial fraction of myocardial tissue [11] [12].

Plasma levels of brain natriuretic peptide (BNP), a marker for estimating ventricular dysfunction, atrial natriuretic peptide (ANP), a cardiac hormone that is synthesized primarily in the cardiac atria in response to cardiac ischemia, were measured using ELISA assay kits [2] [9] [13]. The plasma level of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), was measured using Cusabio ELISA kit (Houston, USA). Myocardial levels of protein arginine methyltransferase 1 (PRMT1) and dimethylarginine dimethylaminohydrolase 2 (DDAH2), both of which catalyze the formation of ADMA. were measured by using ELISA kits from MyBioSource (San Diego, USA) and FineTest (Wuhan, China), respectively [2] [11] [12]. Myocardial endothelial nitric oxide synthase (eNOS) activity was measured using Cusabio ELISA assay kit (Houston, USA) [2] [9] [13].

The caspase-3 activity of myocardial cytosolic fraction was measured by an assay kit (Abcam, Cambridge, United Kingdom) [14]. Protein kinase C-epsilon (PKCε) translocation was assessed by measuring both cytosolic and mitochondrial PKCε levels using an ELISA kit (MyBioSource, San Diego, USA).

2.6. Assessment of Exercise Capacity

The non-ISO-challenged and ISO-challenged rats without or with HECP pre-/co-treatment were assessed for exercise capacity (EC), which is an indirect measure of the capacity of heart function. To evaluate the EC, rats were made running on an open treadmill (Model: EXER-4, Columbus Instruments, Columbus, USA). The treadmill is equipped with an electrified grid at the rear of the belt to motivate continuous running. Both adaptation training and assessment of EC were performed in the period from 14:00 to 18:00. Different groups of animals were assigned to each round of assessment. During the first 3 days of adaptation training, rats were put on a treadmill at 10 m/min, 5˚ inclination for 8 min/day, with the electrical stimulus at an intensity setting of 19.5 and a repetitive rate of 9.7. The adaptation training was followed by a 4-day continued training of treadmill running at 10 m/min, 5˚ inclination for 5 min/day. For the assessment of workload, rats started running at 10 m/min, 5˚ inclination for 5 min and repeated once. Thereafter, the speed was increased at a rate of 1 m/min every minute until fatigue (*i.e.*, when the rats were unable to maintain the running cadence for over 10 s or avoid the electric stimulus for a period longer than 15 s or refuse to run even after sound/force stimulation). The time of the run and the maximum speed that the animal had achieved until fatigue were recorded. Workload (W), which was used as a marker for EC, was calculated from the following equation: W = animal weight (kg) × time until fatigue (min) × maximum running speed (m/min) × sine ɵ (treadmill inclination) [15].

2.7. Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS statistical software. p-Values < 0.05 were regarded as statistically significant.

3. Results

3.1. Effects of Pre-/Co-Treatment with HECP on ISO-Induced Chronic Myocardial Damage in Rats

A long-term, low dose of ISO challenge caused chronic myocardial damage in rats, as evidenced by increases in plasma cTnI level (↑65%) and plasma activities of CK-MB (↑50%) and CK (↑48%) (**Figure 1**). Plasma cTnI level appeared to be a more sensitive as well as specific marker of myocardial damage. Pre-/co-treatment with HECP protected against ISO-induced chronic myocardial damage in a dose-dependent manner (27% - 76%), with the extent of protection being 61% at the human equivalent dose, as assessed by plasma cTnI level.

3.2. Myocardial Mitochondrial Antioxidant Status

The ISO-induced chronic myocardial damage was associated with an increased myocardial mitochondrial MDA level (31%) (**Figure 2**). While the mitochondrial glutathione redox status was enhanced in ISO-challenged rats, the Mn-SOD activity was decreased by 31%. Pre-/co-treatment with HECP at the human

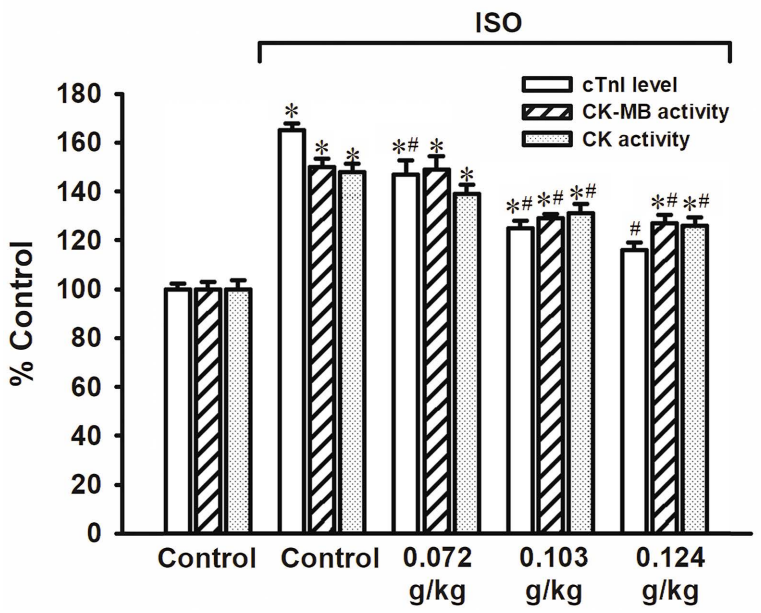

Figure 1. Effects of pre-/co-treatment with HECP on ISO-induced chronic myocardial damage in rats. HECP was orally administered as described in Materials and methods. ISO challenge at a daily dose of 0.1 mg/kg (sc) was commenced on Day 16 following the sample pretreatment and was continued until Day 30. The number of animals in each group ranged from 6 to 8. Data were expressed in percent non-ISO control as mean ± S.E.M. Control values of plasma parameters are shown as follows: cTnI level (pg/mL), 4.0 ± 0.11; CK-MB activity, 26 ± 0.85 (mU/mL); CK activity. *Significantly different from non-ISO control. #Significantly different from the ISO control.

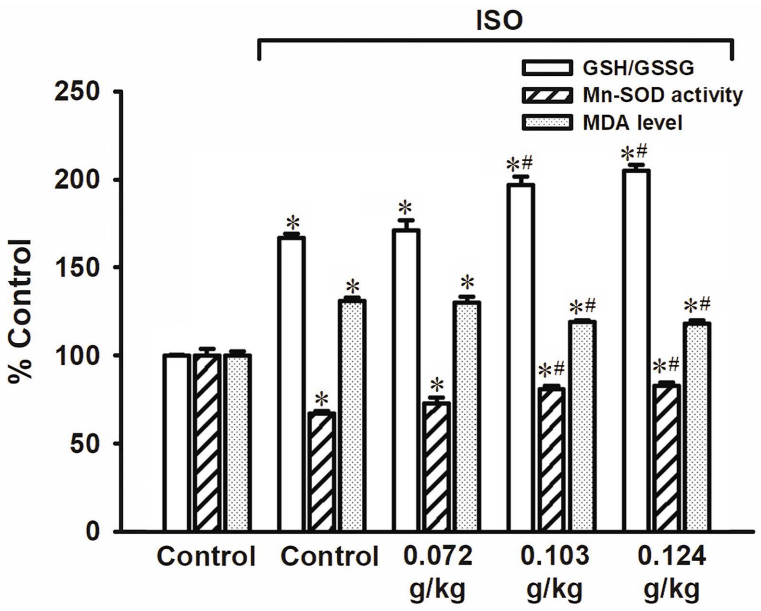

Figure 2. Effects of pre-/co-treatment with HECP on myocardial mitochondrial antioxidant/oxidative status in ISO-challenged rats. Data were expressed in percent non-ISO control as mean ± S.E.M. Control values of parameters are shown as follows: myocardial mitochondrial GSH/ GSSG ratio, 0.68 ± 0.01; myocardial Mn-SOD (U/ mg protein), 36 ± 2.04; myocardial mitochondrial MDA (μg/mg protein), 0.0082 ± 0.0003. *Significantly different from the non-ISO control. #Significantly different from the ISO control.

equivalent dose suppressed the ISO-induced increase in mitochondrial MDA levels (39%). The mitochondrial glutathione redox status was further enhanced by 45%, but the decrease in Mn-SOD activity was inhibited (by 42%).

3.3. Plasma Markers of Myocardial Hypertrophy

Plasma BNP and ANP levels were increased by 51 and 75%, respectively, in ISO-challenged rats (Figure 3). HECP pre-/co-treatment at the human equivalent dose inhibited the ISO-induced increases in plasma BNP (84%) and ANP (75%) levels.

3.4. Plasma and Myocardial Markers of Myocardial eNOS Status

ISO challenge caused increases in myocardial PRMT1 (56%) and plasma ADMA (55%) levels in rats, but the myocardial DDAH2 level was decreased by 21% (Figure 4). Myocardial eNOS activity was also suppressed (28%) in ISO-challenged rats. HECP pre-/co-treatment at the human equivalent dose decreased the ISO-induced elevation in the PRMT1 (by 61%) and ADMA (by 69%) levels. The DDAH2 level and eNOS activity were restored by 81 and 85%, respectively.

3.5. Myocardial Parameters of Apoptosis

Myocardial caspase 3 activity was increased by 26% in ISO-challenged rats (Figure 5). While the cytosolic PKCε level decreased (16%) in rat hearts, the mitochondrial level was increased by 32%. HECP pre-/co-treatment at the human

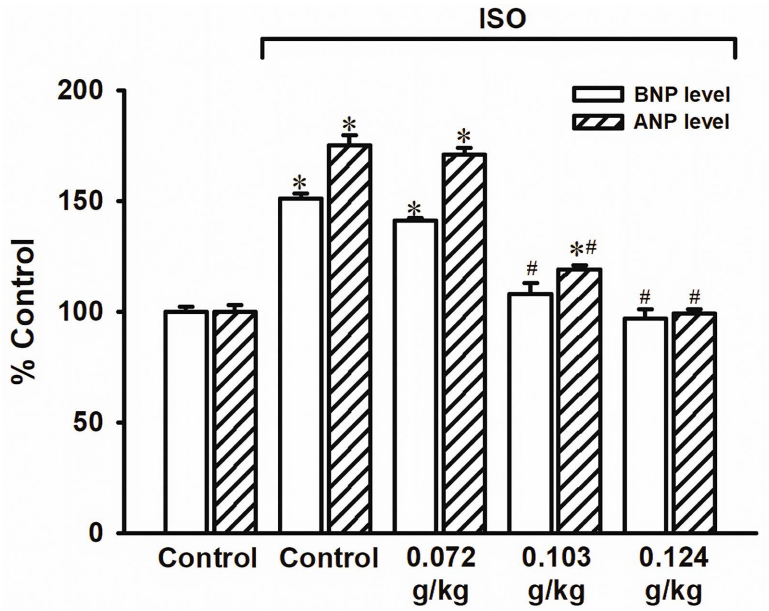

Figure 3. Effects of pre-/co-treatment with HECP on biomarker levels of myocardial dysfunction/hypertrophy in ISO-challenged rats. Data were expressed in percent non-ISO control as mean ± S.E.M. Control values of parameters are shown as follows: plasma brain natriuretic peptide (BNP) level, 95 ± 3.27 pg/mL; plasma atrial natriuretic peptide ANP level, 19 ± 0.73 pg/mL. *Significantly different from the non-ISO control. #Significantly different from the ISO control.

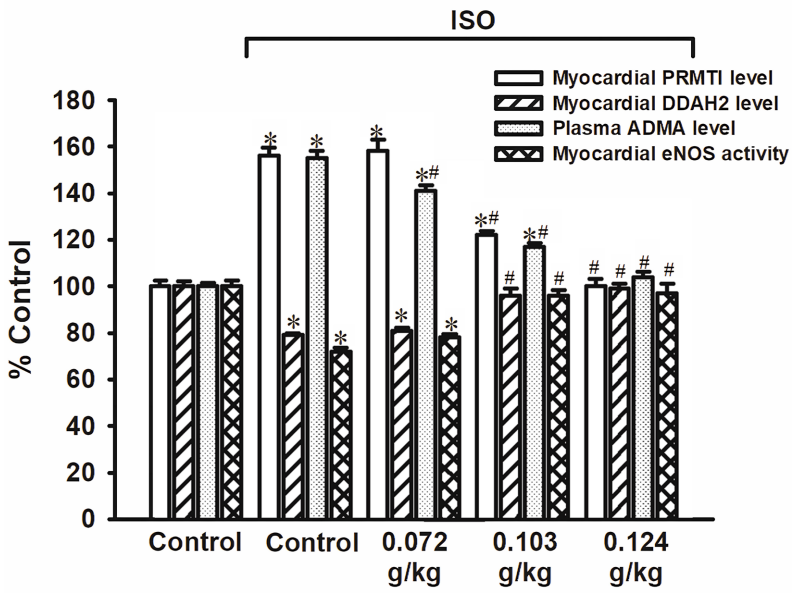

Figure 4. Effects of pre-/co-treatment with HECP on changes in eNOS-related myocardial/plasma signaling protein levels/activity in ISO-challenged rats. Data were expressed in percent non-ISO control as mean ± S.E.M. Control values of parameters are shown as follows: Myocardial PRMT1 level, 287 ± 7.39 pg/mg protein; myocardial DDAH2 level, 3.0 ± 0.34 ng/mg protein; plasma ADMA level, 91 ± 1.89 ng/mL; myocardial eNOS activity 60 ± 2.79 μIU/mg protein. *Significantly different from the non-ISO control. #Significantly different from the ISO control.

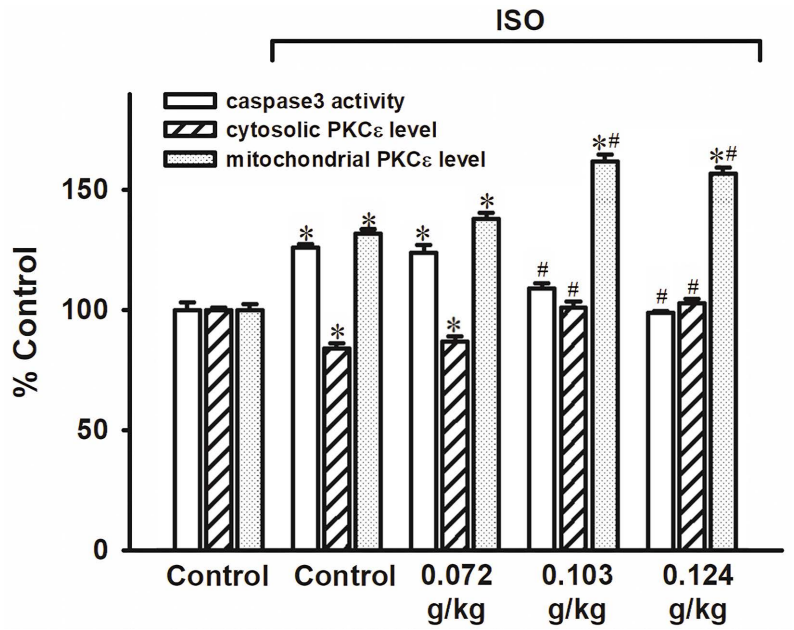

Figure 5. Effects of pre-/co-treatment with HECP on changes in apoptosis-related levels/activity of myocardial cytosolic/mitochondrial signaling proteins in ISO-challenged rats. Data were expressed in percent non-ISO control as mean ± S.E.M. Control values of parameters ware shown as follows: myocardial cytosolic caspase3 activity, 331 ± 4.34 signal/mg protein; myocardial cytosolic PKCε level, 3.20 ± 0.21 ng/mg protein; myocardial mitochondrial PKCε level, 3.95 ± 0.16 ng/mg protein. *Significantly different from the non-ISO control. #Significantly different from the ISO control.

equivalent dose suppressed the ISO-induced increase in caspase 3 activity by 65%. While the cytosolic PKCε level was returned to the normal level, the mitochondrial level was further increased by 94% in HECP-pre-/co-treated and ISO-challenged rats.

3.6. Exercise Capacity

ISO challenge caused a 51% decrease in exercise capacity in rats (**Figure 6**). HECP pre-/co-treatment restored the exercise capacity well beyond the normal level in ISO-challenged rats.

4. Discussion

In the present study, long-term ISO treatment was found to cause myocardial injury in rats, as evidenced by increases in plasma cTnI level and CK-MB activity. As the pathological changes of myocardial injury caused by acute or multiple ISO treatments resemble the clinical manifestations of myocardial infarction [16] [17] [18], the ISO-induced leakage of cTnI and CK-MB from damaged or necrotic cardiomyocytes to blood is a sensitive and reliable assessment for the extent of ISO-induced myocardial injury [13]. The myocardial injury was associated with a decrease in Mn-SOD activity and an increase in mitochondrial MDA level, indicative of oxidative stress-induced inactivation and oxidation of membrane lipids. However, the mitochondrial glutathione redox status was enhanced in myocardial tissue. While the development of ISO-induced myocardial injury involves reactive oxygen species-mediated processes [19], the paradoxical enhancement of mitochondrial glutathione redox status in ISO-challenged hearts

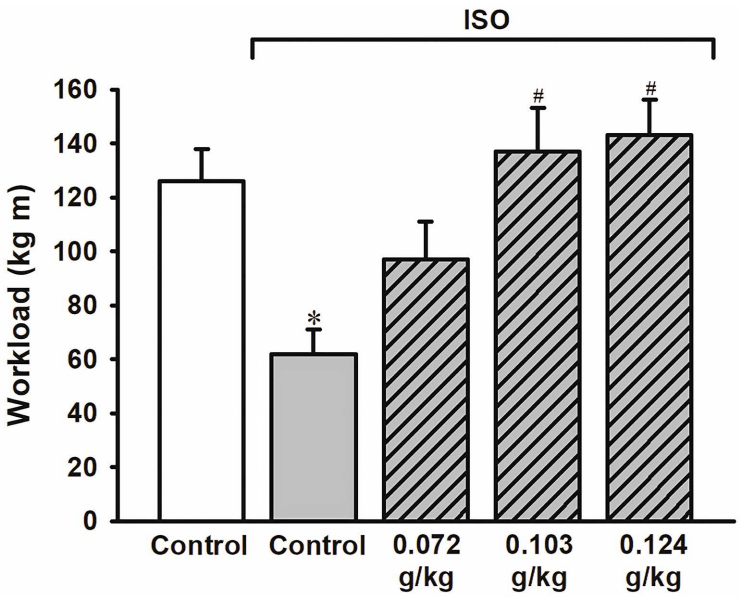

Figure 6. Effects of pre-/co-treatment with HECP on changes on workload in ISO-challenged rats. After 7 days of training for adaptation (Day 23 - Day 30), the workload was assessed 24 h after the last dosing, as described in Materials and Methods. *Significantly different from the non-ISO control. #Significantly different from the ISO control.

may be related to the elicitation of glutathione antioxidant response under oxidative stress conditions. In this connection, the cardioprotection afforded by HECP pre-/co-treatment against ISO-induced chronic injury was accompanied by the further enhancement of mitochondrial glutathione redox status as well as the inhibition of Mn-SOD inactivation and mitochondrial MDA production. Conceivably, the glutathione antioxidant response elicited in HECP-pre-/co-treated myocardium can overwhelm the oxidative stress arising from ISO metabolism, thereby protecting against tissue injury.

The cardioprotective action of HECP was associated with the amelioration of plasma parameters on cardiac dysfunction and hypertrophy. HECP pre-/co-treatment was found to suppress the ISO-induced increases in plasma BNP and ANP levels. BNP is a useful marker for estimating ventricular dysfunction, which is synthesized in the ventricles and released in response to ventricular myocardial contraction [20]. ANP is a cardiac hormone involved in the homeostasis of plasma volume, cardiovascular remodeling, and counter-regulation of the renin-angiotensin-aldosterone system [21]. It is synthesized primarily in the cardiac atria in response to cardiac ischemia, myocardial stretch, or cardiac hypertrophy due to pressure or volume overload [21]. The cardioprotection was also accompanied by the preservation of myocardial eNOS activity. Nitric oxide (NO), which is a widespread signaling molecule in the cardiovascular system, is crucially involved in the development of cardiovascular diseases [11]. NO is solely generated in biological tissues by NOS that chemically transforms arginine to citrulline with the production of NO. Low levels of NO released by eNOS from endothelial cells are important for the maintenance of basal vascular tone. ADMA, as a competitive inhibitor of NOS, is causally related to endothelial dysfunction in heart failure, suggesting that the inhibition of NOS by ADMA could also be the mechanism underlying the pathogenesis of cardiovascular diseases [11]. In this regard, HECP pre-/co-treatment suppressed the ISO-induced increase in plasma ADMA level, presumably preserving the functional integrity of eNOS and hence NO production. This is consistent with the observation that myocardial levels of PRMT1 and DDAH2, both of which catalyze the formation of ADMA [12] and were shown to be induced by chronic ISO challenge, were decreased in HECP-pre-/co-treated and ISO-challenged rats.

The cardioprotection produced by ischemic post-conditioning, a condition similar to that of HECP co-treatment with ISO challenge, is mechanistically linked to the activation of an adenosine-mediated reperfusion-injury salvage kinase pathway and tumor necrosis factor-α-mediated survivor activating factor enhancement pathway, both of which target mitochondria via the activation of PKCε, thereby opening a mitochondrial ATP-dependent potassium channel and leading to the inhibition of mitochondrial permeability transition and cell apoptosis, with resultant cardioprotection [22] [23]. The involvement of PKCε-mediated signaling pathway, as indicated by PKCε translocation to the mitochondria, was observed in the ISO-challenged rat hearts, wherein the ISO-induced PKCε translocation was further increased in HECP-pre-/co-treated rat hearts. Consis-

tently, the extent of apoptosis in the myocardium, as assessed by the cytosolic caspase 3 activity, was reduced. In addition to the biochemical parameters, the beneficial effect of HECP pre-/co-treatment was paralleled by an increase in exercise capacity, which is an indirect functional index of the myocardium, in ISO-challenged rats.

The protection afforded by pre-/co-treatment with HECP against chronic heart failure, as observed in the present study, is consistent with the previously reported cardioprotective effect of cardiomyopeptin [4] [5] [6]. While whether the supplemented herbal extracts can enhance the cardioprotective action of cardiomyopeptin remains to be investigated, it has been shown that Ginseng Radix and its total saponins have positive effects on heart disease through different mechanisms of action [24]. Polygonati Rhizome polysaccharides could prevent acute heart failure induced by adriamycin in rats, with the underlying mechanism related to its antioxidant and anti-inflammatory actions, as well as the inhibition of cardiomyocyte apoptosis [25]. Bambusa leaf extract (from thorny bamboo) was shown to protect against acute myocardial injury induced by ISO in rats [26]. In addition, orientin isolated from Phyllostachys leaf extract protected against myocardial ischemia-reperfusion injury in H9c2 cardiomyocytes, at least in part through the suppression of mitochondrial permeability transition pore opening [27]. Conceivably, supplementation with the three herbal extracts likely enhances the cardioprotective effect of cardiomyopeptin. In conclusion, HECP may offer a promising prospect in preventing chronic heart failure in human subjects.

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Conflicts of Interest

Zumeng Xia is a staff of Infinitus China Limited.

The other authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

Herbal extract-supplemented cardiomyopeptin preparation (HECP); isoproterenol (ISO); glutathione (GSH); oxidized glutathione (GSSG); cardiac troponin I (cTnI); creatine kinase (CK); manganese superoxide dismutase (Mn-SOD); malondialdehyde (MDA; brain natriuretic peptide (BNP); atrial natriuretic peptide (ANP); asymmetric dimethylarginine (ADMA); nitric oxide (NO); nitric oxide synthase (NOS); endothelial nitric oxide synthase (eNOS); protein arginine methyltransferase 1 (PRMT1); dimethylarginine dimethylaminohydrolase 2 (DDAH2); Protein kinase C-epsilon (PKCε); exercise capacity (EC).