Kai-Xin-San suppresses matrix metalloproteinases and myocardial apoptosis in rats with myocardial infarction and depression

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Abstract. Depression is often triggered by prolonged exposure to psychosocial stressors and associated with coronary heart disease (CHD). Matrix metalloproteinases (MMPs) are involved in the pathogenesis of various emotional and cardiovascular disorders. The purpose of this study was to investigate whether Kai-Xin-San (KXS), which may terminate the signaling of MMPs, exerts antidepressant-like and cardioprotective effects in a myocardial infarction (MI) plus depression rat model. Rats were randomly assigned to five groups: A normal control (control group), a celisc-injection of isopropyl adrenaline group (ISO group), depression (depression group), an ISO + depression (depression + ISO group), and an ISO + depression group treated with intragastric administration of 1,785 mg/kg KXS (KXS group). Behavioral changes, echocardiography, biochemical index, matrix metalloproteinase (MMP) and apoptosis-related proteins were assessed. Compared with the depression + ISO group, KXS significantly improved stress-induced alterations of behavioral parameters and protected the heart by enlarging the left ventricular (LV) fractional shortening (FS) and LV ejection fraction (EF). Moreover, KXS significantly attenuated ISO + depression-induced MMP-2 and MMP-9 expression at the mRNA and protein level and decreased TIMP in the heart compared to the complex model group. Myocardial apoptosis was significantly attenuated by KXS by regulating the Bcl-2/Bax axis. These results indicated that MI comorbid with depression may damage the MMP balance in the central and peripheral system, and KXS may have a direct anti-depressive and cardio-protective effect by regulating the level of MMPs and associated myocardial apoptosis. It is promising to further explore the clinical potential of KXS for the therapy or prevention of MI plus depression comorbidity disease.

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Abbreviations: KXS, Kai-Xin-San; MMP, metalloproteinase; LV, left ventricular; FS, fractional shortening; EF, ejection fraction; CHD, coronary heart disease; CAD, coronary artery disease; HPA, hypothalamic-pituitary-adrenal; BDNF, brain-derived neurotrophic factor; CUMS, chronic unpredictable mild stress; TCM, Traditional Chinese Medicine; M-TMCA, Methyl 3,4,5-trimethoxycinnamate; SD, Sprague-Dawley; RIPA, radioimmunoprecipitation assay; GAPDH, glyceraldehyde-3-phosphatedehydrogenase; ECM, extracellular matrix; IL, interleukin; TNF, tumor necrosis factor; QDS, qi-blood circulation deficiency syndrome; TIMP, inhibitor of metalloproteinase

Key words: KXS, Chinese herbal medicine, myocardial infarction, depression, MMP

Introduction

Coronary heart disease (CHD) and major depression are closely related epidemiologically and biologically (1). Epidemiological studies have revealed that in addition to traditional risk factors (alcoholism, hyperlipidemia, hypertension and diabetes), depression is another important concern in patients with coronary artery disease (CAD) (2). As a risk factor for coronary disease, in 30-40% of the patients with CHD, emotional stressors of daily life have led to myocardial ischemia (3), and induced the high morbidity and mortality rate in patients with CAD (4-6). Consistently, ~40% of patients with depression succumbed to CHD, while the rate for the general population without depression was revealed to be eight times lower (7).

Since it is difficult to examine the impact of stress on human cardiovascular disease, animal models have been used to investigate the underlying mechanisms and develop pharmacotherapy. For example, He *et al* used chronic mild stress (CMS) combined with a blocked left anterior descending artery animal model to verify the effect of Ginseng Fruit Saponins on the serotonin system (8). The effects of escitalopram on myocardial apoptosis and the expression of Bax and Bcl-2 were also examined during myocardial ischemia/reperfusion in a rat model with depression (9). Furthermore, anti-depressive medicine, such as escitalopram, fluoxetine, Shuangxinfang, also

exhibited a cardio-protective effect (9-11). However, the exact mechanisms involved in CHD in combination with depression are still mostly unknown and effective medicines are limited.

Matrix metalloproteinases (MMPs) including matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) are involved in cardiac pathophysiology and function as biomarkers of atherosclerosis, myocardial infarctions (MIs), congestive heart failure, and non-ischemic or ischemic cardiomyopathy (12-14). MMPs also play an essential role in brain function. They are associated with cognitive efficiency, depression (15), and neuroinflammation. However, the role MMPs play in CHD in combination with depression remains elusive.

Traditional Chinese medicine (TCM) usually attenuates physical and mental dysfunctions (16). Kai-Xin-San (KXS), a Chinese herbal medicine formula from ginseng (*Panax ginseng* C.A. Meyer), hoelen (*Poria cocos* F.A. Wolf), polygala (*Polygala tenuifolia* Willd) and acorus (*Acorus tatarinowii* Schott) at the ratio of 3:3:2:2, has been revealed to be effective at treating depression and improve learning and memory (17-19). It has the potential to balance serotonin and increase the expression of brain-derived neurotrophic factor (BDNF) (19-21). Most of its components exhibit protective effects on both the brain and heart, such as Ginsenoside Rd and Re (22-24), Methyl 3,4,5-trimethoxycinnamate (M-TMCA) and oligosaccharide ester (25,26) and extracts of hoelen (27). Recently the effects of Kai-Xin-San in fluoxetine-resistant depressive rats were revealed to influence various inflammatory pathways (19).

Based on these previous results, in the present study, an MI and depression model was applied to i) evaluate whether pharmacological treatment with KXS exerts antidepressant-like activity and cardio-protective effects and ii) explore the molecular mechanisms of KXS in regulating MMP levels and apoptosis.

Materials and methods

Animals. All animal experiments were conducted following the Use of Laboratory Animals by the U.S. National Institutes of Health and approved by the Animal Experimentation Ethics Committee of the Chinese PLA General Hospital. In the present study, 60 male Sprague-Dawley (SD) rats weighing approximately 210-230 g at 6 weeks were obtained from Vital River Laboratory Animal Technology Co., Ltd. [SCXX (Beijing) 2012-0521]. Rats were housed in an animal laboratory at 22±2˚C and 60±3% humidity, under a 12 h dark/light cycle.

KXS extract preparation. Herbal KXS was purchased from the Yu Ye Group Co., Ltd. in 2017, and was authenticated by Professor Ping Liu (PLA General Hospital). The total extract was prepared and standardized in accordance with our previous study (28,29). KXS contains four indigenous medicines: Ginseng, hoelen, polygala and acorus. Ginseng was refluxed with 60% ethanol, after which it was heated and refluxed 4 times, for 1 h each time. The extracts were combined and filtered for use, and the ginseng drug residue was also used. Acorus was soaked in water 6 times for 12 h, and volatile oil was extracted for 8 h. The obtained volatile oil was added to ethanol to achieve a 50% oleyl alcohol solution. The ginseng and acorus drug residue with hoelen and polygala were added to 7.4 l of water. They were extracted 3 times for 1 h each time and combined with liquid medicine, after which they were concentrated and added to 50% ethanol, and then chilled overnight and filtered. The filtrate was combined with ginseng extract, and ethanol was recovered. The mixture was concentrated and dried under reduced pressure.

Experimental protocol. Fig. 1 displays the timeline of all procedures. After 3 days of adaptation, the rats were randomly divided into five groups (10 animals each), receiving the following treatments respectively: Normal control rats treated with intragastric administration of saline (control group); celisc-injection of isopropyl adrenaline (ISO)-induced MI rats with intragastric administration of saline (ISO group); depressive rats treated with 4 weeks of chronic mild stress (CMS) and intragastric administration of saline (depression group); ISO-induced MI plus CMS rats and intragastric administration of saline (model group); and ISO-induced MI plus CMS rats with intragastric administration of 1,785 mg/kg KXS daily for 14 days (KXS group). The rats received a standard diet, and free access to water. All rats were sacrificed 3 days after the last day of behavioral testing, and the levels of MMPs in the brain and heart were examined.

ISO-induced MI. The ISO-induced MI animal model was obtained by i.p. administration of ISO (150 mg/kg body weight; Sigma-Aldrich; Merck KGaA) for 3 days (30).

Depression. Chronic unpredictable mild stress (CUMS) is widely used to establish depressive animal models, and this experiment adopted a modified protocol as previously reported (19,26). Rats received 4 weeks of stress stimulations, which consisted of water deprivation (24 h), food deprivation (24 h), restraint (1 h), isolation (24 h), forced cold water swimming (10 min), flashing light (3 h) and were group-housed in a soiled cage overnight, in a random and unpredictable order for 42 days.

ISO combined with depression. The ISO + depression group, the group of rats with ISO-induced MI, and the depression group were then treated by CUMS for four weeks.

Sucrose-preference test. The tests were performed at 0, 28, and 42 days of the experiment. Before the sucrose-preference test, rats were deprived of water and food for 24 h and then fed with two pre-weighted bottles containing water for 1 h and 1% sucrose solution. Intake was measured by weighing the bottles before and after each test. The sucrose preference was calculated as sucrose intake/total water intake (sucrose intake + water intake) (31).

Forced swim test. An adapted version of the forced swim test originally described by Porsolt *et al* (32) was used. Twenty-four hours after the last KXS treatment, rats were forced to swim individually for 5 min in a Plexiglas cylinder (height: 40 cm, diameter: 30 cm) filled with water (temperature: 24±1˚C; depth: 30 cm). The behavior of the rats was videotaped. The overall time spent in (i) immobility (floating and making only those movements necessary to keep the head above

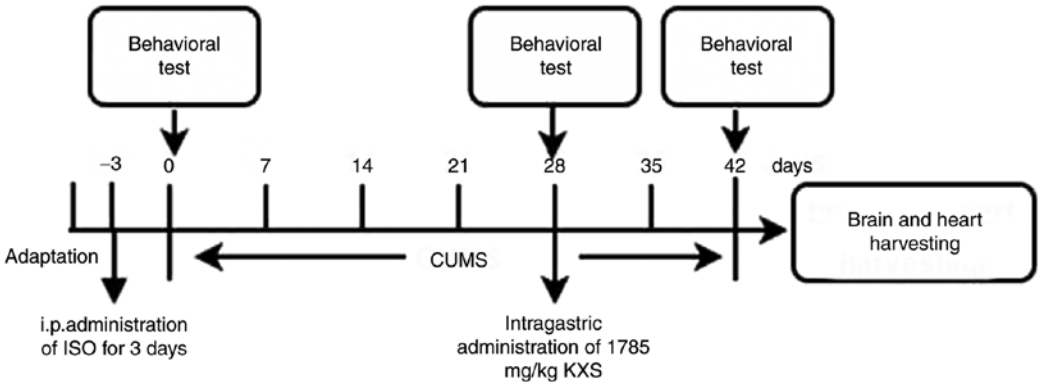

Figure 1. Time line of experimental procedures for the behavioral adaptation and the behavioral tests. ISO, isopropyl adrenaline; KXS, Kai-Xin-San; CUMS, chronic unpredictable mild stress.

water), (ii) swimming (active swimming motions that moved the animal across the center or in circles within the center of the cylinder), and (iii) climbing (attempts to climb the wall of the cylinder) was scored by an experienced experimenter blind to the different treatment groups.

Analysis of cardiac function by echocardiography. Rats were anesthetized in a chamber with a mixture of 4.0 to 5.0% isoflurane and oxygen and maintained by a mixture of 1.2 to 2.0% isoflurane and oxygen. After the hairs on their chest were removed, the rats were fixed in a supine position on the scanning platform of a high-resolution ultrasound system with a 40-MHz transducer (Vero 770; FUJIFILM VisualSonics, Inc.). LV end-diastolic and -systolic dimensions (LVID and LVIS, respectively) were measured on an M-mode obtained from a parasternal short-axis view at the mid papillary level. The fractional shortening (FS) was defined as (LVID-LVIS)/LVID). The ejection fraction (EF) was calculated from the M-mode (LVID3-LVIS3) (33).

Tissue preparation and ELISA analyses. After the final behavioral test, all rats were anesthetized by 10% chloral hydrate (350 mg/kg), and decapitated with a standard rodent guillotine. Brain and hearts were excised, rinsed in ice-cold isotonic saline, accurately weighed, and then stored at -80˚C prior to further analysis. For ELISA analyses, 9 times the volume of normal saline with tissue was added and a homogenate was prepared in an ice water bath which was then centrifuged at 1509.3 x g for 10 min. The level of MMP-2 and MMP-9 in heart and brain tissues was analyzed by ELISA (Beijing Andy Huatai Technology Co., Ltd.). Extracted supernatants of each sample were added into MMP-2 and MMP-9 ELISA Assay Kit plates. The absorbance value was measured at 450 nm to analyze cardiac fibrosis responses and each procedure was performed according to the manufacturer's instructions.

Real-time PCR analysis. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Then, reverse transcription and real-time PCR reactions were performed with Revert Aid™ first strand cDNA synthesis kit (Thermo Fisher Scientific, Inc.) and SYBR® Green PCR Master Mix (ABI; Thermo Fisher Scientific, Inc.), respectively. All primers were purchased from TSINGKE Co, Ltd. The primers used in the present study were: Bax forward, 5'-GCTGATGGC AACTTCAACTGGG-3' and reverse, 5'-TTCTTCCAGATG GTGAGCGAGG-3'; Bcl-2 forward, 5'-TACCGTCGTGAC TTCGCAGAGAT-3' and reverse, 5'-AGGAGAAATCAA ACAGAGGTCGC-3'; and GAPDH forward, 5'-CTGCCT TCTCTTGTGACA-3' and reverse, 5'-TGTAGACCATGT AGTTGAGG-3'. The PCR thermocycling conditions were: Pre-denaturation at 95˚C for 30 sec, 39 cycles of denaturation at 95˚C for 3 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 15 sec. Quantification of mRNA levels relative to GAPDH (a housekeeping gene) was performed with the $2^{-\Delta\Delta Cq}$ method (34).

Western blotting. Proteins were obtained from the whole heart or brain. The frozen animal tissues were homogenized in ice-cold lysis radioimmunoprecipitation assay (RIPA) buffer (Applygen Technologies, Inc.) for 10 min on ice, and then centrifuged at 12,000 x g at 4˚C for 15 min. Total amounts of proteins in each sample were determined by BCA kit, and the protein concentration so fall samples were adjusted to be the same. Supernatants containing 20 μg proteins were separated on 10% SDS-PAGE and electrotransferred onto NC membranes. The blots were probed with a primary antibody overnight after blocking with 5% non-fat milk. Glyceraldehyde-3-phosphatedehydrogenase (GAPDH) and β-actin were used as controls. The antibodies and dilutions were as follows: MMP-2 (1:300, ab51075), TIMP1 (1:300, ab61224), Bcl-2 (1:500, ab196495) and Bax (1:500; ab32503; all from Abcam). The membranes were blocked with 5% non-fat dry milk and incubated with primary antibodies overnight at 4˚C, followed by a secondary horseradish peroxidase-conjugated antibody (1:6,000, ab205718, Abcam) for 2 h. The blots were developed using a electrochemiluminescence system and determined using an image analysis system (Bio-Imaging Analyzer, UVP). Blot quantification was performed with ImageJ version 1.43u (National Institutes of Health). All western blot analyses were performed in duplicate.

Statistical analysis. The data were expressed as the mean ± standard deviation (X ± SD), statistically evaluated by one-way analysis of variance with Tukey-Kramer's test for post hoc analysis and a Bonferroni correction for multiple

comparisons for each outcome variable separately. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of KXS on bodyweight, sucrose preference and immobility. Body weight (BW) was measured before the onset of the CUMS regimen and then weekly until the end of the procedure and treatment with KXS weeks later (Fig. 2A). There was no difference observed between the body weight of the ISO and control group, however, the body weight of the rats in the depression group and the depression + ISO group was increasingly decreased. KXS treatment restored the depression + ISO-induced BW reduction in the rats. The results of the sucrose preference test are presented in Fig. 2B. After 4 weeks of CUMS, the three model groups all exhibited a decrease of sucrose intake, and among them, the depression + ISO group exhibited the most significant decrease of sucrose preference compared with the control group. Two weeks of KXS treatment significantly increased sucrose preference up to the baseline. Behavior during the forced swimming test is presented in Fig. 3. The depression + ISO rats exhibited a greater amount of immobility and a smaller amount of climbing behavior compared to the control and the KXS-treated rats.

Effects of KXS on cardiac function. Echocardiography revealed markedly increased LVIDd and LVIDs and decreased EF and FS in the treated groups, compared with the control group. Among them, the depression + ISO group induced the highest LVIDd and LVIDs and the lowest EF and FS compared to the other groups. Notably, compared with the depression + ISO group, the KXS group increased the EF and FS and decreased the LVIDd and LVIDs (Table I). The heart weight (HW) corrected for BW was significantly increased in the depression + ISO group compared to the control group and was significantly reduced by KXS compared to the depression + ISO group (Table I).

Effects of KXS on the level of MMP-2 and MMP-9 in the brain and heart of rats with MI and depression. The levels of MMP-2 and MMP-9 mRNA expression were significantly increased in both the heart and brain in the depression + ISO group compared with the control group. Among them, the depression + ISO group induced higher MMP levels compared with the ISO group alone in the heart. Also, treatment with KXS significantly alleviated the depression + ISO-induced increase of MMP-2 and MMP-9 levels in the heart and brain (Fig. 4). The levels of MMP-2 and MMP-9 proteins in the heart were consistent with the mRNA levels, however, the levels of protein of MMP-2 and MMP-9 in all groups in the brain were not significantly different (Fig. 5).

Effects of KXS on the level of MMP-2 and TIMP-2 in the heart of rats with MI and depression. As demonstrated in Fig. 6, compared with the control group, MMP-2 and TIMP-2 were both upregulated in the depression + ISO group at the protein level. The changes in MMP-2 protein levels in different groups were examined by western blotting, which was consistent with the results observed using ELISA. The TIMP-2 expression in the depression group was not as significantly altered as the ISO or depression + ISO group. KXS decreased the MMP-2 and TIMP expression compared with the depression + ISO rats.

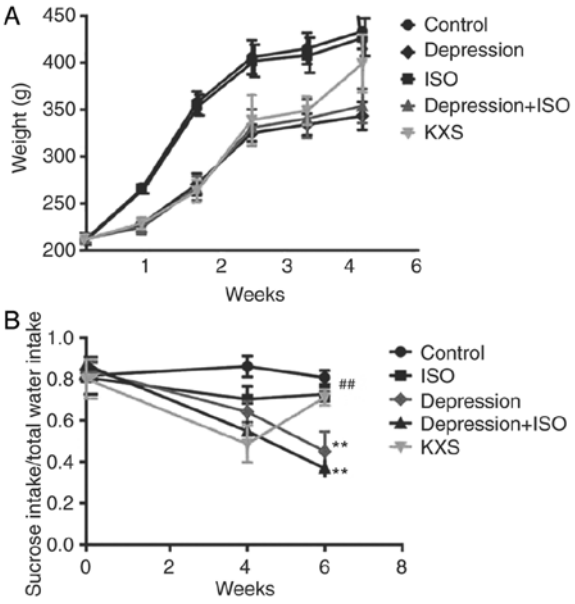

Figure 2. Effect of KXS on the bodyweight and sucrose consumption of rats with MI and depression. (A) Body weight and (B) sucrose intake/total water intake. (n=8-10 in each group). **P<0.01, vs. the control; ##P<0.01 vs. the depression + ISO group. Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MI, myocardial infarction; ISO, isopropyl adrenaline.

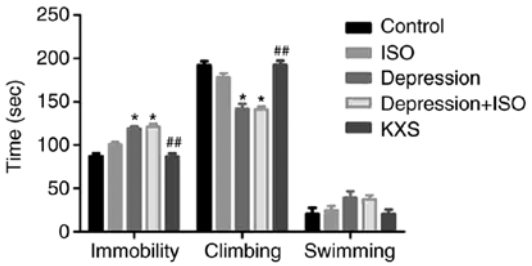

Figure 3. Effect of KXS on the forced swim test. *P<0.05 vs. the control; ##P<0.01 vs. the depression + ISO group. (n=8-10 in each group). Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; ISO, isopropyl adrenaline.

Effect of KXS on Bcl-2, and BAX expression levels in the heart of rats with MI and depression. Compared with the control group, in the depression + ISO group, Bax was significantly increased while Bcl-2 was significantly decreased. Compared with the depression + ISO group, in the KXS group, Bax expression was significantly decreased, while Bcl-2 expression was significantly increased, and the ratio of Bax/Bcl-2 in the KXS group was significantly decreased (Fig. 7 and Table II).

Discussion

In the present study, the effect of KXS, a traditional Chinese medicine, was investigated on MI plus stress-induced

Table I. KXS effect on cardiac function in rats with MI and depression.

Groups	LVIDd (mm)	LVIDs (mm)	EF (%)	FS (%)	HW/BW
Control	3.76±0.25	3.69±0.30	87.46±4.28	52.50±3.94	0.0030±0.0001
ISO	5.54±0.28	4.69±0.51	50.78±4.78[b]	25.59±3.25[b]	0.0034±0.0001[b]
Depression	5.19±0.25	5.01±0.28	77.31±5.05[a]	45.82±6.07[a]	0.0037±0.0004[b]
Depression + ISO	5.71±0.42	5.48±0.33	49.97±6.11[b]	24.71±3.38[b]	0.0039±0.0001[b]
KXS	4.54±0.33	4.36±0.5	58.70±7.66[c]	31.59±6.09[c]	0.0031±0.0002[c]

[a]P<0.05 and [b]P<0.01, vs. the control; and [c]P<0.01 vs. the depression + ISO group. (n=5 in each group). Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MI, myocardial infarction; LVIDd, left ventricular dimensions at end-diastole; LVIDs, left ventricular dimensions at end-systole; EF, ejection fraction; FS, fraction shortening; HW/BW, heart weight/body weight; ISO, isopropyl adrenaline.

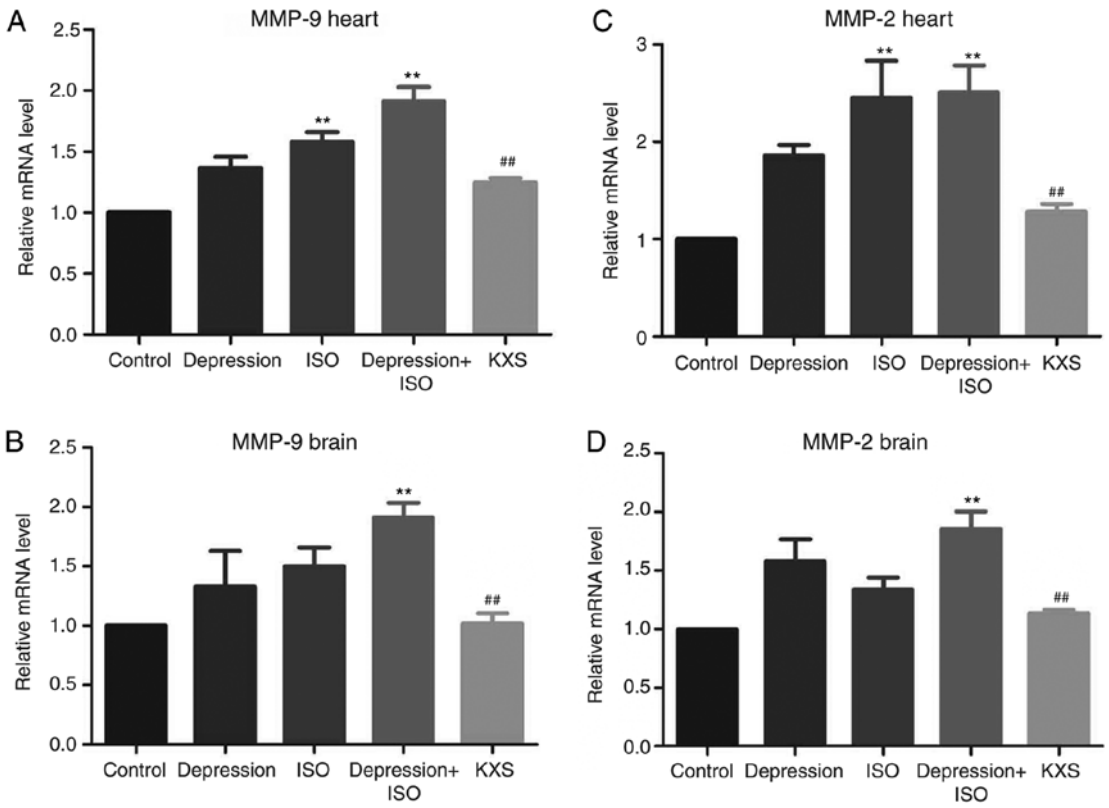

Figure 4. Effect of KXS on the MMP-2 and MMP-9 mRNA levels in the brain and heart. The mRNA levels of MMP-9 in the (A) heart and (B) brain and MMP-2 in the (C) heart and (D) brain were assessed by real-time PCR. (n=5 in each group). **P<0.01, vs. the control; ##P<0.01 vs. the depression + ISO group. Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MMP, matrix metalloproteinase; ISO, isopropyl adrenaline.

depression and cardiac damage. KXS not only significantly improved depressive behavior, as previously reported (19,21), but also improved cardiac function, inhibited MMP-2 and MMP-9 expression, and reduced myocardial apoptosis.

The complex model mimicking coronary heart disease complicated by depression is very limited. It is commonly established with the ligation of the left anterior descending branch of the rat to induce the MI, plus stress to induce the depressive behavior (9,10,35). In the present study, a comprehensive disease model of ISO-induced MI plus CUMS-induced depression was established. After ISO injection and 4-weeks of continuous various stresses, MI plus depression resulted in even worse EF and FS and the decreased consumption of sucrose, compared with the depression or MI groups alone, which indicated that MI plus depression induced even worse cardiac function and depressive behaviors. KXS significantly improved depression-like symptoms and displayed its cardio-protective effect.

MMPs are an endogenous family of proteolytic enzymes implicated in the pathophysiology and function as biomarkers of atherosclerosis, MIs, congestive heart failure, and non-ischemic or ischemic cardiomyopathy (12-14). Previous studies demonstrated that the gene expression and gelatinolytic activity of MMPs in the left ventricles were significantly increased

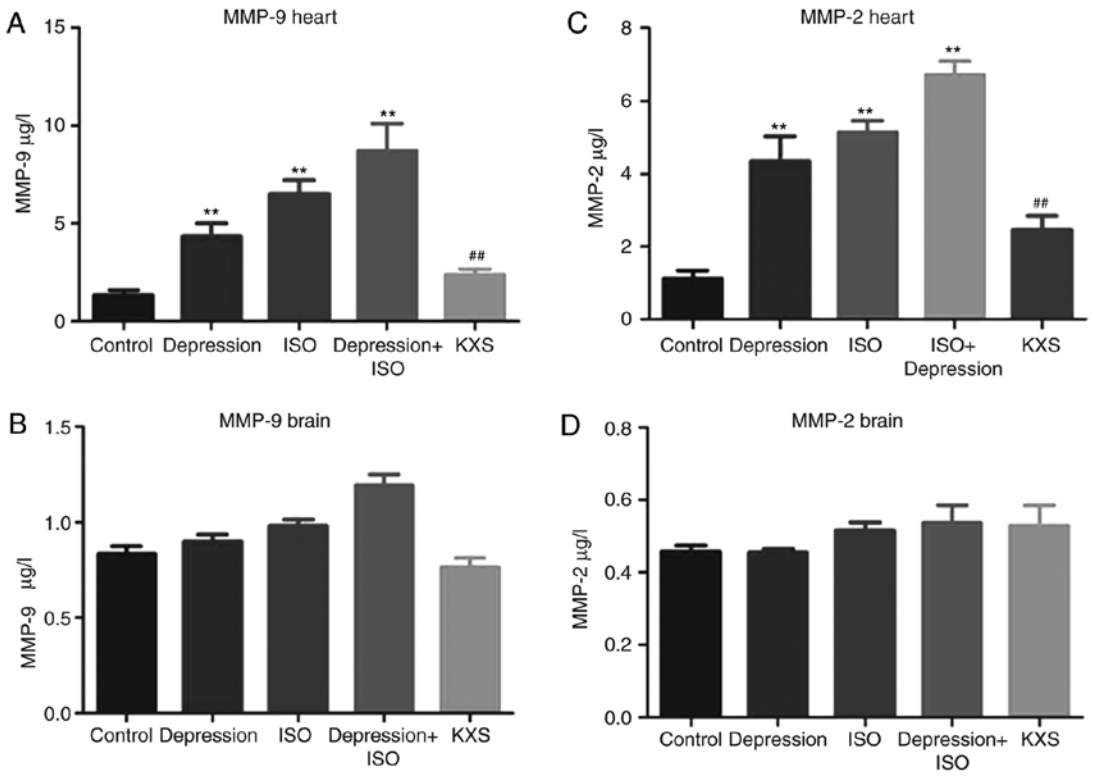

Figure 5. Effect of KXS on the protein level of MMP-2 and MMP-9 in the brain and heart. The protein levels of MMP-9 in the (A) heart and (B) brain and MMP-2 in the (C) heart and (D) brain were assessed by ELISA. (n=5 in each group). **P<0.01, vs. the control; $^{\#\#}$P<0.01 vs. the depression + ISO group. Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MMP, matrix metalloproteinase; ISO, isopropyl adrenaline.

in experimental MI in mice (36), and the gene deletion of the MMP-9 gene attenuated cardiac remodeling post-MI by reducing macrophage infiltration and collagen accumulation through increased apoptosis and reduced inflammation (37).

In addition, myocardial apoptosis has been causally linked to the pathogenesis of MI (38,39). Certain apoptosis-related proteins, including Bcl-2, Bax and caspase-3, are involved in the development of myocardial apoptosis (40), and level of caspase-3 and Bax (as a pro-apoptotic protein) were significantly reduced and Bcl-2 (as an inhibitor of apoptosis) was enhanced in the myocardial tissues of MI rats compared to an MI group (41). A decreased Bcl-2/Bax ratio has also been revealed to increase the probability for myocardial cell apoptosis (42). Several studies have demonstrated the association between MMPs and apoptosis. The downregulation of the expression of MMP-2 has been revealed to be associated to attenuation of apoptosis in several pharmacotherapies for cardiac dysfunction (43,44). Also, the increased MMP-2 expression and imbalance of Bcl-2/Bax expression may be associated with the development and maintenance of atrial fibrillation (45). Collectively, these data indicated that MMPs play a crucial role in promoting cardiac protection through apoptosis. These results are consistent with previous research that revealed that cardio-protection is associated with the modulation of the cardiac levels of MMP-2, MMP-9 and Bcl-2 or Bax. In the present study, KXS significantly attenuated the expression of MMP-2 and MMP-9 and myocardial apoptosis by downregulating Bax expression and upregulating Bcl-2 expression in cardiocytes.

According to previous studies, KXS improved depression by influencing various inflammatory pathways (19), and played a neuroprotective role and enhanced cognitive function by reducing apoptosis and oxidative stress (46). Proteomic analysis of the samples of patients demonstrated that the anti-depressive effect of KXS may also involve the alterations in platelet activation and inflammatory regulation (47). MMPs/TIMPs (inhibitor of metalloproteinase) imbalance plays an important role in the transformation of the vessel wall into pathologic thrombus formation by the release of platelets or induced inflammation (48,49). Changes in MMP and TIMP expression may also be a common element in, or perhaps even a marker for, recurrent depressive disorders and somatic diseases (50). The effect of KXS on inflammation and apoptosis has been documented, whereas there are few studies demonstrating that the activation of MMPs is associated with inflammation and apoptosis (51,52). Thus, the observed MMP-2 and TIMP-2 downregulation in cardiac myocytes may be the result of the anti-inflammatory and anti-apoptosis effect of KXS. However, more studies are required to further corroborate this hypothesis.

MMPs are the main interfering agent of the neural extracellular matrix (nECM), and nECM is usually affected by central nervous system (CNS) disorders (53), both in chronic dysfunction such as neurodegenerative diseases and in acute/subacute disorders with chronic sequelae, such as cerebrovascular and inflammatory pathology (53-55). The present data did not detect a significant difference in MMP-2 and MMP-9 protein levels between the KXS and MI plus depression groups, however, a

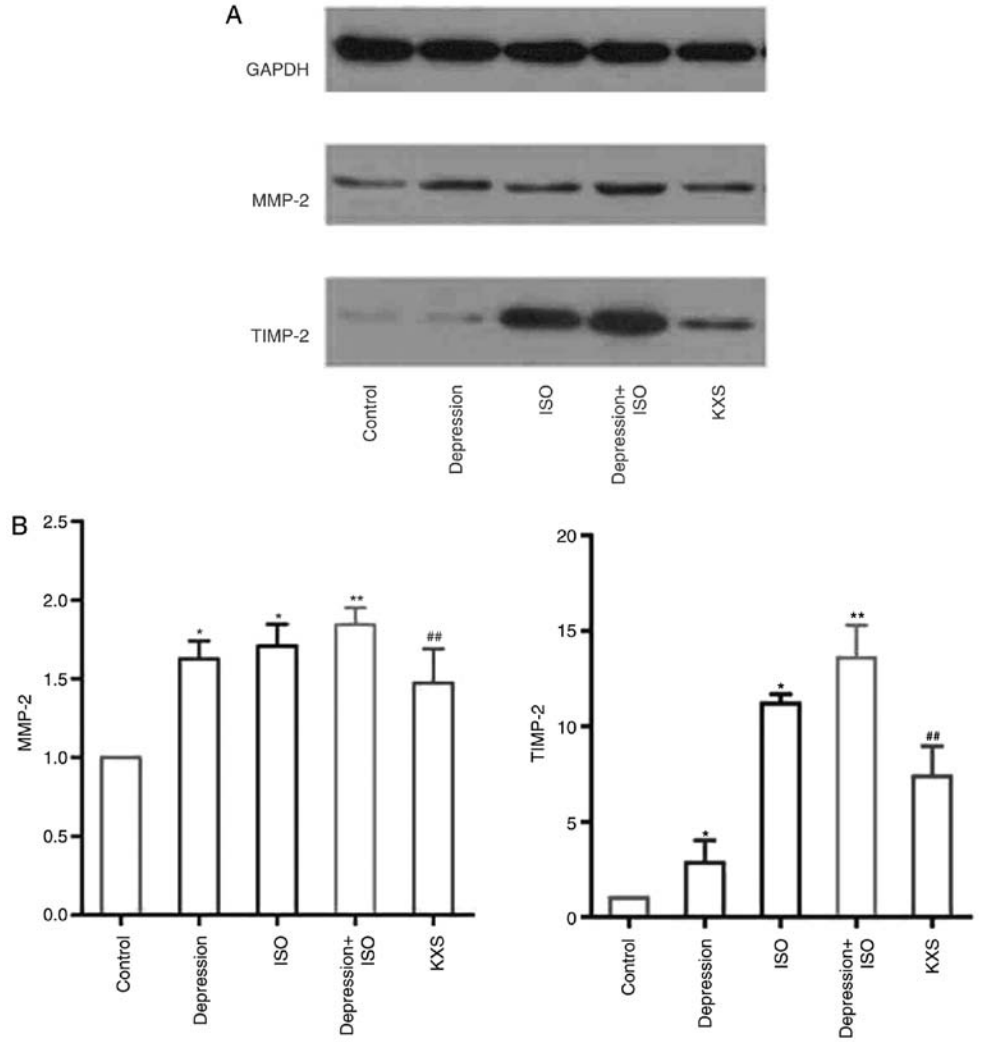

Figure 6. Effect of KXS on MMP-2 and TIMP-2 detected by western blotting in the heart of the rats with MI and depression. (A) Representative western blots, revealing bands of TIMP-2 and MMP-2. (B) Bar graphs, showing the densitometric data corresponding to A. (n=5 in each group). *P<0.05 and **P<0.01, vs. the control; $^{\#\#}$P<0.01 vs. the depression + ISO group. Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MMP, matrix metalloproteinase; MI, myocardial infarction; ISO, isopropyl adrenaline.

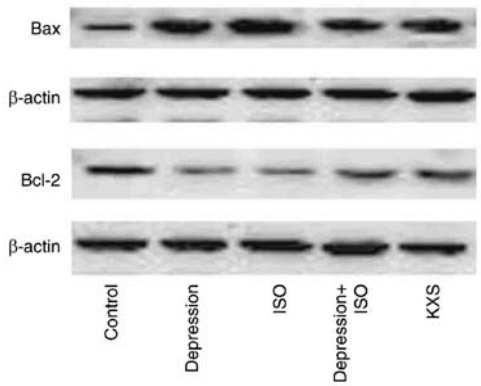

Figure 7. Effect of KXS on Bax and Bcl-2 in the heart of the rats with MI and depression detected by western blotting. (n=5 in each group). Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MI, myocardial infarction; ISO, isopropyl adrenaline.

Table II. Effect of KXS on the expression of protein Bax and Bcl-2 in rats with MI and depression (mean ± SEM, IOD).

Groups	Bax	Bcl-2	Bax/Bcl-2
Control	25.97±6.79	116.5±10.09	0.22±0.06
ISO	114.1±16.67[a]	56.32±7.35[a]	2.05±0.36[a]
Depression	93.73±3.89[a]	83.19±5.76[a]	1.13±0.13[a]
Depression + ISO	128±10.44[a]	52.4±4.62[a]	2.47±0.36[a]
KXS	75.21±4.90[b]	90.47±2.96[b]	0.93±0.06[b]

[a]P<0.01, vs. the control; and [b]P<0.01 vs. the depression + ISO group. (n=5 in each group). Control, normal rats; ISO, injected ISO; depression, chronic mild stress rats; depression + ISO, depression with ISO; KXS, KXS + depression with ISO. KXS, Kai-Xin-San; MI, myocardial infarction; IOD: Integral optical density; ISO, isopropyl adrenaline.

difference was observed in the mRNA expression of MMP-2 and MMP-9 in the brain at the end of the experiment (6 weeks). It is possible that the observed MMP mRNA in the brain modified the nECM in chronic modification during MI plus depression.

KXS could work well on the patients with qi-blood circulation deficiency syndrome (QDS), which is the key point of the relationship between brain and heart disease in the TCM theory. In this study, it was further observed that KXS could significantly decrease the depression-like behavior and effectively protect from cardiac damage the MI plus depressive rats, which indicates that it may be more helpful for the patients with MI and are comorbid with major depressive disorder. The beneficial effect of KXS in animal models may be mediated at least partially by the inhibition of increased MMP-2 and MMP-9 activities as well as myocardial apoptosis. In the future, more stable complex models are required to be established to understand the exact neurobiological pathways by which KXS regulates MMPs and how they are related to oxidative stress, inflammation, or the platelet pathway.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YH, XD and PL contributed to the study design, TZ, HM, WY and YW performed the experiments and analyzed the data. YH and XD contributed to the experiments and writing and performed the secondary data analyses and revising the manuscript for intellectual and scientific content, and PL and YC contributed to the conception of the study. All authors read and approved the manuscript.

Ethics approval and consent to participate

All animal experiments were conducted following the Use of Laboratory Animals by the U.S. National Institutes of Health and approved by the Animal Experimentation Ethics Committee of the Chinese PLA General Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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