Compound Dragon's blood capsule alleviates the degree of myocardial ischemia by improving inflammation and oxidative stress

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Abstract

Background: Compound Dragon's blood capsule (CDC) is a patent medicine mainly composed of dragon's blood (*Dracaena cochinchinensis* (Lour.) S. C. Chen), notoginseng (*Parmex notoginseng* (Burk.) F. H. Chen) and borneol (C₁₀H₁₈O) for the treatment of stabilize coronary heart disease (CHD) and myocardial ischemia (MI). This paper is to investigate the anti-myocardial ischemia properties of CDC both *in vivo* and *vitro*.

Methods: The fingerprint of CDC was established by UPLC-Q/TOF-MS. The hypoxia/reoxygenation (H/R) model was established by using H9c2 cells. The levels of LDH, SOD and MDA were detected by colorimetric method. Moreover, the MI model of rats was established by isoprenaline hydrochloride (ISO), the mortality rate was recorded, the changes in J point of electrocardiogram were determined, the expressions of the myocardial markers, oxidative stress markers (CK, CK-MB, LDH and SOD) and inflammatory mediators (TNF- α , IL-6, IL-10, IL-1 β and NO) in serum were detected.

Results: The fingerprint of CDC was established and 10 mainly active components were identified: 7,4'-dihydroxyflavone, resveratrol, loureirin A, loureirin B, pterostilbene were identified from Dragon's blood, notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, oleanolic acid, ginsenoside Rd were identified from notoginseng. *In vitro* study, CDC significantly improved H9c2 cell viability and SOD level ($P < 0.05$), decreased LDH and MDA level ($P < 0.05$). *In vivo* study, CDC increased survival rate and SOD level of serum, decreased J-point of ECG, CK-MB, LDH, TNF- α , IL-6, IL-10 level ($P < 0.05$).

Conclusions: CDC had a significant anti-myocardial ischemia effect by alleviating inflammation and oxidative stress, suggesting that CDC is a suitable adjuvant to treat CHD, dragon's blood has the prospect of developing other new drugs.

1. Background

Compound Dragon's blood capsule (CDC) is a patent compound formulation three traditional Chinese medicine in China: dragon's blood (*Dracaena cochinchinensis* (Lour.) S. C. Chen), notoginseng (*Parmex notoginseng* (Burk.) F. H. Chen) and borneol (C₁₀H₁₈O) in the ratio of 130: 70: 1. It is used against stable exertional angina and acute myocardial infarction (MI) caused by heart blood stasis. Dragon's blood is commonly used as a holy drug in Vietnam, Laos, Yunnan and Guangxi provinces of China to arrest bleeding, as well as to promote blood circulation in these regions. It is reported that dragon's blood has good properties on promoting the blood circulation and removing the blood stasis^[1]. Notoginseng also known as Sanqi or Sanchi, is one of the most popular Chinese herbal medicine. The anti-MI effects of its active ingredients have also been extensively studied^[2, 3, 4]. Borneol promotes the active components of dragon's blood and notoginseng to through the blood-brain barrier to get a better effect^[5].

Coronary heart disease is one of the diseases with the highest morbidity and fatality rates in the world, which is accompanied with hypertension, diabetes and other related diseases. The deposition of lipid-

loaded macrophage-derived foam cells in the intima of the arterial wall, which causes atherosclerosis (AS), leads to plaque formation and then lumen stenosis or obstruction, resulting in tissue ischemia, hypoxia and eventually necrosis^[6]. As the basic physiological process of CHD, myocardial ischemia is caused by the decrease of coronary blood flow per unit time. Therefore, CHD is primarily treated by reducing or eliminating the degree of MI. The overload of reactive oxygen species (ROS) and the activation of inflammatory cascades are the main causes of cardiomyocyte abnormality^[7]. There are expressions of many cytokines and infiltration of inflammatory cells in the ischemic injury area, which is the basis of the change from ischemic injury to inflammatory injury. Moreover, the over-production of ROS can activate the Toll-like receptor (TLR) signaling pathway and promote the phosphorylation of NF- κ B pathway^[8], and the activation of NF- κ B pathway promotes the over-production of IL-1 β , IL-6 and TNF- α , leading to the exacerbated MI.

Apart from inflammation, MI is also closely related to apoptosis. In normal organism, the signals of apoptosis and anti-apoptosis remain in a dynamic balance. However, when ischemia occurs, the balance will shift to apoptotic signaling, leading to myocardial cell damage and promoting the occurrence of excessive apoptosis. MI can inhibit the production of cytokines to suppress the activation of PI3K, resulting in reduced phosphorylation of Akt and its activation. Akt can inhibit the apoptosis of cells through a series of downstream proteins^[9, 10]. Therefore, when its production is reduced, apoptotic factors will be generated in large quantities, and the level of apoptosis will be increased. The lack of Akt can also affect the activation of rapamycin target protein (mTOR) in two ways^[11], and improve the levels of apoptosis factors in cells^[12], thus accelerating the apoptotic process.

At present, there are a few studies on CDC, which mainly focus on quality control, such as qualitative analysis some components by HPLC^[13, 14, 15]. However, there are no direct evidence showing that CDC has a significant anti-myocardial ischemia properties. This study aimed to evaluate the effect of CDC on myocardial ischemia both in cell culture and rats.

2. Methods

2.1 Drugs and Chemicals

CDC was provided by Yunhe Co.,Ltd (Yunnan, CHN). Notoginsenoside R1 (191226), ginsenoside Rg1 (191207), ginsenoside Rf (190927), ginsenoside Rd (190927), oleanolic acid (190912), loureirin A (191006), loureirin B (191005), pterostilbene (190729), resveratrol (200316), borneol (190813) were all purchased from Shanghai Winherb Medical Technology Co.,Ltd (Shanghai, CHN). 7,4'-Dihydroxyflavone (111787-201002) was purchased from National Institutes for Food and Drug Control (Beijing, CHN). The purity of all standards chemicals $\geq 98\%$. The Acetonitrile used in the experiment is reagent grade, purchased from Merck KGaA (Darmstadt, GER). Distilled water purchased from A.S. Watson Group Ltd (Hong Kong, CHN).

Cell Counting Kit-8 (CA1210-1000T) was purchased from Solarbio Life Sciences (Beijing, CHN). LDH kit (C0016), SOD Activity Assay (S0101), MDA kit (S0131), NO kit (S0021) were all purchased from Beyotime Biotechnology (Shanghai, CHN). LEGEND MAX™ Rat TNF- α ELISA Kit (438207) and LEGEND MAX™ Rat IL-6 ELISA Kit (437107) were purchased from Biolegend (San Diego, USA). Rat IL-1 beta ELISA Kit (ab100768) and Rat IL-10 ELISA Kit (ab100764) were purchased from Abcam (Cambridge, UK). LDH kit (20190925), CK kit (20190910) and CK-MB kit were purchased from BioSino Bio-Technology & Science Inc.(Beijing, CHN). Isoprenaline hydrochloride (ISO, 190201, Harvest Pharmaceutical Co., Ltd., Shanghai, China), 0.9% physiological saline (190201, Shanghai YSRIBIO Industrial Co., Ltd, China).

2.2 Preparation of CDC

The preparation of CDCs refers to the Ch.P. I, 2020: grind 140 g of notoginseng into coarse powder, add appropriate 70% ethanol in airtight conditions for 60 min, add 70% ethanol to soak for 24 h, percolate until the mixture is colorless, filter, concentrate at 60 °C until the mixture becomes a clear cream with a relative density of 1.10-1.12, fully mix with 260 g dragon's blood powder and 2 g borneol powder, then dry at 60 °C, grind and deposit into capsules. Altogether, this process creates 1000 capsules. each capsule is 0.3 g. The proportion of dragon's blood, notoginseng and borneol in a CDC is 130: 70: 1.

The CDC extraction preparation refers to Ch.P. I, 2020: grind the contents of a CDC, weigh 0.2 g precisely and place in a 50 ml measuring flask, add an appropriate amount of ethanol, sonicate (power 250 W, frequency 50 kHz) for 20 minutes, add ethanol to the scale after cooling, shake well, filter and remove the filtrate.

2.3 Establishment of the fingerprint of CDC with UPLC-Q/TOF-MS

UPLC-Q/TOF-MS experiments were performed on a Waters SYNAPT G2 HDMS system equipped with a Waters RP18 column (2.1×150 mm, 1.7 μ m, Waters, USA). The column temperature was maintained at 25 °C. The mobile phase was water containing formic acid (99.9/0.1, v/v, solvent A) and acetonitrile (solvent B). The gradient program was set as follows: 0-2 min, 75% solvent A, 15 min, 60% solvent A; 22 min, 57% solvent A; 33 min, 50% solvent A; and 35 min, 35% solvent A. The flow rate of the mobile phase was set to 0.2 mL·min⁻¹. The injection volume was 1 μ L, except for 7,4'-Dihydroxyflavone, which was 3 μ L. Detection was performed under electrospray ionization (ESI) mode with positive full scan mode (190–400 nm). Solutions were infused from the ESI source at 0.5 mL·min⁻¹ with the following parameters: capillary 3,000 V, drying gas 50 L·h⁻¹, and drying gas temperature 400 °C. Nitrogen was used as the nebulizing and drying gas. All MS conditions were optimized to achieve the maximal detection sensitivity.

2.4 In *vitro* experiments

2.4.1 Cell culture and hypoxia/reoxygenation (H/R) model

Rat cardiomyocytes (H9c2) were purchased from the Cell Resource Center, Institute of Basic Medical Sciences, CAMS/PUMC. The cells were maintained in Dulbecco's modified Eagle medium supplemented

with 1% penicillin/streptomycin (DMEM, 12100, Solarbio Life Sciences, CHN) and 10% fetal bovine serum (FBS, PAN Seratech, GER). All cells tested negative for mycoplasma contamination before use. The cells were divided into seven groups as follows: control, model, CDST, CDC 25, 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$. Cells were cultured in 96-well plates with high-glucose DMEM at 37 °C in an atmosphere containing 5% CO_2 until the degree of cell fusion was more than 80%. The old medium was removed, and 100 μL glucose-free DMEM (90113, Solarbio Life Sciences, CHN) was added. The detailed culture medium recipe for each group is as follows: Control and H/R groups: 100 μL of high-glucose DMEM was added; CDST group: 100 μL of high-glucose DMEM containing 100 $\mu\text{g}\cdot\text{mL}^{-1}$ CDST was added; CDC 100 group: 100 μL of high-glucose DMEM containing 100 $\mu\text{g}\cdot\text{mL}^{-1}$ CDC was added; CDC 50 group: 100 μL of high-glucose DMEM containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ CDC was added; and CDC 25 group: 100 μL of high-glucose DMEM containing 25 $\mu\text{g}\cdot\text{mL}^{-1}$ CDC was added. Except for the control group, the remaining groups were all subjected to anoxic incubation (37 °C, 5% CO_2 , 5% H_2 , 90% N_2) for 6 h. The protocol of H/R establishment is shown in Fig. 1.

2.4.2 Cell viability assay using CCK-8

The Cell Counting Kit-8 (CCK-8, Solarbio Life Sciences, Beijing, CHN) was used to determine cell viability. The culture medium of each well was removed, 100 μL of 10% CCK-8 solution was added, and the cells were incubated at 37 °C for 1 h. Subsequently, the absorbance at 450 nm was determined using a spectrometer. Only 10% of the CCK-8 solution without cells was used as a blank.

Cell viability (%) = $[A(\text{Dosing}) - A(\text{blank})] / [A(0 \text{ dosing}) - A(\text{blank})] \times 100\%$

2.4.3 LDH, SOD and MDA assays

The levels of lactate dehydrogenase (LDH), superoxide dismutase (SOD) and malondialdehyde (MDA) were determined using colorimetry according to the manufacturer's instructions (SOD, MDA and NO kit, Beyotime Biotechnology, Shanghai, CHN).

2.5 *In vivo* experiments

2.5.1 MI Model induced by ISO in rats

A total of 90 healthy SD rats (male, body weight of 180-200 g) were provided by the Experimental Animal Center of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences (IMPLAD, CAMS). All animal-related experiments were approved by the Institutional Review Boards of IMPLAD, CAMS (No. slxd-20190710001). All animals were bred in an animal facility at a temperature of 23 ± 1 °C, a relative humidity of $55 \pm 5\%$ and a 12/12 h day/night cycle. All rats were acclimatized to the environment for 1 week prior to the experiment. Subsequently, the rats were randomly and evenly divided into six groups: the control (distilled water), model (distilled water), CDST group (compound Danshen tablet aqueous solution at a dose of $259.2 \text{ mg}\cdot\text{kg}^{-1}$), L-CDC group ($121.5 \text{ mg}\cdot\text{kg}^{-1}$ CDC), M-CDC group ($243.0 \text{ mg}\cdot\text{kg}^{-1}$ CDC) and H-CDC group ($486.0 \text{ mg}\cdot\text{kg}^{-1}$ CDC). The abovementioned doses were converted from the clinical dose to the rat dose according to the body surface area. All rats were administered for 4 weeks, and the administration volume was $10 \text{ mL}\cdot\text{kg}^{-1}$. On days 27 and 28, all groups were

hypodermically injected with 5 mg·kg⁻¹ isoprenaline hydrochloride (ISO, 190201, Harvest Pharmaceutical Co., Ltd., Shanghai, China), except for the control group, which was hypodermically injected with 0.9% physiological saline (190201, Shanghai YSRIBIO Industrial Co., Ltd, China). All rats were in abrosia in the afternoon on day 27, while access to water was given.

2.5.2 Mortality and electrocardiogram (ECG)

On the 27th day, the mortality rate of animals in each group was recorded after hypodermic injection of ISO. On the 28th day, rats were anesthetized with urethane (1.3 g·kg⁻¹, 20101026, Sigma) 1 h after the last administration, kept in the supine position and monitored with an MP150 data acquisition and analysis system (BIOPAC, USA). ECG was recorded for 5 min. Abnormal rats were discarded. After stabilization, all rats except the control group were hypodermically injected with 5 mg·kg⁻¹ ISO, and the control group was hypodermically injected with 0.9% physiological saline. Then, we recorded the ECG at 0.5, 1, 2, 5, 10, 15 and 20 minutes after injection and observed mV changes at the J point (the junction of the end of QRS and T waves) (Wojciech et al., 2017). ΔJ (change in J point) = J point value at each time point after injection (MV) - J point value before injection (MV). One hour after injection, we collected blood samples from the abdominal aorta, stored them at 20 °C for 60 min, centrifuged them at 2,500 rpm for 15 min, and separated the serum, which was then stored at -20 °C.

2.5.3 Assays of myocardial and oxidative stress markers in serum

The serum SOD and MDA levels were tested using colorimetry according to the manufacturer's instructions (SOD and MDA kit, Beyotime Biotechnology, Shanghai, CHN). The CK, CK-MB and LDH levels were detected using an automatic biochemical analyzer according to the manufacturer's instructions (LDH, CK and CK-MB kit, BioSino Bio-Technology & Science Inc., Beijing, CHN).

2.5.4 Assays of inflammatory mediators in serum

The activity of TNF- α , IL-6, IL-10, and IL-1 β in serum was determined by enzyme-linked immune sorbent assay (ELISA) according to the manufacturer's instructions (LEGEND MAX™ Rat TNF- α ELISA Kit and LEGEND MAX™ Rat IL-6 ELISA Kit, Biolegend, San Diego, USA) (Rat IL-1 beta ELISA Kit and Rat IL-10 ELISA Kit, Abcam, Cambridge, UK). The serum NO level was determined using colorimetry according to the manufacturer's instructions (Beyotime Biotechnology, Shanghai, CHN).

2.6 Statistical analysis

All experiments were independently repeated at least three times. Data were presented as the mean $\pm$ SD. Quantitative data were analyzed using one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant (* $P < 0.05$; ** $P < 0.01$).

3. Results

3.1 Establishment of CDC fingerprint

The CDC fingerprint is shown in Fig. 2. There were a total of 73 peaks. Ten components were identified through comparison with standard materials, including six components from dragon's blood and four components from notoginseng. (Table 1).

Table 1
Ten compounds identified in CDCs using UPLC-Q/TOF-MS

No	RT (min)	Molecular formula	Compound	Source
1	2.64	C ₄₇ H ₈₀ O ₁₈	Notoginsenoside R1	Notoginseng
2	3.25	C ₄₂ H ₇₂ O ₁₄	Ginsenoside Rg1	Notoginseng
3	8.77	C ₁₅ H ₁₀ O ₄	7,4'-Dihydroxyflavone	Dragon's blood
4	9.19	C ₁₄ H ₁₂ O ₃	Resveratrol	Dragon's blood
5	9.91	C ₅₄ H ₉₂ O ₂₃	Ginsenoside Rb1	Notoginseng
6	10.96	C ₃₅ H ₅₆ O ₇	Oleanolic acid	Notoginseng
7	12.47	C ₄₈ H ₈₂ O ₁₈	Ginsenoside Rd	Notoginseng
8	19.59	C ₁₇ H ₁₈ O ₄	Loureirin A	Dragon's blood
9	20.21	C ₁₈ H ₂₀ O ₅	Loureirin B	Dragon's blood
10	25.61	C ₁₆ H ₁₆ O ₃	Pterostilbene	Dragon's blood

3.2 In vitro experiments

3.2.1 Effect of CDCs on cell viability

As shown in Fig. 3, 3-A shows that the survival rate of H9c2 cells in the H/R group was only 34.66%, indicating that certain cell damage was caused by H/R. Compared with the H/R group, the CDST group exhibited an obviously higher survival rate ($P < 0.05$) in a dose-dependent manner. For example, 25 $\mu\text{g}\cdot\text{mL}^{-1}$ CDCs demonstrated a trend of improved cell viability, while there was no significant difference between the 25 $\mu\text{g}\cdot\text{mL}^{-1}$ group and the H/R group. In addition, 50 $\mu\text{g}\cdot\text{mL}^{-1}$ and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ CDCs significantly improved the cell survival rate ($P < 0.05$).

3.2.2 Effect of CDCs on LDH, SOD and MDA levels

Figure 3-B shows that compared with the control group, H/R significantly enhanced the LDH release rate ($P < 0.01$). Moreover, compared with the H/R group, 25 and 50 $\mu\text{g}\cdot\text{mL}^{-1}$ CDCs significantly reduced the LDH level ($P < 0.05$), and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ CDCs also significantly reduced the LDH level ($P < 0.01$). Fig. 3-C and Fig. 3-D illustrate the effects of CDCs on the levels of the oxidative stress markers SOD and MDA. Compared with the control group, H/R significantly enhanced the SOD level ($P < 0.01$), while the MDA

level was decreased ($P < 0.05$). Compared with the H/R group, 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ CDCs significantly enhanced the SOD level, while the MDA level was reduced ($P < 0.05$). However, 25 and 50 $\mu\text{g}\cdot\text{mL}^{-1}$ CDCs had no effect on either SOD or MDA levels.

3.3 *In vivo* experiments

3.3.1 Effect on the rat mortality rate

Figure 4-A shows the mortality rate of MI rats after injection of ISO. Among the control group, the CDST group and the CDC group, ISO treatment resulted in the highest mortality rate, reaching 52.94%. These groups are followed by CDST treatment group, which exhibited a mortality rate reaching 46.15%. Treatment with different CDC concentrations effectively reduced lethality. The mortality rates in the high-dose, middle-dose and low-dose CDC groups were 23.08%, 35.71% and 30.77%, respectively. No deaths were observed in the control group. See supplementary material S1 for full details.

3.3.2 Effect on ECGs

The changes in the J point of the ECGs were determined by recording the ECG at 0, 0.5, 1, 2, 5, 10, 15 and 20 min after ISO injection. Fig. 4-B shows some ECG results, and the change in the J point is shown in Fig. 4-C. After ISO injection, the J point was significantly increased ($P < 0.01$) (Fig. 4-B-2). Compared with the ISO group, the administration groups' results did not demonstrate an increasing trend in the J point (Fig. 4-B-3). The improvement effect was ranked as high-dose group > low-dose group > medium-dose group, and the effect on the CDST group was between the low-dose and medium-dose groups.

3.3.3 Effects on myocardial markers and oxidative stress markers in rats

CK-MB and LDH are commonly used diagnostic indices for the clinical detection of MI. Fig. 5 reveals the effects of CDCs on CK, CK-MB, LDH and SOD levels in rat serum. Fig. 5-A shows that 5 $\text{mg}\cdot\text{kg}^{-1}$ ISO did not increase the release of serum CK, while the high-dose CDCs significantly reduced the CK serum level ($P < 0.05$). CK-MB is the most specific enzyme in the myocardial enzyme spectrum for clinical diagnosis of myocardial injury. Fig. 5-B shows that after injection of ISO, serum CK-MB was significantly increased ($P < 0.05$), that the high-dose CDCs significantly reduced the serum CK-MB level ($P < 0.01$), and that the CK-MB level was significantly reduced in the medium-dose and CDST groups ($P < 0.05$). Low-dose CDCs also reduced the serum CK-MB level, but not significantly. Fig. 5-C shows that ISO significantly increased LDH levels ($P < 0.05$). LDH levels were significantly reduced by CDCs and CDST ($P < 0.05$), especially in the high-dose group ($P < 0.01$). Fig. 5-D shows that the SOD level after ISO injection was significantly reduced ($P < 0.05$), while this value in the CDC and CDST groups was significantly increased ($P < 0.05$), especially in the high-dose group ($P < 0.01$).

3.3.4 Effect on inflammatory mediators

Figure 6 shows CDCs' intervention effect on the levels of TNF- α , IL-6, IL-10, IL-1 β and NO in the serum of rats with ISO-induced acute MI. After injection of ISO, the release of TNF- α in serum was significantly

increased (Fig. 6-A, $P < 0.01$), which was significantly reduced by CDST ($P < 0.05$), and the level of TNF- α in the serum of the low-dose, medium-dose and high-dose CDC groups was also significantly reduced ($P < 0.01$), indicating that the effect of CDCs was slightly better compared with CDST. Similarly, ISO significantly increased the release of IL-6 in serum ($P < 0.01$), while CDST and CDCs at different doses significantly reduced the level of IL-6 (Fig. 6-B, $P < 0.05$). Fig. 6-C depicts the effect on IL-10. The release of IL-10 in serum was significantly decreased after injection of ISO ($P < 0.01$), while CDST or CDCs at all three doses significantly rise the level of IL-10. Fig. 6-D and 6-E indicate that the levels of IL-1 β and NO in serum were not significantly affected by ISO, but they were still significantly reduced by CDCs at the medium dose.

4. Discussion

It is reported that cardiovascular disease has been the leading cause of death in the world^[16]. The gradual accumulation of asymmetric focal thickening of the arterial intima, which forms AS, can cause MI and ischemia by preventing blood flow through the coronary artery. The process of AS is always accompanied by infiltration of T cells, especially CD4⁺ T cells^[17]. When antigen binds to antigen receptor of T cells, it will activate the cascade reaction, leading to the expressions of a series of cytokines, enzymes, cell surface molecules and so on^[18]. Studies have shown that such cascade reaction can cause inflammation, which is similar to delayed-type hypersensitivity reaction, thus indirectly promoting the synthesis of inflammatory cytokines, tumor necrosis factors and interleukin. These inflammatory factors will further accelerate the inflammatory changes, aggravate the atherosclerotic lesions, and cause more serious MI^[19]. Therefore, anti-MI by inhibiting inflammatory response has become a hot topic.

CDC is a compound composed by dragon's blood, panax notoginseng and borneol, which has been included in the latest version Chinese Pharmacopoeia. The main component—dragon's blood, as a ethnic medicine, is widely used in Yunnan Province (China), Thailand, Laos and other Southeast Asian countries. At present, there are some compound preparations of dragon's blood on the market, CDC is the most famous, which has been used in clinic for CHD and acute MI in China and got a better effect than other drugs^[20]. However, there are few pharmacological data of CDC, and its efficacy and mechanism have not been confirmed. Our study fill the blank of material standard and pharmacodynamics of CDC.

The establishment of fingerprint by UPLC can reflect the complex components and interaction well, find the qualitative and quantitative relationships between different components, and provide a strong guarantee for the comprehensive and in-depth quality control^[21]. In Chinese Pharmacopoeia, Loureirin A, Loureirin B and saponins in CDC are used as indicators, while other components identified by UPLC-Q/TOF-MS, such as 7,4'-dihydroxyflavone, Resveratrol and Pterostilbene, are not listed as characteristic indicators. Flavonoids can improve blood circulation, symptoms of cardiovascular disease and reduce cholesterol. As a polyphenol compound, resveratrol can regulate the cholesterol level in blood by binding with estrogen receptor in humans, and inhibit the platelet formation and blood clot adhesion to vascular wall, thus inhibiting the occurrence and development of cardiovascular disease and reducing the risk of

cardiovascular disease^[22, 23]. Pterostilbene, as the next generation of resveratrol, has significant anti-inflammatory effect. It can improve MI by regulating the expressions of TNF- α , IL-1 and other inflammatory factors^[24]. The results have suggested that besides the two types of loureirins which are specified in the Pharmacopoeia, other active components can also be used as index components to improve the quality evaluation system of CDC.

The mechanism of MI injury is complex and diverse, involving processes such as the destruction of Ca⁺ homeostasis and mitochondrial injury. At present, H/R is most commonly used *in vitro* model, which provides a better simulation of MI injuries^[25]. H9c2 cells are widely used in H/R models^[26, 27] due to their stable source, easy availability and simple culture procedure. In addition, H9c2 cells have a higher ATP level, mitochondrial function and respiratory capacity and are more sensitive to H/R^[28]. Therefore, H9c2 cells are more suitable for this study. In the present study, the viability of H9c2 cells in the H/R group was greatly decreased, and the levels of LDH, SOD and MDA were also significantly changed with those in the control group. CDC can improve the damage of H9c2 cells caused by H/R, indicating that CDC has a good anti-myocardial ischemia effect *in vitro*. ISO, as a strong β receptor stimulant, can be used to construct acute myocardial ischemia model in rats by i.p.^[29], which causes physiological and pathological changes in rats similar to human acute myocardial ischemia^[30]. In our study, the ISO group increased mortality (52.94%) sharply, the ST segment of the ECG was dramatically higher, and the serum levels of myocardial markers were significantly higher, too. The reason may be that ISO increases myocardial oxygen consumption through accelerating heart rate, enhancing myocardial contractility and other links, resulting in cardiac overload in rats, myocardial microcirculation disorders, coronary artery spasm, and thus myocardial infarction and ischemia^[31]. In addition, the levels of serum oxidative stress markers and inflammatory factors were detected, and the levels of LDH, SOD, TNF- α , IL-6 and IL-10 in ISO group were significantly increased or decreased compared with control. These changes indicated that myocardial ischemia occurred in rats. However, CDC treatment improved these abnormalities in a similar way to Compound Danshen tablet. These results suggest the remarkable effect of CDC in the protection of myocardial ischemia.

5. Conclusions

In this study, we found that CDC had a notable therapeutic effect on MI both *in vitro* and *vivo*. The results indicated that CDC was composed of many active components for anti-MI effect, which could restore the biochemical indicators and antioxidants parameters to the normal levels. However, further studies are needed to clarify the more specific mechanism of CDC, such as metabonomics, network pharmacology and so on.

Abbreviations

CDC	Compound Dragon's blood capsule
CDST	Compound Danshen tablet
CHD	coronary heart disease
LDH	<i>lactate dehydrogenase</i>
SOD	<i>Superoxide dismutase</i>
MDA	<i>Malondialdehyde</i>
H/R	hypoxia/reoxygenation
ECG	electrocardiogram
CK	Creatine Kinase
CK-MB	Creatine Kinase, MB Form
TNF- α	Tumor Necrosis Factor- α
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-1 β	Interleukin-1 β
CCK-8	Cell Counting Kit-8
NO	Nitric Oxide
ISO	isoprenaline hydrochloride
MI	acute myocardial infarction
AS	atherosclerosis
ROS	<i>reactive oxygen species</i>
TLR	Toll-like receptor
FBS	fetal bovine serum
DMEM	Dulbecco's Modified Eagle Medium

Declarations

Ethics approval and consent to participate

All animals were provided by the Experimental Animal Center of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences (IMPLAD, CAMS). All experimental protocols for animal were approved by the Institutional Review Boards of IMPLAD, CAMS with ethics code of SLXD-

20190710001. The study was carried out in compliance with the ARRIVE guidelines. The authors declare that all of the experiments comply with the current guidelines and legislation of P.R. China and CAMS. For consent to participate, not applicable.

Consent for publication

Not applicable for this submission.

Availability of data and materials

The data presented in this manuscript belong to Dr. Xinkai Lyu and has not been deposited in any repository yet. However, the materials are available to the researchers upon request from professor Juan Lu on reasonable request.

Competing interests

None to declare.

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Authors' contributions

YH Li, J Lu, and X Chen: design experiments scheme; MG Hu, and Juan Lu: UPLC-Q/TOF-MS; X Mi and XK Lyu: Cell experiments; XK Lyu, Y Yu, and XY Chang: rats experiments and measurement of biochemical indicators; JC Wang and SM Hu: Provision of medicines. XY Chang and XK Lyu: data processing and analysing; YH Li and J Lu: redaction and supervisor of the work.

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Not applicable.

Authors' information

Not applicable.

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Figures

Figure 1

Protocol of establishment of H/R

Figure 2

CDC fingerprint from UPLC-Q/TOF-MS. Notoginsenoside R1 (peak 1), ginsenoside Rg1 (peak 2), 7,4'-Dihydroxyflavone (peak 3), resveratrol (peak 4), ginsenoside Rb1 (peak 5), oleanolic acid (peak 6), ginsenoside Rd (peak 7), loureirin A (peak 8), loureirin B (peak 9), and pterostilbene (peak 10).

Figure 3

Effect of CDCs on the viability of H/R H9c2 cells and the levels of LDH, SOD and MDA

A. Mortality rate, B. LDH, C. SOD, D. MDA; Mean $\pm$ SD, $n=3$; * $P < 0.05$, compared with control group; ** $P < 0.01$, compared with control group; # $P < 0.05$, compared with H/R group; ## $P < 0.01$, compared with H/R group; H/R: model group; CDST: Compound Danshen tablet; CDC 25, 50 and 100: CDC 25, 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ groups

Figure 4

Effect of CDCs on mortality rate and ECG in rats with acute MI induced by ISO. A. Mortality rate, B. ECG of different groups (B-1: control, B-2: ISO, B-3: ISO+CDC), C. Changes in J point; Mean $\pm$ SD, n=6; H/R: model group; CDST: Compound Danshen tablet; ISO+L/M/H-CDC: low-/middle-/high-dose CDC groups

Figure 5

Effects of CDCs on the levels of CK, CK-MB, LDH and SOD in rats with acute MI induced by ISO; A: CK, B: CK-MB, C: LDH, D: SOD; Mean $\pm$ SD, n=6: H/R: model group; ISO+CDST: Compound Danshen tablet; ISO+L/M/H-CDC: low-/middle-/high-dose CDC groups

Figure 6

Effects of CDCs on the levels of TNF- α , IL-6, IL-10, IL-1 β and NO in rats with acute MI induced by ISO; A: TNF- α , B: IL-6, C: IL-10, D: IL-1 β , E: NO; Mean $\pm$ SD, n=6; H/R: model group; ISO+CDST: Compound Danshen tablet; ISO+L/M/H-CDC: low-/middle-/high-dose CDC groups.

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