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Cardioprotective Effects of Curcumin Co-treatment in Rats With Establishing Chronic Variable Stress Stereology Study

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Abstract

Objectives: Prolonged and recurrent exposure to chronic variable stress (CVS) may lead to cardiovascular dysfunction. It is a basic cause of heart failure. The aim of current study was to determine the effect of curcumin (CUR) on the treatment of cardiac dysfunction in rats with established CVS.

Materials and Methods: Thirty-five adult male Sprague-Dawley rats were divided into 3 groups as control, CVS, CVS+CUR (100 mg/kg/d dissolved in 0.5 mL of distilled water). All of the control animals and half of the animals in CVS and CVS+CUR groups were sacrificed after 15 days and the rest of animals were allowed to recover for 50 days. The relaxin (RLX), cortisol, adrenocorticotropic hormone (ACTH), oxytocin serum levels, and heart stereological structure were evaluated.

Results: Significant deviations from the normal range occurred in RLX, cortisol, ACTH, and oxytocin serum levels in CVS and CVS + recovery groups compared to the control rats ($P<0.01$). Furthermore, weight and heart weight, volume of the hearts and ventricles, total number of the nuclei reduced, and also volume of the connective tissue and diameter of the total vessels increased in the CVS animals in comparison to the control ones ($P<0.01$). These parameters changed to a lesser extent in CVS+CUR animals compared to the CVS rats with or without a recovery period ($P<0.01$).

Conclusions: Findings of this study suggested that CUR might have a therapeutic potential for heart structure and function following the established CVS by regulation of stress and cardiovascular-related hormones and outcomes related to the change of this hormone.

Keywords: Cortisol, RLX, Oxytocin, CVS, CUR

Introduction

Symptom heart failure (SHF) is a multifarious syndrome described as the incapability of cardiac muscle to preserve peripheral tissue blood supply. Metabolic inefficiency of the cardiac muscle and the whole body of the patients may lead to heart failure (1). Surprisingly, reduction of cardiomyocyte and substitution of collagen (connective tissue) leads to increased heart stiffness and impaired contractile function; besides, they altogether result in poor health status and quality of life (2). Exposure to chronic stress for a long term brings about changes in cardiovascular and related cardiovascular hormone function and other central and peripheral systems. Chronic stress involves sensitive organs like heat. Heart is one of the sensitive target organs to stress attack; chronic variable stress (CVS) changes psychological, behavioral, biological factor, and cardiovascular-related hormone (3). Nonetheless, little is known about the effect of stress on the heart structure and function (1), for example, resting tachycardia with reduced heart rate, increased rate of plaque in narrowing the artery, an inadequate blood supply to myocardial, heart fibrosis, coronary heart disease because of CVS disruptive (2-4). Besides, other studies have shown that dysfunction of heart is the results of functional of stress (1). Cardiovascular-related hormone regulates the heart function (5). Oxytocin and relaxin (RLX) may be protected myocardial ischemia, cardiac fibrosis by endogenous mechanisms (6,7). The RLX may be involved in endogenous mechanisms of cardiac protection against ischemic injury and fibrosis (7).

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One of the key factors of survival of cardiomyocyte is RLX (8). In addition of RLX, oxytocin is another cardiovascular hormone that shows protective anti-hypertrophic effects in rat ventricular myocytes (2) and its concentrations increases during chronic stress (9). Cardiac dysfunction is one of the most important cause of death in the world (4). For hundreds of years, curcumin (CUR), as an active turmeric fraction, has been used to reduce the symptoms of diseases in Asia (10). Most researchers have indicated that CUR is not toxic and can change biological function by anti-inflammatory, anti-oxidant, anti-carcinogenic, and anti-thrombotic effects (11-15). Heart failure and CVS have been evaluated in previous studies but their effects have not been investigated on the related hormone and the relationship between them. Therefore, the first goal of the present research was to estimate the effect of CVS on heart histology and physiology. The second aim was to initiate a protective agent, which could be easily available to the public and able to prevent the side effects of CVS on cardiovascular function. To achieve these goals, CUR was considered.

Material and Methods

Animals and Experimental Design

In this study, 35 adult male rats (Sprague-Dawley 220-250 g, 10 weeks) were selected from comparative research center of Guilan University and were kept in unbroken humidity and temperature and also on unrestricted diet. All the rats were kept in a standard position, then were divided into 3 groups: the first control group (no treatment) (n = 7); the second group (CVS), according to the previous method with some modifications (n = 14) (16,17); and the third group, CVS+CUR (CUR, Sigma-Aldrich St. Louis, MO): Curcuma longa (Turmeric) C7727) solved in distilled water (n = 14). All the administrations including CUR (100 mg/kg/d) were carried out by oral gavages (18). All of the control animals and half of the animals in CVS and CVS+CUR groups were sacrificed after 15 days and the rest were allowed to live for a 50-day recovery period free from any manipulations. After the last experimental day, their blood samples were collected through the cardiac puncture, and then they were euthanized by cervical dislocation.

CVS Model

The second and third groups were exposed to CVS for 15 days; other animals were allowed to recover for 50 days without stressor operation in their cages (17,19) (Table 1).

Table 1. The Protocol for Induction of CVS in 15 Days for Rat Model

Stressor	Days
Cold restraint (1.5 h)	1
Inclination of home cages (3 h)	2
Flashing light (1.5 h)	3
Restraint (1.5 h)	4
Isolation	5
Damp bedding (3 h)	6
Inclination of home cages (2 h)	7
No stressor applied	8
Flashing light (1.5 h)	9
Isolation	10
Water deprivation (24 h)	11
Restraint (2 h)	12
Damp bedding (2 h)	13
Cold restraint (2 h)	14
Inclination of home cages (3 h)	15

Hormonal Assay

At first, to collect serum 5 mL, blood samples were centrifuged at 3000 rpm for 15 minutes. The sera were then frozen at -20°C up to 2 weeks. The concentration levels of oxytocin (20,21), adrenocorticotropic hormone (ACTH) (22) cortisol (23) and RLX (24) were measured by radio immune assay (RIA).

Stereological Method

Following the last day of treatment, dissection of heart weight and primary volume were measured by the immersion method (25). For estimation of Cavalieri's principle, the degree of shrinkage was calculated. The area of circular piece was measured again and the degree of shrinkage "d(shr)" was computed as follows: 1- (AA/AB)1.5 (Figure 1) (26). Then, their hearts were randomly sliced by the orientation method (Figure 1). Finally, nine–twelve pieces were collected from a heart. After embedding the slabs in paraffin and sectioning (3 and 25 μm thick), they were stained with haematoxylin & eosin (H&E) and Heidenhain's Azan for stereology study (27-33). The heart volume (right [Rt] and left [Lt] ventricles) was measured by V=V(primary)×(1−d[shr]) (Figure 1) (28-32).

Vessels and Connective Tissue Volume Estimation

Moreover, after estimating the heart volume,, the researchers used the point-counting method to measure the volume density of the vessels and connective tissue (26-32).

Numerical Density Estimation of Cardiomyocyte Nuclei

The researchers used objective lens (100 oil immersion) at magnification of 1300x and also a microcator. Using the optical disector method to estimate the numerical density and NV of the cardiomyocyte nuclei were, an unbiased counting was used on the 25 μm thick sections (Figure 1) (33,34).

Estimation of the Cell Number

The density of numerical cardiomyocyte nuclei of heart, nuclei/heart = (total number of the nuclei -/[total number of points. area of the counting frame. height of

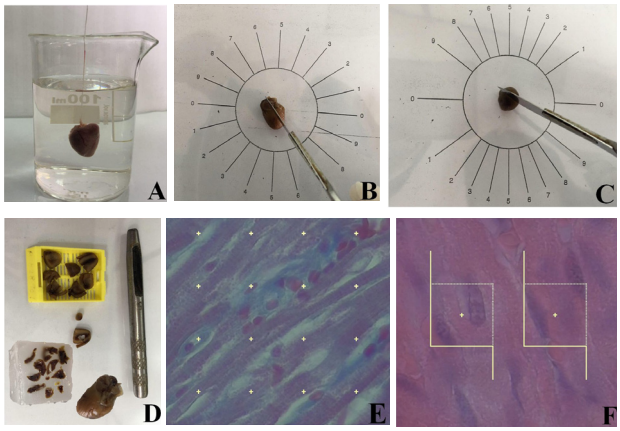

Figure 1. Application of Stereological Techniques: **A**. Immersion method; **B**. Obtaining isotropic uniform random sections by placing the heart on the equally divided circle; **C**. Placing the two halves of the heart on the cosine-weighted divided circle and slicing each half; **D**. Punching out of a circle through a random slice, embedding, and tissue slide preparation; **E**. The point-counting method to obtain the volume density of the components; **F**. Optical sections of the heart to estimate the numerical density of the cells.

the dissector] (Figure 1) (12,34).

Statistical Analysis

The data of this study were reported as the mean ± standard deviation (SD). For hormone analysis, one-way ANOVA and for stereology study, Mann-Whitney and Kruskal-Wallis were applied. Meanwhile, the *P* value <0.05 was an indicator of a statistical difference.

Results

Sample Collection and Biomarker Measurement

The sera ACTH, cortisol, and oxytocin concentration in the CVS and CVS + recovery animals showed an increase compared to the corresponding control animal, respectively ($P<0.01$). These parameters diminished to a lesser extent in the CVS+CUR treated animals compared to the CVS rats with or without the recovery period ($P<0.01$) (Table 2).

Moreover, RLX serum concentration decreased in the CVS and CVS + recovery animals compared to the controls ($P<0.01$). The RLX serum concentration also decreased, but to the lowest rate, in CVS+CUR animals in comparison to the rats that received CVS with or without the recovery period ($P<0.01$) (Table 2).

Stereology Measurements

Statistically significant differences were observed in different parameters of stereology analysis.

Animals' body and heart weight and also total number of cardiomyocytes nuclei significantly decreased in CVS animals in comparison to those of the control rats with or without recovery period ($P<0.01$). These changes were found to be in a lesser extent in the CVS+CUR rats, with or without recovery period, compared to control animals ($P<0.01$) (Figures 2-4).

Another finding of the study showed that the volume of each heart (Rt & Lt ventricle), connective tissue, and the percent of capillary increased in CVS rats in comparison to those of the control rats with or without recovery period ($P<0.01$). Additionally, these parameters changed to a lesser extent in CVS+CUR as compared to the control rats with or without recovery period ($P<0.01$) (Figures 2-4).

Discussion

The findings of the present study indicated that CVS, with or without the recovery period, could change the structure and function of the rats' heart. The finding also showed an important key factor of RLX and oxytocin in creation of autonomic responses to CVS. Significantly, the interference and interaction of these factors were required for proper cardiovascular responses to CVS. Moreover, HPA (hypothalamic-pituitary-adrenal) axis activation was also needed for development of ACTH and cortisol responses (10). The main findings of the present study in CVS were as follows:

1. The animals' heart weight and the number of cardiomayocyte cells decreased.
2. The heart volume (Lt & Rt ventricles), the percentage of vessel profiles and connective tissue in CVS rats were increased in comparison to the control group without or with the recovery period.

The above-mentioned results revealed that variable stressors increased oxytocin concentration and that chronic stress elevated oxytocin that may led to the activation of HPA, increasing of ACTH, and

Table 2. Mean ± Standard Deviation of the Serum, ACTH (ng/mL), Cortisol (ng/mL), RLX (ng/mL), and Oxytocin (ng/mL) in Control, Stress and CVS Groups, With or Without Subjection to 50 Days of Recovery Period (n = 7)

Groups	ACTH	Cortisol	RLX	Oxytocin
Control	45.4±7.02	26.34±1.2	36.4±6.7	11.2±1.07
CVS	96.3±3.5[a]	71.24±4.12[a]	16.3±3.7[a]	35.4±4.2*
CVS + CUR	78.10±3.47[a]	41.72±3.06[a]	34.4±4.4[a]	23.8±2.8*
CVS (Recovery)	84.08±6.01[b]	58.54±5.4[b]	21.2±2.6[b]	31.06±3.6**
CVS + CUR (Recovery)	55.18±5.61[b]	32.3±3.39[b]	35.49±5.03[b]	13.4±6.3**

[a] $P < 0.01$ (Control vs. CVS) or (CVS+ CUR vs. CVS) without recovery period (n=7).
[b] $P<0.01$ (Control vs. CVS) or (CVS + CUR vs. CVS) with 50 days of recovery (n=7).

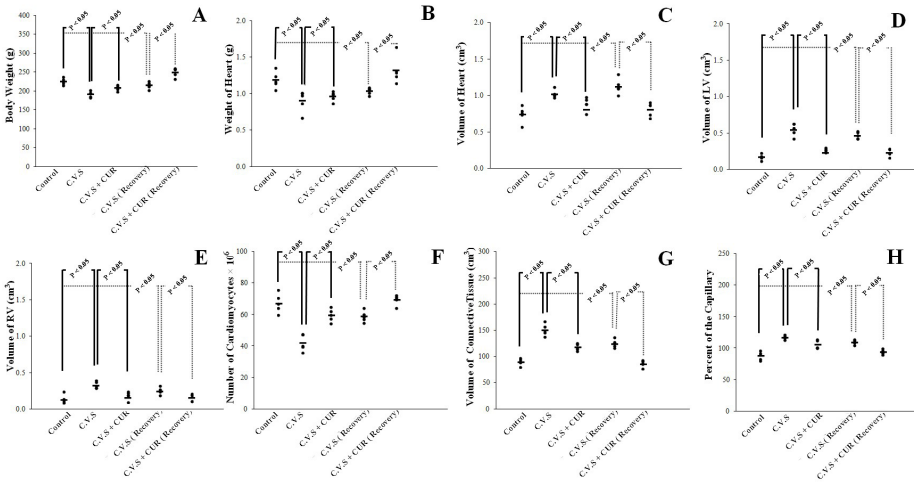

Figure 2. The Scatter Plots of: **A.** Body weight; **B.** Heart weight; **C.** Heart volume; **C.** Volume of left ventricle; **D.** Volume of right ventricle; **E.** Volume of connective tissue; **F.** Number of cardiomyocyte; and **G.** Percentage of capillary after 15 days of exposure to CVS and recovery for 50 days in control, CVS, CVS+CUR, CVS (Recovery) and CVS+CUR (Recovery) rats. Note: Each dot represents an animal and the horizontal bar is the mean number of the animals in each group.

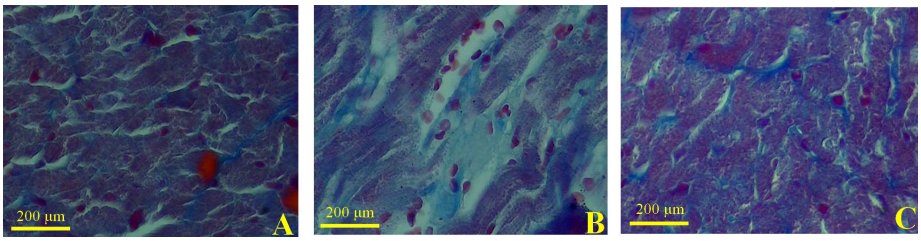

Figure 3. Comparison of Changes in Connective Tissue and Atrophic Changes of the Cardiomyocyte in Comparison to the Control Group and Histology Scattering Cells After 15 Days of Exposure to CVS in Cntrol, CVS, CVS+CUR Rats.

corticosteroid concentration (10). In addition, another researcher showed the effect of anti-inflammatory and cardio protective of oxytocin in the heart of humans and rats (7,34,35). Skopek et al, in rats' heart exposed to acute stressor, showed mRNA expression of oxytocin receptor by real-time quantitative polymerase chain reaction (PCR) (6). Experimental data demonstrated that RLX had anti-apoptotic as well as anti-hypertrophic effects on rodent cardiomyocytes and led to their viability and survival in heart failure. Furthermore, it had anti-fibrotic actions in the heart (9). Boccalini et al reported the protection and anti-apoptotic effect of RLX in oxidative stress model (36). Importantly, much evidence suggests that an important reason for losing the cardiomyocyte cells is the apoptosis (1). In another study, it was found that exposure to forced swim stressor for 10 minutes (every day) led to an increase in RLX concentration (7,37). Marin et al also reported that elevation of the extent of corticosterone level was dependent on environment and type of stressors (38). In addition, Jankord et al reported that chronic stress significantly decreased oxytocin expression in late adolescent animals (39); perhaps the release of RLX was age-related, as oxytocin and RLX secretion might be dependent on each other so that after condition adjustment by initial oxytocin secretion, RLX release reduced. Decreased cardiomyocytes' nuclei may be due to reduced RLX. The present investigation showed elevation of heart volume (Rt & Lt ventricle) and the volume of connective tissue; this might be a result of the reduction in cardiomyocyte and that replacement of collagen (2,40) increased and occupied the area between the myocyte and created fibrosis that was initially a compensatory mechanism and might change stiffness, ventricular function, and ventricular modeling (41). Then, cardiac fibrosis induced significant left ventricular hypertrophy

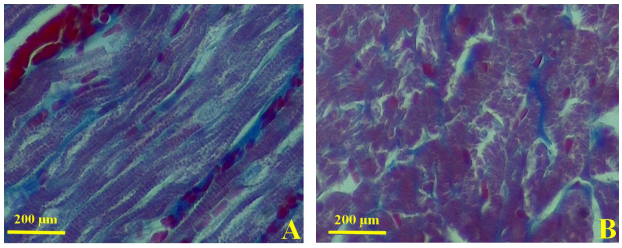

Figure 4. Comparison of Changes in Connective Tissue and Atrophic Changes of the Cardiomyocyte in Comparison to the Control Group and Histology Scattering Cells at Exposure to CVS (Recovery) and CVS + CUR (Recovery).

(42). Therefore, the present study demonstrated that animals which were exposed to new stresses could be prone to heart damage. As it is known, fibrosis has the significant role in impaired cardiac contractility and heart failure (2). Elevation of oxygen demand and spasticity of coronary vessels occur as a results of chronic stress (42). Increased vessels observed in this investigation may be used to correct and repair damaged cardiomyocyte. Fukuda et al reported similar results as well. They demonstrated that ischemic stressor induced angiogenic signaling in rats (43). The CUR has been used to reduce the symptoms of a variety of diseases (44,45). The results of the current study suggested that CUR moderated changes of ACTH, cortisol, and oxytocin concentration and also the structure and function of cardiovascular system against CVS. The CUR decreased the process of reducing cardiomyocyte in group treatment, that this justified, and increased the weight that was observed in CVS+ CUR group. It was found that oxytocin concentrations decreased in response to CUR. Moreover, an increase was observed in maximal corticosterone responses to ACTH associated with the increase in oxytocin. This is in accordance with the previous result from Ondrejcakova et al (9). In addition, Yau and Potenza (46) reported that frequent or prolonged activation of ACTH and cortisol could change the functional tone of these systems. As a result, decreased oxytocin by CUR would somehow modify this system. However, further studies are suggested to be conducted in this regard to regulate the underlying molecular bases of these results.

Conclusions

Exposure to 15 days of CVS led to cardiac dysfunction and these changes were not fully recovered even after 50 days. The results of this study showed that CUR could moderate changes of cardiovascular structure and function in animals' exposure to CVS.

Conflict of Interests

None to be declared.

Ethical Issues

The investigation procedures were executed according to the Guilan University of Medical Sciences and ethics guidelines of the committee (ethical No. IR.GUMS.REC.1395.235).

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