



ORIGINAL ARTICLE

Lactobacillus paracasei Impact on Myocardial Hypertrophy in Rats with Heart Failure

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ABSTRACT: Cardiac hypertrophy and cardiac dysfunction are important complications of heart failure. Cardiovascular, immunological, and hormonal players are involved in the pathogenesis of heart failure. Current evidence suggests that probiotics may have fruitful effects on the heart function. This was our aim. To this end, effects of oral administration of *Lactobacillus paracasei* subsp. *paracasei* 8700:2 on isoproterenol-induced heart failure were investigated. Forty male Wistar rats weighing 200 g were randomly assigned to five groups; the control group (saline-treated group), probiotic-treated group, heart failure group (isoproterenol-introduced group), pretreatment group (treating them by probiotic for 20 days then induced heart failure) and treatment group (following heart failure-induced, treating them by probiotic for 20 days). The groups were studied for 30 days. Serum levels of atrial natriuretic peptide (ANP) and chemerin were measured by ELISA. Finally, the hearts were removed for histopathological evaluation. Compared to the control group, isoproterenol caused cardiac hypertrophy and increased ANP ($P < 0.05$) and chemerin levels. Treatment with *Lactobacillus paracasei* significantly reduced the levels of ANP ($P < 0.01$) and decreased the pathological damages to the myocardium. It caused a small reduction in chemerin level, as well. Pretreatment with probiotics had no positive effects on cardiac hypertrophy and related parameters. Our findings indicate that treatment with *Lactobacillus paracasei* subsp. *paracasei* 8700:2 reduces cardiac hypertrophy in rats. In addition, this probiotic reduces the serum levels of chemerin and ANP.

INTRODUCTION

Heart failure (HF) is a complicated syndrome and may be considered as an outcome of several manifestations of cardiovascular disease (CVD). In spite of advances in

clinical practice, HF still has a poor prognosis with high rate of mortality and morbidity [1-5]. CVD is accompanied by a myriad of symptoms and dysfunctions, from

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hypertension to cardiomyopathy. Myocardial infarction (MI) or heart attack, coronary heart disease, hypertension, and chronic inflammations are all examples of confounding factors instigating and perpetuating HF. Immune cells, cytokines, and compensatory mechanisms are involved in pathogenesis of HF [6-10].

Findings from several lines of studies have provided evidence for beneficial effects of probiotics in various conditions including heart diseases. Probiotics are defined as live microorganisms that can promote health status of the host [11]. Probiotics can alter the inflammation responses in different conditions. *Lactobacillus paracasei* subsp. *paracasei* 8700:2 is a probiotic isolated from human feces and healthy human gastrointestinal mucosa. *Salmonella enterica* and *Helicobacter pylori*, two pathogens commonly traced in the gastrointestinal tract are inhibited by *Lactobacillus paracasei*. This probiotic could break oligofructose and inulin, and produce lactic acid as the end product [12, 13].

A hormone-mediated recovery process takes place to compensate the mechanical function of the heart. These hormones are often considered as biomarkers of HF and represent the compensatory responses [14, 15]. Atrial natriuretic peptide (ANP) is a hormone secreted primarily by the atria with important diuretic, and vasodilator effects [16, 17]. Expression of ANP may be induced in conditions like hypertension, wall stress and ventricular load, and increased ventricular mass [17]. ANP production is strongly correlated with cardiomyocyte size, where a higher rate of ANP expression is found on cells with large diameters [16-19]. ANP reduces cardiac load and wall stress through its anti-hypertensive properties. Accordingly, serum levels of ANP are linked with cardiac hypertrophy [18-20].

Chemerin is an adipokine mainly secreted by adipose tissue and the liver. It has an important role in energy and

metabolism. Evidence shows that in addition to some inflammatory diseases, high levels of chemerin are usually associated with an increased risk of coronary heart disease (CHD) [21-23]. Current findings show that coronary and aortic atherosclerosis lesions are positively associated with local chemerin expression in pericoronary and periaortic adipose tissue, suggesting a predictor role for chemerin in CHD [22-26]. Limited studies have been done to explore the effects of probiotic on cardiac hypertrophy. The present study was aimed at evaluating effects of *L. paracasei* on HF in rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (n=40, 185 ± 15 g), were purchased from Baqiyatallah University of Medical Sciences. They were kept (temperature of 23.5 ± 1.5 °c and 12 h light/ 12 h dark cycle) in the animal house of Semnan University of Medical Sciences, with free access to water and standard food. This study was performed with the approval of Ethical Committee of Semnan University of Medical Sciences.

Chemical reagents

Lactobacillus paracasei subsp. *paracasei* 8700:2 was presented by Takgene Zist Company (Tehran, Iran). Isoproterenol was purchased from Sigma Aldrich (St. Louis, MO, USA; CAS No. 51-30-9).

Induction of heart failure

Heart failure was induced by isoproterenol dissolved in normal saline and injected subcutaneously to study animals (5 mg/kg) daily for 10 consecutive days [27-29].

Table 1. Study groups.

Groups	1-10	Days (11-20)	21-30
Normal Control	#	##	##
Probiotic control	*		
Isoproterenol control	**		
Pretreatment	*	*	**
Treatment	**	*	*

Normal saline (5 mg/kg/day, subcutaneous), ## Normal saline (0.25 mg/kg/day, oral),

* Probiotic (0.25 mg/kg/day, oral),** Isoproterenol (5 mg/kg/day, subcutaneous)

Study protocol

As shown in Table 1, rats were randomly divided into five groups (n=8). After 30 days, all animals were euthanized by ether; and blood samples were collected by jugular vein puncture. *L. paracasei* subsp. *paracassie* 8700:2 (10^9 CFU/g) was dissolved in normal saline and were given orally (0.25 mg/kg) by gavage at 24-hour intervals for 20 days. The blood samples were placed at ambient temperature for a while, and then centrifuged at 4000 g for 10 min at 25 °c and sera were collected. For histopathological evaluation, the sections of cardiac apex were fixed in 10% (w/w) neutral buffered formalin. The standard histological techniques (H & E staining) were done for all groups. The histopathological changes were graded as follows; 1, 2, 3, and 4 for low, moderate, high, and intensive pathological changes, respectively. To evaluate the degree of heart hypertrophy and congestion, the wet to body weight ratios and the wet to dry weight ratios of the cardiac tissues were studied. Cardiac hypertrophy was measured through comparing the LV weight (LVW) to total body weight (BW) for each animal. A significant increase in the LVW/BW ratio indicated left ventricular hypertrophy.

Measurement of ANP and chemerin

Serum levels of ANP and chemerin were measured by ELISA according to manufacturers' instructions; ANP (Abcam, Cambridge, UK), and chemerin (Hangzhou East Biopharma, Torrance, California, USA).

Statistical analysis

Data were shown as mean \pm SEM. The analyses were conducted by one way ANOVA, followed by the Tukey-Kramer multiple comparison test. Data analyses were performed with SPSS software version 19 (USA). $P < 0.05$ was considered to denote significant differences.

RESULTS

Effect of treatments on the body and tissue weights

As shown in Table 2, weight gain had a significant rise in the treatment group compared to isoproterenol group ($P < 0.01$). The left ventricle to heart weight ratio, left ventricle wet to dry weight ratio, and heart weight to body weight ratio were higher in isoproterenol group. Probiotic therapy caused a small and significant reduction in left ventricle to heart weight and left ventricle wet to dry weight ratios ($P < 0.05$).

Table 2. Weight gain in different study groups.

Groups	Baseline BW (g)	Δ BW (g)	HW to BW (g/kg)	LV wet to dry weight (g/g)	LV to HW (g/g)
Control	294 \pm 12	23 \pm 2	3.25 \pm 0.28	4.29 \pm 0.03	0.65 \pm 0.03
Probiotic	300 \pm 8	24 \pm 1.4	3.19 \pm 0.25	4.33 \pm 0.04	0.68 \pm 0.03
Isoproterenol	286 \pm 13	12 \pm 0.9 ^a	4.87 \pm 0.33	4.83 \pm 0.1	0.65 \pm 0.02
Pretreatment	278 \pm 7	13 \pm 1.1	4.26 \pm 0.12	4.41 \pm 0.5	0.59 \pm 0.04
Treatment	285 \pm 11	21 \pm 1.7 ^b	3.48 \pm 0.4 ^b	4.09 \pm 0.01 ^b	0.66 \pm 0.04

Data are presented as mean \pm SE. *n*=5. BW: body weight. HW: Heart weight; LV: Left ventricular.

^a P < 0.01 vs control group, ^b P < 0.05 vs isoproterenol group

Serum levels of chemerin and ANP

As shown in Figure 1A, isoproterenol administration reduced the serum levels of chemerin. Pretreatment and treatment with probiotics could raise the levels of chemerin; however, the differences were not significant compared to isoproterenol group. As presented in Figure 1B, isoproterenol administration significantly increased the

serum levels of ANP compared to control group (P < 0.05, Figure 1). Both treatment and pretreatment with probiotics groups could reduce ANP levels; however, the changes were significant in treatment group compared to isoproterenol group (P < 0.01).

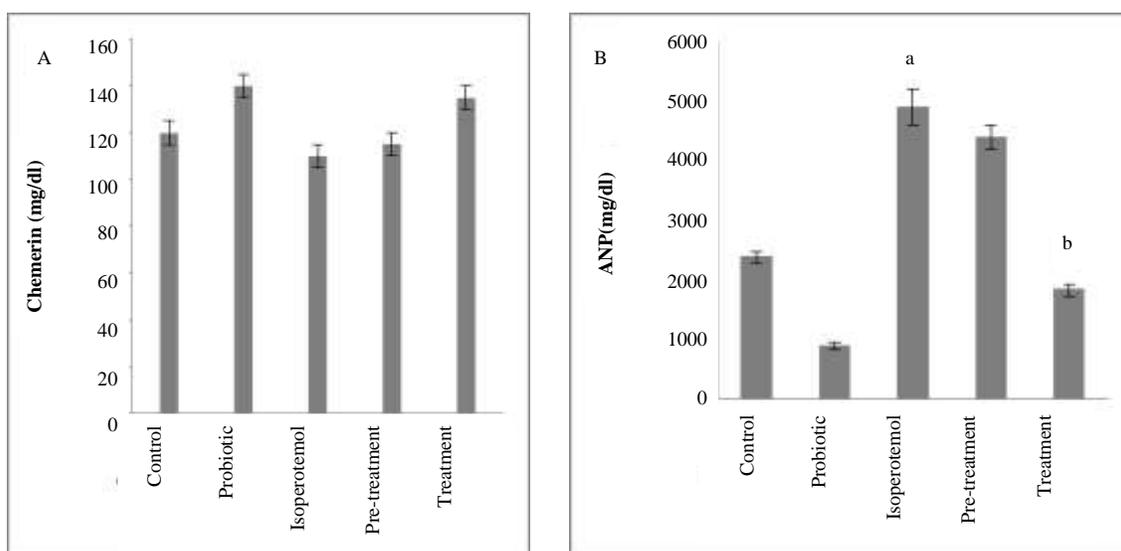


Figure 1. A: Serum levels of chemerin (mg/dl) in different study groups. B: Serum levels of ANP (mg/dl) in different study groups. ^a P < 0.05 vs. control group, ^b P < 0.01 vs. isoproterenol group.

Histopathological examination

As shown in Figure 2, there was no level of heart degeneration in control group and myocardial tissues were normally arranged. Necrosis, hypertrophy, dilation of capillaries, and infiltration of the inflammatory cells were observed in isoproterenol group. According to the grading

of histopathological changes, infiltration of the inflammatory cells, fibrosis, and necrosis were significantly improved in probiotic treatment group ($P < 0.05$). Compared to isoproterenol group, lower grade of myocardial changes were seen in pretreatment group.

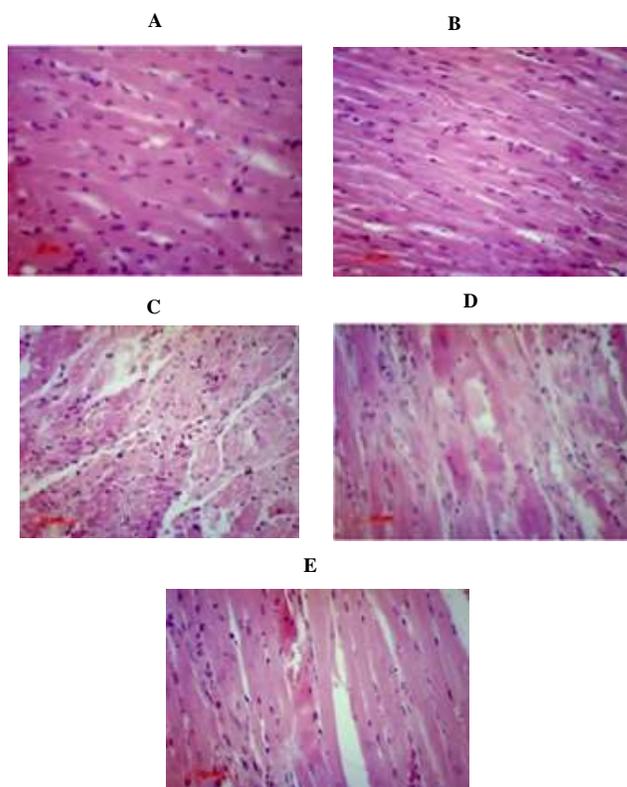


Figure 2. Photomicrographs of rat cardiac apexes sections with H & E (40X); A: Control; B: Probiotic; C: Isoproterenol; D: Pretreatment; E: Treatment. Heart tissue of isoproterenol control showed a marked cardiomyocyte necrosis, hyperemia, and focal mononuclear cell infiltration. Treatment group showed a significant improvement but pretreatment with probiotic group revealed an intensification of low grade myocardial damages.

DISCUSSION

In this report, we showed the beneficial effects of *L. paracasei* on isoproterenol-induced heart failure in rats. In the present study, administration of *Lactobacillus paracasei* subsp. *paracasei* 8700:2 improved myocardial necrosis and massive fibrosis. In addition, it could increase the levels of ANP which its reduction is usually associated with exacerbation of heart failure. Paradoxically, probiotic administration caused a small increase in chemerin level compared to isoproterenol group. It can be partially explained by time manner of damages and administered agents. Continuous treatment with probiotics in a larger time frame might lead to a reduction in chimera level. Atrial

natriuretic peptide (ANP) is released from myocardial cells in the atria and the ventricles in response to volume expansion and it is likely to increase wall stress [13]. Plasma concentration of ANP increases in patients with asymptomatic and symptomatic left ventricular dysfunction. ANP promotes diuresis, natriuresis, and vasodilation in early chronic heart failure (CHF). As CHF progresses, beneficial effects of ANP and other natriuretic peptides are diminished.

Interestingly, pretreatment with *L. paracasei* initiated the myocardial damages. Therefore, the positive effects of *L. paracasei* were achievable only in treatment group.

Helpful effects of probiotics on heart failure were first reported by Gan et al. [24]. They showed that *Lactobacillus rhamnosus* GR-1 administration could improve heart failure in rats with an increase in ANP gene expression and improvement of hemodynamics. Compared to our study, they used a different method for induction of heart failure and did not investigate the pretreatment effects of the probiotic. It is worth noting that we studied another strain of *Lactobacillus* and did not investigate the hemodynamic parameters. Duration of treatments was not similar in these two works, as well. In addition, another study showed that probiotic-fermented purple sweet potato yogurt could reduce cardiomyocyte apoptosis and improve myocardial remodeling in rats with hypertension [28].

The first pilot randomized clinical trial on heart failure and probiotics was conducted by Costanza et al. [29]. This trial showed that 3-month daily add-on therapy with *Saccharomyces boulardii* could improve ejection fraction and reduce left atrial diameter in patients with CHF.

Mechanism of action of probiotics has been widely investigated over recent years and has been a subject of debate. Evidence suggests that probiotics have anti-oxidant and anti-inflammatory properties. Some studies have shown that *L.casei*, *L. acidophilus*, and *Bifidobacterium lactis* are associated with an increase in the synthesis of anti-oxidant compounds [30]. Some others have shown that anti-inflammatory effects of probiotics are attributable to the inhibition of inflammatory cytokines [31]. Another study has proposed that the mechanism of action might be an interaction between probiotics and toll-like receptors (TLRs), particularly TLR2 and TLR4, which are well understood players in the activation of immune cells [32]. TLRs are involved in many inflammatory situations including heart diseases [33]. A recent work by Sadeghzadeh has shown the positive results of pretreatment effects of probiotics on acute myocardial infarction [34]. In contrast, we showed that pretreatment could exacerbate damages with a small increase in ANP levels. It could be partially explained by induction methods of heart failure and MI. Such differences in methods may lead to damages that may be accelerated by pretreatment of investigational drugs.

Study limitations

This study has several limitations that should be acknowledged. We could not detect hemodynamic parameters in our study groups. In addition, we could not compare the effects of probiotics on immunosuppressive agents.

CONCLUSIONS

Our study shows that *Lactobacillus paracasei* subsp. *paracasei* 8700:2 improves cardiac hypertrophy and some complications of heart failure.

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Conflict of interests

The authors declare no conflict of interests.

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