

*Full Length Research Paper*

# Effect of jujube extract on oxidative injury in heart muscles of exhausted training rats

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**Jujube is an important plant in traditional Chinese medicine and is recommended for the treatment of some diseases such as tumors and cardiovascular disease related to the production of radical species resulting from oxidative stress. The objective of this study was to investigate effect of jujube extract on oxidative injury in heart muscles of exhausted training rats. Four groups of animals were studied: (a) sedentary control (n = 8); (b) vehicle-treated control (n = 8); (c) low dose of jujube extract-treated rats (n = 8); (d) high dose of jujube extract-treated rats (n = 8). Jujube extract-treated rats were orally given 100 or 300 mg/kg body weight jujube extract for 30 days, respectively. Then, all the rats (except for sedentary rats) were submitted twice to 15-min swimming bouts on two different days. Results showed that jujube extract could reduce heart lipid peroxidation level and Bax expression, increase heart antioxidant enzymes activities and Bcl-2 expression, and improve heart function.**

**Key words:** Exhausted training, rat, jujube, oxidative.

## INTRODUCTION

Chinese jujube has been commonly used as a traditional Chinese medicine and as food for thousands of years. It is mainly distributed in the subtropical regions of Asia. The Chinese share of world jujube production is about 90%. Jujube is an important plant in traditional Chinese medicine and is recommended for the treatment of some diseases such as tumors and cardiovascular disease related to the production of radical species resulting from oxidative stress. The peels and pulps of jujube are commonly used as foods, food additives and flavorings as a supplement for promoting health (Li et al., 2007). The seeds of jujube are used in traditional Chinese medicine for their sedative and hypnotic effects (Jiang et al., 2007). Therefore, the study of the antioxidant activity of different tissue types of jujube may explain some of the empirical uses in folk medicine.

Exhausting (Khanna et al., 1999; Gul et al., 2003) or moderate (Alessio, 1993; Gul et al., 2001) exercise in rats may increase ROS production exceeding the

antioxidant defences. Oxidative stress is the imbalance capacity of of pro- and anti-oxidants in favor of the former. Exercise-induced oxidative stress was also reported in thoroughbred racehorses after a 1000 m race at maximum velocity (White et al., 2001). Increased oxidative stress can be harmful to all cellular macromolecules such as lipids, proteins and DNA (Halliwell and Gutteridge, 1984).

In this study, we investigated the effect of jujube extract on oxidative injury in heart muscles of exhausted training rats.

## MATERIALS AND METHODS

Thirty-two male Wistar rats (2 months old) were used in this study. Four groups of animals were studied: (a) sedentary control (n = 8); (b) vehicle-treated control (n = 8); (c) low dose of jujube extract-treated rats (n = 8); (d) high dose of jujube extract-treated rats (n = 8). Jujube extract-treated rats were orally given 100 or 300 mg/kg body weight jujube extract for 30 days, respectively. Then, all the rats (except for sedentary rats) were submitted twice to 15-min swimming bouts on two different days.

The heart muscles were quickly excised after death of the animals by decapitation with both rest and exhausted rats. These tissues were immediately introduced into liquid N<sub>2</sub>, and maintained

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at  $-80^{\circ}\text{C}$  until determination.

### Biochemical analysis

The tissues malondialdehyde (MDA) concentration was determined using the method described by Jain et al. (1989), based on TBA reactivity. Briefly, 0.2 ml supernatant obtained from tissues, 0.8 ml phosphate buffer (pH 7.4), 0.025 ml BHT and 0.5 ml 30% TCA were added to the tubes and mixed. After 2 h incubation at  $-20^{\circ}\text{C}$ , the mixture was centrifuged ( $4000 \times g$ ) for 15 min. After this, 1 ml supernatant was taken and added to each tube, and then 0.075 ml of 0.1 M EDTA and 0.25 ml of 1% TBA were added. These tubes with Teflon-lined screw caps were incubated at  $90^{\circ}\text{C}$  in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 for tissues MDA concentration.

GSH was determined by its reaction with 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically (Sedlak and Lindsey, 1968). Total superoxide dismutase (SOD) was assayed by monitoring the rate of inhibition of reduction of nitroblue tetrazolium (NBT) by the enzyme (Asada et al., 1974). One unit of the SOD represents the amount of enzyme required to produce 50% inhibition of NBT reduction per minute. Catalase (CAT) activity was assayed by monitoring the disappearance of  $\text{H}_2\text{O}_2$  at 240 nm (Aebi, 1984). One unit of CAT represents the decrease of 1  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  per minute. Activity of glutathione peroxidase (GSH-Px) was determined according to the method of Lawrence and Burk (1976). GSH-Px activity for tissues was expressed as  $\mu\text{moles}$  of NADPH oxidized to  $\text{NADP}^+$   $\text{min}^{-1} \text{mg}^{-1}$  protein. BP value was measured according to the literature (Fritz and Rinaldi, 2008). Cardiac output value was measured according to the literature (Champion et al, 1997).

The left ventricular systolic pressure (LVSP) of the rat heart was measured using a pressure transducer.

### Flow cytometric evaluation of Bcl-2 and Bax levels

The levels of Bcl-2 and Bax were measured by flow cytometry as described previously (Antonella et al., 1992; Liu and Zhu, 1999), with minor modifications. Briefly, thymocytes were cultivated in CM/10% FCS without or with different concentrations of MSG (ranging from 1 to 100 mM) for 24 h. Thereafter, the cells were collected, washed twice with PBS containing 5% FCS. Permeabilization of thymocytes was done using saponin-based permeabilization reagent IntraPrep™ (Immunotech, Marseille, France), according to the manufacturer instructions. Cells were incubated in the darkness for 45 min at room temperature with anti-rat Bcl-2 monoclonal antibody (final concentration, 2  $\mu\text{g}/\text{ml}$ ) and anti-rat Bax monoclonal antibody (final concentration, 10  $\mu\text{g}/\text{ml}$ ). After incubation, cells were washed twice in PBS containing 5% FCS and incubated in the darkness, at room temperature, for 30 min with PEconjugated anti-mouse IgG monoclonal antibody (diluted 1:100). Non-specific binding was detected by the control cells which were incubated with the secondary antibody (PE-conjugated anti-mouse IgG) alone. Labeled cells were fixed in 4% formalin and analyzed (5000 analyzed cells/per sample) on a flow cytometer.

### Statistical analysis

Results were expressed as mean  $\pm$  SD. Means of two groups were compared with the Student's t test, after testing normality and equality of variances with an F-test. The one way-ANOVA test was used when more than two groups were compared, followed by the Newman-Keuls test for multiple comparison. In all cases, values of

**Table 1.** Effect of jujube extract on MDA and GSH levels in heart muscles of exhausted training rats.

Group	MDA	GSH (mol/mg protein)
I	11.63 $\pm$ 1.05	34.17 $\pm$ 1.63
II	18.32 $\pm$ 1.43	20.51 $\pm$ 1.69
III	16.39 $\pm$ 1.29	27.39 $\pm$ 1.83
IV	13.27 $\pm$ 1.33	32.26 $\pm$ 2.07

$p < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

During the past decade, the reactive oxygen species (ROS) generation and oxidative stress have been implicated in the development of many diverse diseases including hypertension, cardiac dysrhythmia and myocardial damage (Sato and Nishida, 2004), all of which are present in scorpion envenomation (Ismail, 1995).

Exhausted exercise resulted in elevation of myocardium MDA by about 1.58 folds of exhausted exercise model control (Table 1). Parallel to the elevation in MDA, a concomitant depletion was observed in the myocardium GSH levels (Table 1). The pretreatment of rats with jujube extract (100 and 300 mg/kg body wt.) for 10 days, dose dependently, restored MDA levels and GSH contents which is comparable to the exhausted exercise model controls (Table 1).

To protect themselves against the adverse effects of the ROS, these cells present a complex machinery of antioxidant compounds and enzymes, such as SOD, CAT and glutathione peroxidase (GSH-Px) (Harris, 1992). The activities of these enzymes have been shown to be regulated by nutrients (Miyasaka et al, 1998; Harris, 1992) and hormones (Pereira et al., 1998). There is substantial evidence that estrogen presents antioxidant properties (Gomez-Zubeldia et al., 2000; Lacava and Luna, 1994). The antioxidant effect of estrogen has been regarded as the main mechanism for this hormone to protect skeletal and cardiac muscles (Persky et al., 2000), uterus (Diaz-Flores et al, 1999) and liver (Huh et al., 1994) from damage.

Myocardium SOD, CAT and GSH-Px activities were significantly decreased in exhausted exercise model rats. Pretreatment with jujube extract (100 and 300 mg/kg body wt.) for 10 days afforded a significant and dose dependent protection against exhausted exercise induced decrease in activities of all the antioxidant enzymes studied (Table 2). At the lower dose (100 mg/kg body wt.), the recovery in enzyme activities ranged from 12 (for SOD), 34 (for GSH-Px) to 21% (for CAT) (Table 2), while with higher dose of extract (300 mg/kg body wt.), the recovery of enzyme activities ranged from 40 (for SOD), 74 (for CAT) to 102% (for GSH-Px) of the

**Table 2.** Effect of jujube extract on SOD, CAT and GSH-Px activities in heart muscles of exhausted training rats.

Group	SOD	CAT	GSH-Px
I	231.62±16.35	42.18±2.05	28.19±2.05
II	163.92±15.28	23.18±1.95	11.83±1.14
III	189.41±12.95	37.93±1.57	17.38±1.63
IV	228.64±23.81	40.05±2.83	24.09±1.73

**Table 3.** Effect of jujube extract on BP, cardiac output and LVSP in heart muscles of exhausted training rats.

Group	BP (kPa)	Cardiac output (ml·min <sup>-1</sup> ·kg <sup>-1</sup> )	LVSP (kPa)
I	18.32±1.32	431.51±28.46	21.53±1.74
II	18.27±1.28	401.21±31.83**	18.59±1.53*
III	18.33±1.62	422.17±33.09 <sup>§</sup>	20.14±1.77
IV	18.97±1.25	430.51±37.11 <sup>§§</sup>	21.42±1.49 <sup>§</sup>

**Table 4.** Effect of jujube extract on Bax and Bcl-2 expression levels in heart muscles of exhausted training rats.

Group	Bax	Bcl-2
I	78.34±3.28	67.24±3.61
II	95.53±4.29	89.37±4.29
III	93.61±5.18	91.43±4.77
IV	86.39±5.56 <sup>§</sup>	91.27±5.82

exhausted exercise control value (Table 2).

On the basis of the results obtained from the heart function test, the BP, cardiac output and LVSP values in exhausted exercise model rats were reduced. The exhausted exercise rats pre-treated with jujube extract (100 and 300 mg/kg body wt.) for 10 days were rapidly increased in a dose-dependent manner (Table 3).

The common view on how cardiomyocytes die during or after myocardial infarction has altered in recent years. For a long time, necrosis was regarded as the sole cause of cell death in myocardial infarction. Now, recent studies indicate that apoptosis also play a role in the process of tissue damage subsequent to myocardial infarction (Kajstura et al., 1996; Saraste et al., 1997; Zhu et al., 2001; Yin et al., 2003).

Bax is a member of the Bcl-2 family and, when over-expressed, it accelerates cell apoptosis by competing with Bcl-2 (Misao et al., 1996; Tsujimoto, 1998). Bcl-2 is the most important gene that inhibits apoptosis. It can inhibit cardiomyocyte apoptosis caused by both oxygen free radicals and P53.

To further elucidate the biochemical mechanism of the inhibition of jujube extract against myocardial tissue injury, its effects on the expression of Bax and Bcl-2 were determined. The treatment of jujube extract resulted in a

significant reduction of Bax expression levels in rats' myocardium tissue (Table 4). Bcl-2 expression level was unchanged.

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