

Full Length Research Paper

# ***Morinda officinalis* How enhances exercise endurance and possesses protective effects against oxidative stress of the rats after exercise**

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The aim of this study was to investigate the effect of *Morinda officinalis* How (RMO) on exercise endurance capacity and exercise-related changes in lipid peroxidation. Thirty-two male Wistar rats were taken in the study, and the animals were divided into four groups (n = 8 per group) including one control group and three RMO administered groups (200, 400 and 800 mg/kg body weight). The RMO extracts were administered every day orally using intragastric tube for 30 days. Forced swimming test was performed with a weight corresponding to 3% body weight attached to the tail. Forced swimming time and biochemical parameters were measured. The result indicated RMO could enhance exercise endurance and possessed protective effects against oxidative stress of the rats after exercise. The study also revealed that RMO elevated superoxide dismutase (SOD) concentrations, suggesting that RMO were able to up-regulate antioxidant enzymes to protect against oxidative stress-induced injury after exercise. In addition, RMO also possessed the ability to retard and lower the blood lactate produced after exercise.

**Key words:** *Morinda officinalis* How, endurance capacity, lipid peroxidation, exercise.

## INTRODUCTION

Free radicals are capable of independent existence and are produced in all living cells. Reactive oxygen species (ROS) or reactive nitrogen species (RNS), e.g., superoxide (O<sub>2</sub><sup>•-</sup>), hydroxyl (OH<sup>•</sup>), alkoxy (RO<sup>•</sup>), peroxy (ROO<sup>•</sup>), and hydroperoxide (ROOH) can oxidize other biological molecules, including carbohydrates, amino acids, fatty acids and nucleotides (Leelarugrayub et al., 2005). During the process of normal metabolism ROS is produced and if ROS is present in excess, it can lead to cumulative tissue damage. It has been shown over the past three decades that strenuous exercise can lead to the acute production of ROS (Armstrong, 1990; Reid et al., 1992; Bejma et al., 2000; Ji et al., 2004). During and after strenuous exercise, ROS production is increased in several cellular sources such as mitochondrial respiratory

chain, xanthine oxidase, NADPH oxidase, and activated phagocytes, challenging the endogenous antioxidant defense system and causing oxidative stress and acute tissue damage (Sen, 1995; Niess et al., 1999; Ji et al., 2004; Wang et al., 2006). Oxidative stress increases during exercise and the removal of free radicals depends on the presence of a well-developed antioxidant system (Alessio et al., 1997; Dekkers et al., 1996; Jenkins, 1993). If the rise in the level of oxygen free radicals exceeds the antioxidant defense potential of cells, then oxidative damage could occur (Duthie et al., 1990; Tsai et al., 2004; Groussard et al., 2003).

*Morinda officinalis* How (Rubiaceae) is a small vine that grows widely in tropical and subtropical regions (Yang et al., 1992; Li et al., 2001). The roots of this plant, named "Ba-ji-tian", have been used as a traditional Chinese herbal medicine for thousands of years (Xu et al., 2003). The earliest known Chinese medicinal monograph, documented medicinal use of *M. officinalis* How (RMO) around 2500 years ago. It has been recorded in pharmacopeia of

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**Figure 1.** Dried roots of *M. officinalis* How (RMO).

the People's Republic of China and used to help strengthen the bones and kidneys and enhance the immune system function. This plant has also been used to treat impotence, menstrual disorders, and inflammatory diseases such as rheumatoid arthritis and dermatitis (Wu et al., 2009). Recently, RMO has been shown to have antioxidant, antistress, antidepressive, antifatigue and other biological actions (Zhang et al., 2001; Li et al., 2001; Cheng et al., 2004; Ramesh and Okigbo, 2008; Zhang et al., 2009). In south China, Hong Kong and Macao, this plant has been developed into various health foods, such as "Ba-ji-tian wine", "Ba-ji-zi-bu Gao" (Li et al., 2009). This study sought to investigate the effect of RMO on exercise endurance capacity and exercise-related changes in lipid peroxidation in forced swimming rats.

## MATERIALS AND METHODS

### Plant material

The dried roots of *M. officinalis* How (RMO) were purchased from the Huai'an Herbal Medicine Market, Jiangsu Province, China, and the plant was identified by Prof. Zhang, Huaiyin Normal University, China (Figure 1). The voucher specimens of this plant were deposited at the Herbarium, Huaiyin Normal University.

### Preparation of *M. officinalis* How (RMO) extracts

RMO was extracted as described previously (Soon and Tan, 2002). The roots (1 kg) were ground to powder and extracted with 80% denatured ethanol (20 L) till exhaustion at room temperature. After filtration with cotton wool, the filtrate was concentrated at 65°C by a R-215 rotavapor (Buchi Labortechnik AG, Postfach, Switzerland). The concentrate was then freeze-dried to yield 276 g of dark brown powder. The ethanolic extract was dissolved in distilled water before being used.

## Experimental animals and their care

Experimental procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomed Biomedical Research as adopted and promulgated by the World Health Organization (1985).

Male Wistar rats, aged 10 weeks (220 - 250 g body weight) were obtained from the laboratory animal centre, Huaiyin Normal University, after ethical approval was granted by the ad hoc ethical committee of the university. The rats were housed in polypropylene cages containing locally produced sterile paddy husk as bedding. The rats were maintained at uniform temperature ( $25 \pm 1^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ), light/dark periodicity (12/12 h), with free access to water and standard rat chows. Rats were adapted to conditions for 1 week before the experiment began. The animals were randomly divided into the following four groups, each group consisting of eight rats (Table 1). The volume of administration was 1 mL, and the RMO extracts were administered every day orally using intragastric tube for 30 days. Body weights were measured before and after the experiment.

## Experimental design

The exercise endurance capacity was assessed 10 h after the last administered by forced swimming test, and it was modified according to previous researches (Yalcin et al., 2000; Nayanatara et al., 2005; Niu et al., 2008). Rats were forced to swim in plastic tanks (length 100cm, width 40 cm, depth 60 cm) containing tap water maintained at a temperature of  $36 \pm 2^\circ\text{C}$ . The water depth, 35 cm, was set so that the rats could not rest by supporting the tail on the bottom of the tank. Each of the rats had a weight attached (3% body weight) to the tail for the duration of the swim-to-exhaustion exercise. The animals were assessed to be exhausted when they failed to rise to the surface of the water to breathe within 7 s. At this moment, the animals were removed from the tank.

Blood samples were withdrawn through the retro-orbital plexus under light ether anesthesia using a glass capillary and collected in tubes. Blood plasma was collected and stored at  $-80^\circ\text{C}$  in a deep freeze for future analysis for lactate, malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations. Blood lactate concentrations were measured using a bench-top analyzer (YSI 1500 sport I-lactate analyser, YSI Inc, USA). Serum MDA and SOD concentrations were measured using standard test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

## Statistical analysis

All the data were expressed as mean  $\pm$  S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Turkey post hoc test. The criterion for statistical significance was  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Effects of *M. officinalis* How (RMO) extracts on body weight

Table 2 showed the body weight change of the rats during the experimental period. For the group A, the body weight at day 0 and day 30 were  $421.84 \pm 26.16$  and  $484.85 \pm 39.84$  g, respectively. For the group B, the body weight at day 0 and day 30 were  $437.26 \pm 31.23$  and

**Table 1.** Animals and grouping.

Group A	Control rats administered distilled water
Group B	Administered RMO extracts 200 mg/kg body weight
Group C	Administered RMO extracts 400 mg/kg body weight
Group D	Administered RMO extracts 800 mg/kg body weight

**Table 2.** Effects of RMO extracts on body weight.

Group	Body weight (g)	
	Before experiment (day 0)	After experiment (day 28)
A	421.84 ± 26.16	484.85 ± 39.84
B	437.26 ± 31.23	498.27 ± 34.65
C	429.86 ± 27.81	491.28 ± 36.57
D	423.77 ± 28.19	486.54 ± 30.81

Values are the mean ± S.E.M. (n = 8, respectively).

498.27 ± 34.65 g, respectively. For the group C, the body weight at day 0 and day 30 were 429.86 ± 27.81 and 491.28 ± 36.57 g, respectively. For the group D, the body weights at day 0 and day 30 were 423.77 ± 28.19 and 486.54 ± 30.81 g, respectively. The body weight of all the administered groups with RMO extracts (Group B, Group C and Group D) was not different significantly from that in the control group (Group A) before and after experiment ( $p > 0.05$ ). These results indicated RMO had no significant effect on body weight.

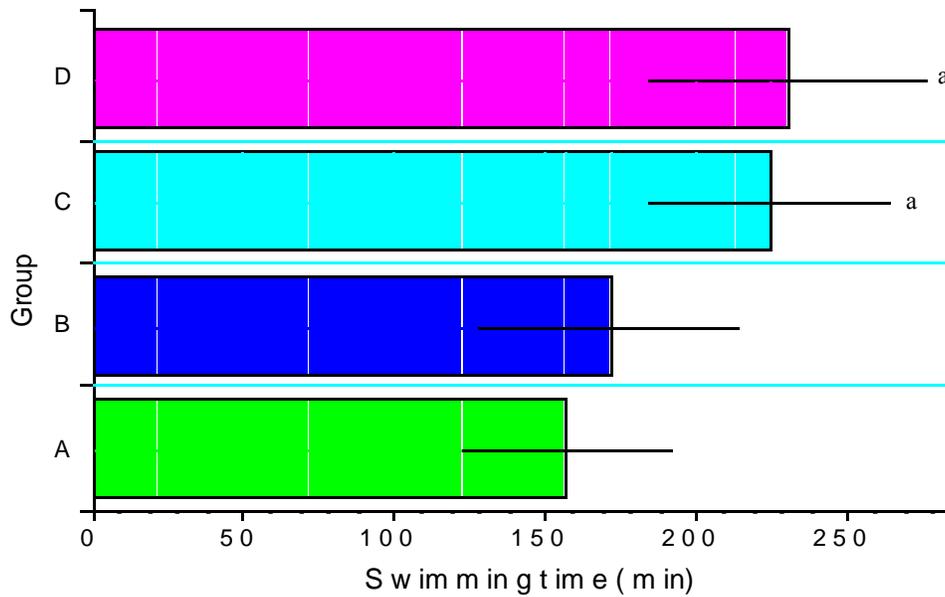
### Forced swimming test

Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress (Greenen et al., 1988; Tan et al., 1992). Swimming has got a number of advantages over other types of exercise such as treadmill running. The amount of work done during swimming exercise is far greater than that during the treadmill running of identical time duration. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated (Kramer et al., 1993; Nagaraja and Jeganathan, 1999). The forced swimming test developed by Porsolt et al. (1977) has now become widely accepted model for studying physical stress in animals. To standardize the workload and reduce the swimming time, weights at specific body weight percentages were added to the chest or tail of the animal (Denadai, 1994; Matsumoto et al., 1996). In this study, the rats had a weight attached (3% body weight) to the tails during the swimming based on the above reason. The swimming time to exhaustion of the rats was measured to investigate the effect of RMO extracts on endurance capacity. As shown in Figure 2, the swimming

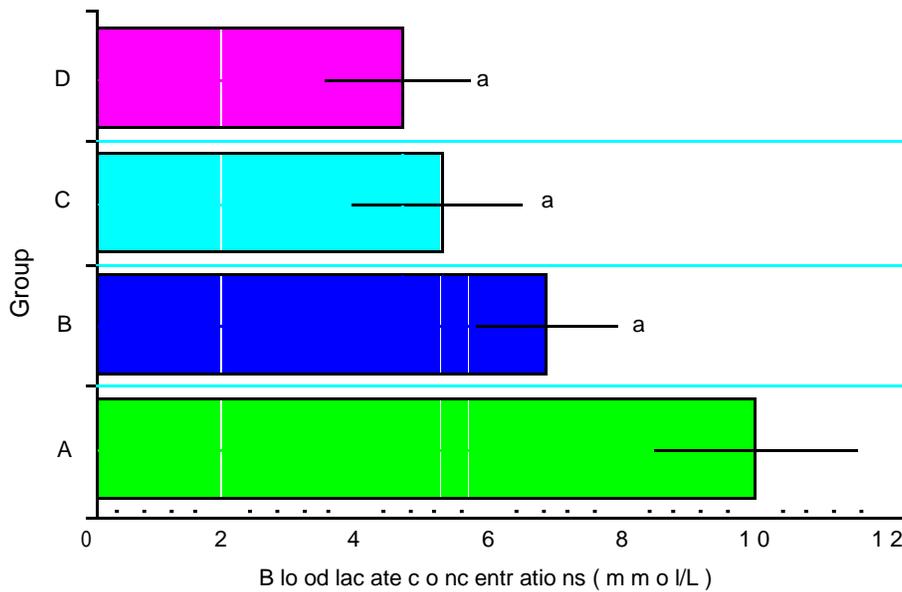
time to exhaustion of the Group A, B, C and D was found to be 158 ± 34, 172 ± 43, 225 ± 39 and 231 ± 46 min, respectively. There were no differences in the swimming time to exhaustion between the Group A (control group) and Group B ( $p < 0.05$ ). The swimming time to exhaustion of the Group C and Group D was longer than that of the Group A ( $p < 0.05$ ), and the increase of the swimming time of the Group C and D were 42.41 and 46.20%, respectively. These results indicated RMO could enhance exercise endurance of the rats.

### Effects of *M. officinalis* How (RMO) extracts on blood lactate

Lactate serves as an energy source in highly oxidative tissues (Dorchy, 2002). During exercise, organs such as liver and heart, and tissues such as skeletal muscle, help to remove lactate from the blood (Bonen, 2000; Brooks, 2000; Lee et al., 2009), but intense exercise can increase lactate production and accumulation, which due to the need of degrading rapidly glucose to obtain energy for the muscle (Wasserman, 1994; Green, 2004). A substantial lactic acidosis in blood and muscle may be a potent factor of oxidative stress (Groussard et al., 2003). As shown in Figure 3, blood lactate concentrations of the Group A, B, C and D were found to be 9.97 ± 1.51, 6.84 ± 1.03, 5.23 ± 1.27 and 4.62 ± 1.10 mmol/L, respectively. The blood lactate concentrations of all the administered groups with RMO extracts (Group B, C and D) were lower than that of the Group A ( $p < 0.05$ ), and the decrease of the blood lactate concentrations of the Group B, C and D were 31.39, 47.54 and 53.66%, respectively. These results indicated RMO possess the ability to retard and lower the blood lactate produced after exercise.



**Figure 2.** Swimming time to exhaustion in forced swimming test. Values are the mean  $\pm$  S.E.M. (n=8, respectively), <sup>a</sup>p < 0.05 indicates significant difference from the Group A (control group).

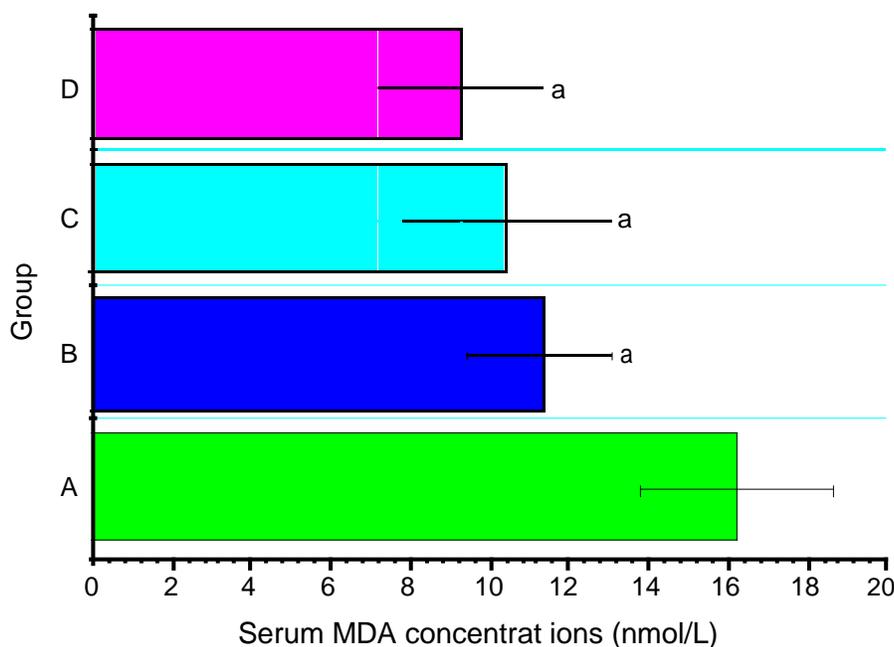


**Figure 3.** Effects of RMO extracts on blood lactate. Values are the mean  $\pm$  S.E.M. (n = 8, respectively), <sup>a</sup>p < 0.05 indicates significant difference from the Group A (control group).

### Effects of *M. officinalis* How (RMO) extracts on serum malondialdehyde

The rise in oxygen consumption associated with exercise has been increasingly implicated in the production of oxygen free radicals (Davis et al., 1982), resulting in the formation of lipid peroxides (Lawrence et al., 1975; Dillard et al., 1978). Lipid peroxidation is a free radical-mediated

chain reaction, initiated by the radicals attacking polyunsaturated fatty acids in membranes and it results in oxidative damage, which ultimately affects membrane stability (Rokizki et al., 1994; Kendall and Eston, 2002). Thus, the MDA, a marker of lipidperoxidation, would increase after exhaustive exercise (Tsai et al., 2004). As shown in Figure 4, serum MDA concentrations of the Group A, B, C and D were found to be  $16.19 \pm 2.43$ ,



**Figure 4.** Effects of RMO extracts on serum MDA. Values are the mean  $\pm$  S.E.M. (n = 8, respectively), <sup>a</sup>p < 0.05 indicates significant difference from the Group A (control group).

11.26  $\pm$  1.79, 10.37  $\pm$  2.57 and 9.21  $\pm$  2.04 nmol/L, respectively. The serum MDA concentrations of all the administered groups with RMO extracts (Group B, C and D) were lower than that of the Group A (p < 0.05), and the decrease of the serum MDA concentrations of the Group B, C and D were 30.45, 35.95 and 43.11%, respectively. Linear regression analysis revealed a significant inverse relationship between swimming time and serum MDA (r = 0.841, p < 0.05). These results indicated RMO possesses the ability to reduce lipid peroxide and suppress tissue damage after exercise.

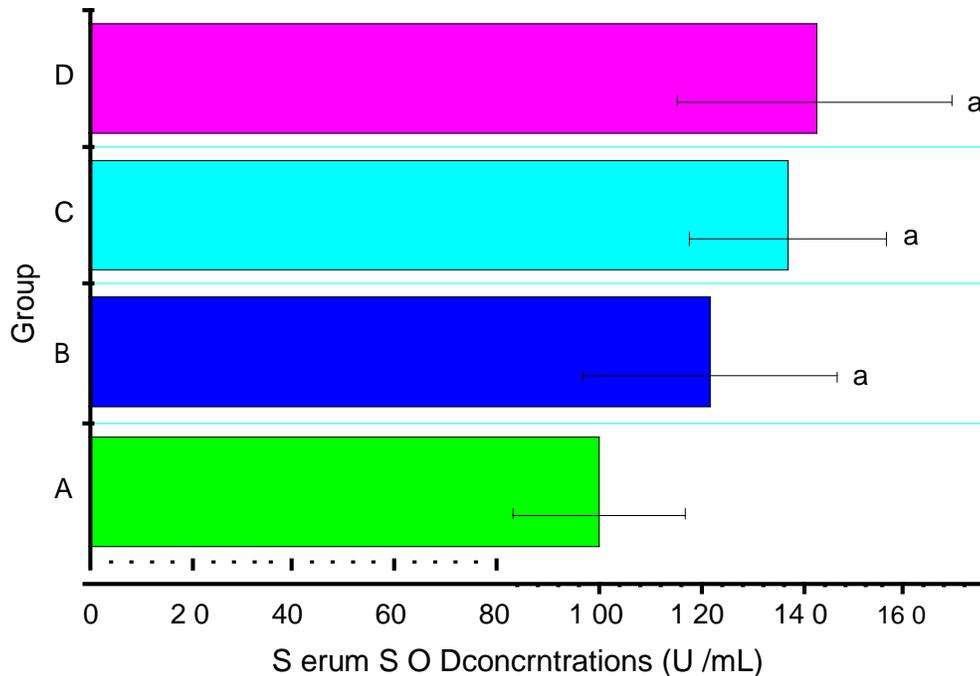
#### Effects of RMO extracts on serum superoxide dismutase

The antioxidant defense systems of the living body consist of antioxidant enzymes and antioxidant nutrients, which may be involved in reducing oxidative stress (Leibovitz et al., 1990; Zoppi et al., 2006; Ciocoiu et al., 2007). As antioxidant enzymes play an important role in the protection against free radical damage, a decrease in the activities or expressions of these enzymes may predispose tissues to the free radical damage (Lee et al., 2009; He et al., 2009). Free radicals are produced by lipid peroxidation derived from oxygen, and the first line of defense against them is SOD. It is well known that SOD is one of the most important enzymes in the antioxidant defense system of the living body, the increase of SOD concentrations would indicate an up-regulation of the defense mechanism to try to cope with an enhanced

production of superoxide anion radicals. This in turn might help to down-regulate the production of lipid peroxides or oxidative stress (Lee et al., 2009). As shown in Figure 5, serum SOD concentrations of the Group A, B, C and D were found to be 99.68  $\pm$  16.87, 121.73  $\pm$  24.86, 137.28  $\pm$  19.25 and 142.36  $\pm$  27.06 U/mL, respectively. The serum SOD concentrations of all the administered groups with RMO extracts (Group B, C and D) were higher than that of the Group A (p < 0.05), and the increase of the serum SOD concentrations of the Group B, C and D were 22.12, 37.72 and 42.82%, respectively. Linear regression analysis revealed a significant direct relationship between swimming time and serum SOD levels (r = 0.945, p < 0.05). These results indicated that RMO were able to up-regulate antioxidant enzymes to protect against oxidative stress-induced injury after exercise.

#### Conclusions

In conclusion, this is the first study to demonstrate that *M. officinalis* How could enhance exercise endurance and possessed protective effects against oxidative stress of the rats after exercise. The study also revealed that *M. officinalis* How elevated SOD concentrations, suggesting that *M. officinalis* How were able to up-regulate antioxidant enzymes to protect against oxidative stress-induced injury after exercise. In addition, *M. officinalis* How also possessed the ability to retard and lower the blood lactate produced after exercise. Therefore,



**Figure 5.** Effects of RMO extracts on serum SOD. Values are the mean  $\pm$  S.E.M. (n = 8, respectively), <sup>a</sup>p < 0.05 indicates significant difference from the Group A (control group).

*M. officinalis* How should be considered as a candidate for future studies on fatigue.

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