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Full Length Research Paper

Modification of oxidative stress in acute exhaustive exercise mice with n-butanol and water fractions from *Pedicularis decora* Franch root treatment

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The current study investigated the effect of n-butanol and water fractions of *Pedicularis decora* Franch on oxidative stress induced by an acute exhaustive exercise. Institute of Cancer Research (ICR) mice were divided into sedentary, exercise and recovery groups. Each group includes control and water fraction as well as n-butanol fraction subgroup. Mice were treated with the two fractions of *P. decora* Franch root and saline for a period of 21 days. After the treatment period, the exercise and recovery groups swam to exhaust. Sedentary and exercise groups animals were sacrificed immediately after swimming exercise and recovery mice were sacrificed after 24 h. In sedentary group, superoxide dismutase (SOD), glutathione peroxidase (GPx) activities were higher and lipid peroxidation (LPO) levels were lower with the two fractions treatment. SOD, GPx, lactate dehydrogease (LDH) activities and LPO levels significantly decreased in exercise and recovery groups with the two fractions treatment. Also, the two fraction could promote energy deposits through an increase in glycogen contents. In addition, n-butanol fraction significantly prolonged swimming time to exhaustion. In short, n-butanol and water fractions of *P. decora* Franch may both protect mice against oxidative stress induced by an acute exhaustive exercise and n-butanol fraction could significantly improve the swimming capacity. Further, n-butanol fraction is more pronounced in ameliorating the oxidative stress induced by an acute exhaustive swimming exercise.

Key words: *Pedicularis decora* Franch, n-butanol fraction, water fraction, lipid peroxidation, antioxidant enzymes, an acute exhaustive exercise.

INTRODUCTION

Regular training has normally been shown to give beneficial effects for a long time. Thus regular or moderate exercise has been proposed to give a protection on the health, including a decrease in risk of the obesity, cardiovascular disease and the metabolic syndrome (Fogelhoil et al., 2000; Sato et al., 2007). On the other hand, some studies show that acute, eccentric and exhaustive exercise or training generates excessive oxidative stress in the body. Excessive oxidative stress causes exceeding free radicals product especially reactive oxygen species (ROS). These free radicals may react with cell components, including proteins, DNA and lipids resulting into cell damage (Terblanche et al., 2001; McArdle et al., 2001; Jackson 1999). In addition, mitochondrial membrane fluidity has been affected during the acute bouts of exercise, including exhaustive, maximal exhaustive and eccentric exercise, so it partly increased excessive ROS (Ascensão et al., 2007).

It has been shown that exhaustive exercise is the most

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likely form of exercise to induce free radicals release (Viña et al., 2000). However, cells have developed different antioxidant systems and nonenzymatic antioxidants to defend themselves against free radicals' attack. For example, it has been reported that the increase in catalase (CAT) and superoxide dismutase (SOD) activity merely counters this increase to maintain the homeostasis in face of a higher rate of ROS production in an acute exercise (Gianni et al., 2005). Exhaustive or eccentric exercise, in rats may increase ROS production exceeding the capacity of antioxidant defenses (Gul et al., 2003; Oztasa et al., 2004; Ozdag, 2010). Some studies have shown that exogenous antioxidants supplementation could reinforce the antioxidant status in response to exercise-induced oxidative stress (Margaritis et al., 2003).Therefore, supplementation of antioxidants may be desirable to reduce oxidative stress and provide a larger protective margin against its possible consequences (Bonina et al., 2005).

Among various antioxidants, medicinal plants still present a large source of natural antioxidants in different parts of the world from time immemorial (Srinivasan et al., 2007; Hemalatha et al., 2010). *Pedicularis decora* Franch is an important species of *Pedicularis* Linn. *P. decora* Franch, a Chinese folk herbal medicine which is used for a wide variety of ethnomedical properties for the treatment of general debility, collapse, exhaustion, invigorate the circulation of blood, aid digestion, full of vitality and so on. The crude extract from *P. decora* Franch root was observed to ameliorating the oxidative stress induced by intense exercise (Li et al., 2002). However, the effect of its successive extracts supplementation on exercise has not been studied clearly in animals especially taking into account the possible benefits. Therefore, this study was conducted to determine the effect of its successive fractions on acute exhaustive exercise training. Specifically, oxidative stress markers such as lipid peroxidation (LPO), antioxidant enzymes such as SOD, glutathione peroxidase (GPx) were determined. Further, exercise associated biomarkers lactate dehydrogenase (LDH), glycogen, glucose and hemoglobin (Hb) were also measured after acute exhaustive exercise.

MATERIALS AND METHODS

P. decora Franch roots were collected at Taibai Mountain, in the region of Shaanxi province, China. All other chemicals used were of analytical grade and were purchased locally.

Preparation of plant extract

The roots of *P. decora* Franch were dried at room temperature and crushed into a coarse powder. The powder was extracted three times with 95% ethanol to yield ethanol extract. After removal of ethanol, the ethanol extract was dissolved in n-butanol-H_2O (1:1). The solvent was completely removed by rotary vacuum evaporator. The two fractions were preserved in a refrigerator for further use.

Animal housing and experiment design

ICR mice (18 to 22 g) were purchased from the Experimental Animal Center of Xi'an Jiaotong University, Shaanxi Province, China. The mice were bred in our animal facility with controlled temperature (18 to 22℃), humidity (50 to 60%) and photoperiod (12:12 h light/dark cycle). Animals were fed with a commercial balanced diet and tap water *ad libitum*. The experiment on the mice was performed based on the animal ethics guidelines of the Institutional Animal Ethics Committee in Xi'an Jiaotong University. Mice were randomly assigned to sedentary (S), exercise (E) and recovery (R) groups. Sedentary group includes sedentary control (SC), sedentary water (SW) and sedentary n-butanol fraction (SB) subgroups. Exercise group includes exercise control (EC), exercise water (EW) and exercise n-butanol fraction (EB) subgroups. Recovery group contains recovery control (RC), recovery water (RW) and recovery n-butanol fraction (RB) subgroups. SC, EC and RC subgroups were given the saline alone (9 g/L, 0.1 ml per day p.o.) for 21 days. SW, EW and RW subgroups received water fraction from *P. decora* Franch. SB, EB and RB subgroups were treated with n-butanol fraction. The two plant fractions were both given at the dose level of 15 g/kg b. w. per day p. o. for 21 days.

Exercise training program

The swimming was selected as a model for exercise performance, because swimming appears to belong to the natural behavior of rodents and humans (Venditti and Di Meo, 1996). The exercise and recovery groups underwent an acute exhaustive swimming exercise after three weeks of treatment with the two fractions of *P. decora* Franch root or saline solution. The swimming exercise was performed in a tank (0.55×0.56×0.48 m) with water kept at 34±1℃. The duration of exercise is determined by making mice to swim in order to be exhausted. The mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7 s period. After exhaustive swimming exercise, the mice were salvaged and towel-dried, recovery mice were returned to their respective cages. No deaths occurred during or after swimming in any of the subgroups. Sedentary group of mice were confined to cage activity and handled only during changing of cages.

Sample collection and preparation

After an acute exhaustive swimming, exercise and control groups were immediately sacrificed. The recovery group was sacrificed after 24 h of the exhaustive swimming exercise. Blood samples were rapidly collected and serum was separated by centrifugation. Immediately after the blood had been collected, liver and red quadriceps were immediately dissected out, frozen in liquid nitrogen and kept at -70℃ until biochemical analyses. Part of the tissues of the liver and red quadriceps were then homogenized in 50 mM phosphate buffer solution (pH 7.4) using a tissue homogenizer at 4℃. The supernatant was collected after centrifuging at 1,000 g for 5 min and stored at -70℃ for further analyses. The content of protein in the tissues homogenate was determined by the method of Lowry et al. (1951).

Determination of LPO

LPO was assayed in mouse liver, red quadriceps and blood according to the method of Wright et al. (1981). Briefly, phosphate buffer 0.58 ml (0.1 M, pH 7.4), 0.2 ml ascorbic acid (100 mM), 0.02

Table 1. Effect of *P. decora* Franch water and n-butanol fraction on the swimming time to exhaustion.

Group	n	Swimming time (min)	Extend percent (%)
Saline control groups	20	193.2±39.1	
Water fraction groups	22	198.6±36.2	2.85
n-butanol fraction groups	23	247.9±41.8	27.94***

All values are expressed as mean ± S.D. ***P < 0.001, when compared to saline control groups.

ml ferric chloride (100 mM) were added to 0.2 ml of tissue homogenate (10%, w/v) and incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by the addition of 1.0 ml trichloro acetic acid (10%, w/v), following which 1.0 ml thiobarbituric acid (0.67 %, w/v) was added and all the tubes were placed in a boiling water bath for 20 min. After centrifugation at 2500 × g for 10 min, the amount of malonaldehyde formed in each of the samples and was assessed by measuring the optical density of the supernatant at 532 nm against a reagent blank. It was calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nanomoles of TBARS/mg of protein.

Analysis of SOD, GPx and LDH activities

SOD activity was analyzed by monitoring the auto-oxidation of pyrogallol described by Marklund and Marklund (1974). SOD catalyzed the superoxide radical to hydrogen peroxide and oxygen, the absorbance of the sample was measured at 420 nm. GPx activity was measured according to the method of Hafeman and Hoekstra (1973) with a slight modification. The reaction mixture contained GSH, NaN_3-PBS pH = 6.5, H_2O_2, TCA (trichloroacetic acid), Na_2HPO_4, DTNB (5, 5′-dithiobis (2-nitrobenzoic acid)). The absorbance at 423 nm was monitored on a UV-220 spectrophotometer at 37°C. One unit of enzyme activity was defined as the amount of enzyme that oxidized 1 mol/L of GSH per min per mg of protein. LDH assay conditions were based on those described earlier (Bass et al., 1969). The assay medium for LDH contained 50 mM Tris-HCl buffer pH 7.5, 0.15 mM NADH and 1 mM pyruvate (omitted for control). The absorptivity of NADH at 340 nm was 6.22 µM/ cm.

Hb measurement

The Hb concentration was determined spectrophotometrically using the potassium ferricyanide and potassium cyanide (Drabkin's reagent). Briefly, 20 µl blood was dissolved in Drabkin's reagent to a concentration of 0.5 to 1.0 mg/ml and incubated for 30 min at room light and at room temperature. Then the reaction mixture was maintained at 4°C in the dark. Hb concentration (Hb) was calculated using a 540 nm of 11.5 $mM^{-1}cm^{-1}$ (Winterbourn, 1990).

Determination of glycogen and glucose concentrations

Glycogen concentrations were determined as previously described by Lo et al (1970). In brief, part of the tissue samples were collected immediately after exhaustive exercise, cut, weighed, and boiled for 30 min in 1 ml of 30% KOH saturated with $NaSO_4$. After the tissue samples were completely digested, glycogen was precipitated in 2 ml of 95% ice-cold ethanol and incubated on ice for 20 min. Tubes were spun for 30 min at 500 g. The pellets were resuspended in 1 ml H_2O, and 1 ml of 5% phenol was added. A colorimetric reaction was obtained by adding 5 ml of sulfuric acid. After incubation on ice for 30 min, the absorbance at 490 nm of the solution in vials was spectrophotometrically determined. The concentrations of serum glucose were determined by the glucose oxidase method (De Sousa et al., 2004).

Statistical analysis

The data were expressed as mean ± SD. Student t-tests were used to compare between sedentary mice, exercise and recovery trained groups. All analyses were performed using SPSS 13.0 for Windows. The statistical significance level was set at P < 0.05.

RESULTS

Effect on the swimming time to exhaustion

A significant difference was observed in the swimming time to exhaustion between the saline control groups and n-butanol fraction groups (Table 1). However, there was no significant difference between the vehicle groups and water fraction groups. Extend percent was 27.94 in n-butanol fraction groups and 2.85 in water fraction groups when compared to saline groups, respectively.

Effect on LPO levels

LPO level of liver, red quadriceps and blood was determined and shown in Figure 1. In sedentary groups, LPO level significantly decreased (P < 0.001) in the two fractions from *P. decora* Franch treated mice (SW and SB groups) when compared to SC group mice. An acute exhaustive swimming exercise significantly increased (P < 0.05, 0.001) LPO levels in all exercise and recovery groups when compared to SC group. In exercise groups, treatment with the two fractions (EW and EB groups) significantly decreased (P < 0.001) LPO levels in liver and red quadriceps but not in blood compared with EC group. In recovery groups, a significant decrease (P < 0.001) in LPO levels was observed in liver, blood and red quadriceps compared with EC group. Also, LPO levels significantly decreased (P < 0.01, 0.001) in the two fractions from *P. decora* Franch treatment groups (RW and RB groups) except in liver of the water fraction treatment compared to RC group.

In addition, LPO level significantly decreased (P < 0.001) in the corresponding group of n-butanol fraction

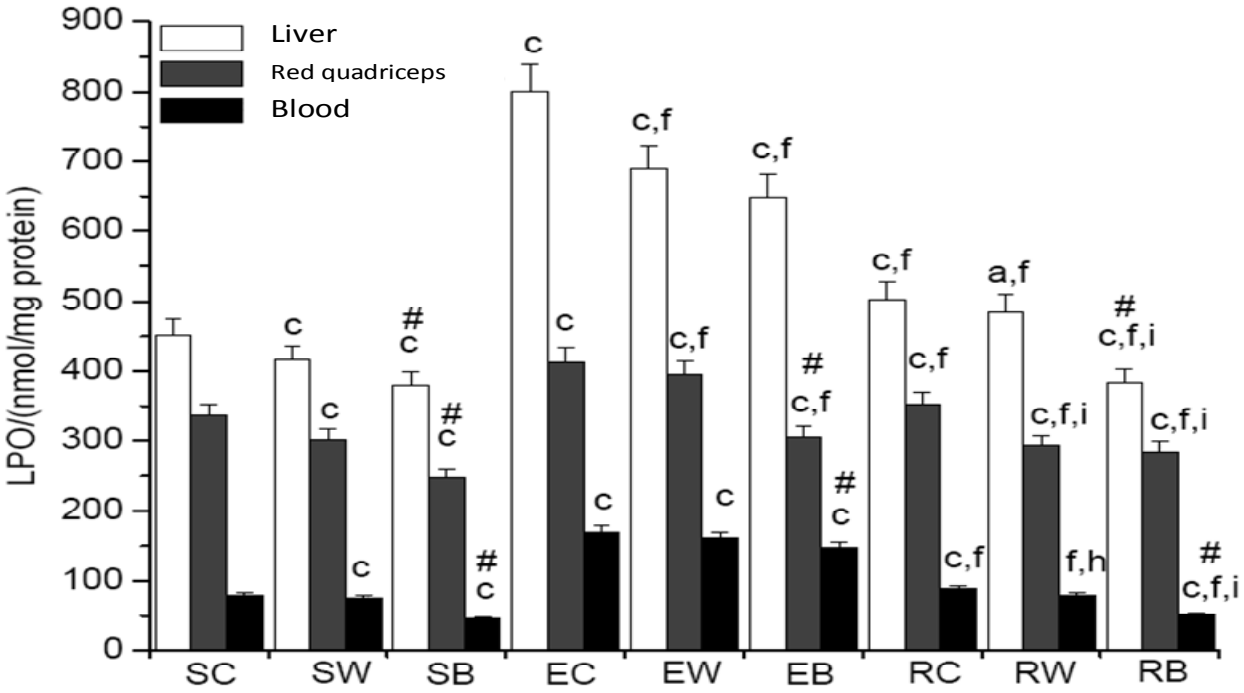

Figure 1. Effect of water and n-butanol fractions from *P. decora* Franch root on LPO levels in liver, red quadriceps and blood in mice. The values are expressed as mean ± S.D. for groups of ten animals each. [a]P < 0.05, when compared to group SC. [c]P < 0.001, when compared to group SC. [f]P < 0.001, for groups EW and EB when compared with group EC. [h]P < 0.01, for groups RW and RB when compared with group RC. [i]P < 0.001, for groups RW and RB when compared with group RC. [#]p < 0.001, when n-butanol fraction treatment group compared with the corresponding water fraction treatment group. S: sedentary groups; E: exercise groups; R: recovery groups; C: mice administered with saline; W: mice administered with water fraction from *P. decora* Franch; B: mice administered with n-butanol fraction from *P. decora* Franch.

treatment except in liver in EB group and in red quadriceps in RB group versus the corresponding EW and RW group (Figure1).

Effect on SOD activities

SOD activities in the liver, red quadriceps and blood were shown in Figure 2. In sedentary groups, a significant increase (P < 0.01) in SOD activity was observed of the n-butanol fraction and water fraction treated groups but not in liver and red quadriceps of the water fraction treated group when compared to SC group. Additionally, an acute exhaustive swimming exercise significantly increased (P < 0.05, 0.001) the activities of SOD in all exercise and recovery groups when compared to SC group. In exercise groups, administration with the two fractions (EW and EB groups) showed a significant decrease (P < 0.001) in SOD activity in red quadriceps but not in liver and blood when compared to EC group. In recovery groups, a significant decrease (P < 0.01, 0.001) in SOD activities was observed when compared with EC group. The two fractions treatment groups (RW and RB groups) significantly decreased (P < 0.01) the activities of SOD in red quadriceps but no significant difference in liver and blood when compared to RC group.

Additionally, SOD activity significantly increased in liver (P < 0.01) and red quadriceps (P < 0.001) in SB group when compared with the corresponding SW group. A significant increase (P < 0.001) was observed in red quadriceps in EB group when compared to EW group. Also, a significant increase was observed in liver (P < 0.01) and red quadriceps (P < 0.05) in RB group when compared with the corresponding RW group (Figure 2).

Effect on GPx, LDH activities and Hb concentrarions

As shown in Table 2, GPx activities were significantly increased (P < 0.001) in the n-butanol fraction treated group (SB group) and in all exercise groups when compared with SC group. In recovery groups, a significant increase in GPx activities (P < 0.001) was observed when compared to SC group and EC group. A significant decrease (P < 0.05) of RW group and a significant increase (P < 0.001) of RB group in GPx activities were observed in blood compared with RC group.

Treatment with the two fractions did not significantly affect LDH activity in sedentary groups (Table 2). LDH

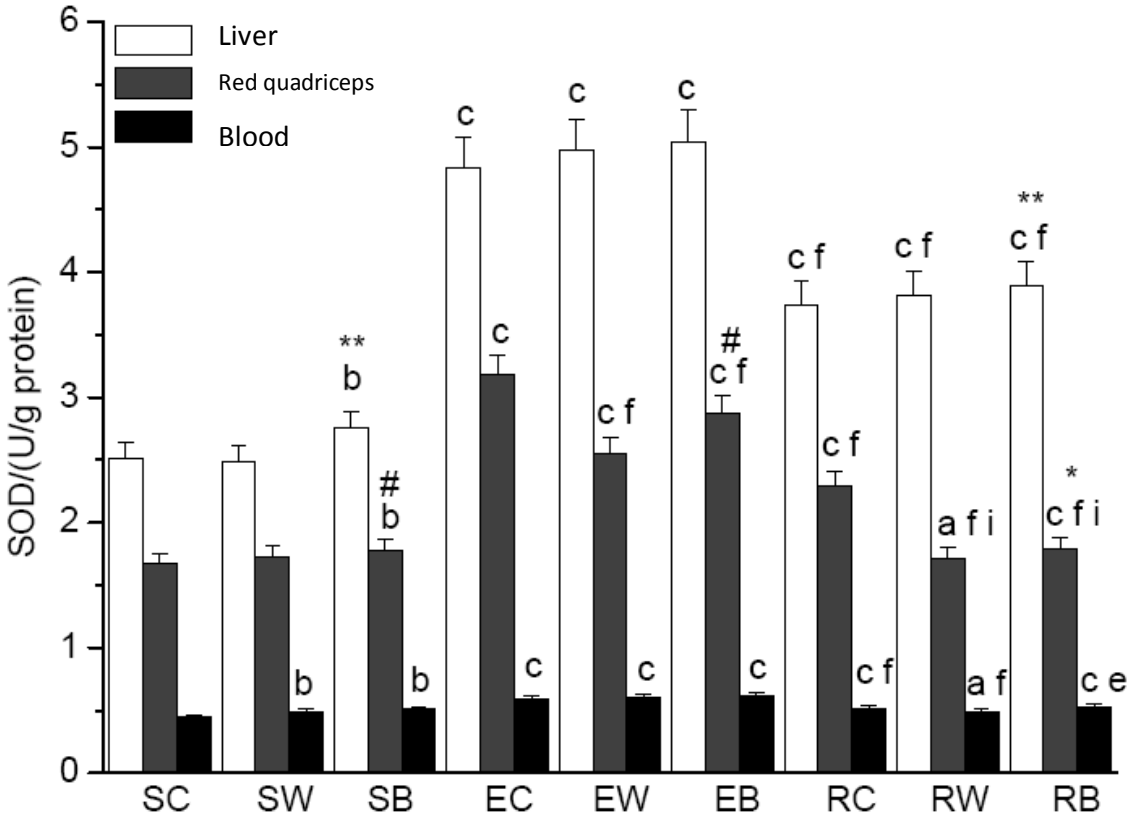

Figure 2. Effect of water and n-butanol fractions from *P. decora* Franch root on SOD activities in liver, red quadriceps and blood in mice. The values are expressed as mean ± S.D. for groups of ten animals each. [a]P < 0.05, when compared to group SC. [b]P < 0.01, when compared to group SC. [c]P < 0.001, when compared to group SC. [e]P < 0.01, for groups EW and EB when compared with group EC. [f]P < 0.001, for groups EW and EB when compared with group EC. [i]P < 0.001, for groups RW and RB when compared with group RC. *p < 0.05, **p < 0.05, [#]p < 0.001, when n-butanol fraction treatment group compared with the corresponding water fraction treatment group. S: sedentary groups; E: exercise groups; R: recovery groups; C: mice administered with saline; W: mice administered with water fraction from *P. decora* Franch; B: mice administered with n-butanol fraction from *P. decora* Franch.

Table 2. Effect of n-butanol and water fractions on Hb concentrations and LDH, GPx activities.

Groups	Hb (g/L)	LDH (U/100 ml)	GPX (nmol/mg.min)
SC	140.7±16.7	125.8±6.0	31.13±2.93
SW	139.3±29.3	126.1±8.2	31.80±1.02
SB	149.5±24.0	124.6±5.7	44.56±1.07[c,#]
EC	105.3±14.7[c]	142.6±8.4[c]	51.91±2.54[c]
EW	120.0±24.7[a]	148.8±10.3[c]	49.43±0.83[c]
EB	123.0±20.8[d]	153.6±10.8[c,d]	47.98±11.4[c]
RC	138.0±15.3[f]	122.5±5.6[f]	36.95±1.70[c,f]
RW	145.3±11.8[f]	120.5±9.4[f]	35.20±1.44[c,f,g]
RB	161.4±19.2[a,f,h,*]	112.2±4.7[c,f,I,*]	46.80±1.44[c,f,I,#]

All the values are expressed as mean ± S.D. for groups of ten animals each. [a]P < 0.05, when compared to group SC. [c] P < 0.001, when compared to group SC. [d]P < 0.05, for groups EW and EB when compared with group EC. [f] P < 0.001, for groups EW and EB when compared with group EC. [g]P < 0.05, for groups RW and RB when compared with group RC. [h] P < 0.01, for groups RW and RB when compared with group RC. [i] P < 0.001, for groups RW and RB when compared with group RC. *p < 0.05, [#]p < 0.001, when n-butanol fraction treatment group compared with the corresponding water fraction treatment group. S: sedentary groups; E: exercise groups; R: recovery groups; C: mice administered with saline; W: mice administered with water fraction from *P. decora* Franch; B: mice administered with *n*-butanol fraction from *P. decora* Franch.

Table 3. Effect of n-butanol and water fractions on glucose levels and glycogen contents.

Groups	Glycogen (mg/g)		
	Red quadriceps	Liver	Glucose (mg/100 ml)
SC	1.23±0.63	6.72±1.87	86.4±19.5
SW	1.69±0.87	8.83±4.20	85.3±18.0
SB	2.10±1.05[a]	8.02±3.34	82.8±23.6
EC	0.44±0.15[c]	4.33±1.20[b]	58.0±17.1[b]
EW	1.38±0.50[c,f]	3.60±2.00[b]	54.1±15.3[c]
EB	0.98±0.60[c,d]	3.49±2.12[b]	47.4±15.3[c]
RC	1.18±0.78[b]	6.58±2.4[d]	80.4±27.9[d]
RW	1.59±0.71[c]	7.22±2.00[e]	80.8±6.7[f]
RB	1.69±0.92[c]	7.82±3.20[e]	91.2±24.5[e]

All the values are expressed as mean ± S.D. for groups of ten animals each. [a]$P < 0.05$, when compared to group SC. [b]$P < 0.01$, when compared to group SC. [c]$P < 0.001$, when compared to group SC. [d]$P < 0.05$, for groups EW and EB when compared with group EC. [e]$P < 0.01$, for groups EW and EB when compared with group EC. [f]$P < 0.001$, for groups EW and EB when compared with group EC. S: sedentary groups; E: exercise groups; R: recovery groups; C: mice administered with saline; W: mice administered with water fraction from *P. decora* Franch; B: mice administered with n-butanol fraction from *P. decora* Franch.

activity significantly increased ($P < 0.001$) in blood in exercise groups when compared to SC group. A significant increase ($P < 0.05$) in LDH activities in blood was observed in EB group when compared with EC group. In recovery groups, a significant decrease ($P < 0.001$) was observed in RB group when compared with SC group. LDH activity in recovery groups was seen to be striking lower than the EC group ($P < 0.001$). Also, a significant decrease ($P < 0.001$) was observed in RB group when compared to RC group.

The concentrations of Hb were not significantly affected in the two fractions treatment groups (SW and SB groups) when compared to SC group (Table 2). Hb concentrations were significantly reduced ($P < 0.05$, 0.001) after the acute exhaustive swimming exercise but not in EB group when compared to SC mice. The reduction of Hb concentrations in acute exhaustive swimming exercise mice was markedly inhibited ($P < 0.05$) in EB group when compared with EC group. Recovery mice also showed a significant ($P < 0.001$) increase in Hb concentrations when compared to EC mice. Further, n-butanol fraction showed a significant inhibition ($P < 0.05$) and a significant increase ($P < 0.01$) effect when compared with SC group, RC group, respectively.

A significant increase ($P < 0.001$) in GPx activity of SB and RB groups was observed when compared to the corresponding SW and RW group. A significant increase in Hb concentration ($P < 0.05$) and a significant decrease in LDH activity ($P < 0.05$) in RB group were observed when compared with the corresponding RW group (Table 2).

Effects on glucose and glycogen contents

Table 3 represented the contents of glycogen and glucose in control and treatment mice. Glycogen contents significantly increased ($P < 0.05$) in red quadriceps of n-butanol fraction treated group and significantly decreased ($P < 0.01$, 0.001) in an acute exhaustive swimming exercise mice as compared with SC group. Administration with the n-butanol and water fractions from *P. decora* Franch significantly increased ($P < 0.05$, 0.001) glycogen concentrations in red quadriceps versus EC group. Treatment with the two fractions markedly restored ($P < 0.001$) in red quadriceps glycogen compared with SC group after an acute exhaustive swimming exercise. A significant increase ($P < 0.05$, 0.01) was observed in recovery groups when compared to EC group but no significant difference compared with RC group. No difference was found on glucose levels in the two fractions treated sedentary mice. A significant decrease ($P < 0.01$, 0.001) in exercise mice and a significant increase ($P < 0.05$, 0.01, 0.001) to near even higher than the level of SC group in recovery mice was found in the experiment, respectively. No significant difference was found in the contents of glycogen and glucose between the n-butanol fraction treatment group and the corresponding water fraction treatment group.

DISCUSSION

In this study, the effects of n-butanol and water fractions of *P. decora* Franch on an acute exhaustive swimming exercise have been estimated. Physical exercise is characterized by an increase in oxygen consumption for the whole body. It is even further associated with a rise in the production of oxidative stress (Cannon et al., 1990). The antioxidant enzymes and LPO are well known markers of oxidative stress. In addition, Hb, glucose and glycogen parameters are associated with exercise training. These parameters would vary to counteract ROS induced by an acute exhaustive exercise. Changes of

these parameters indicated that the two fractions may reduce the excessive oxidative stress. Generally, the swimming time to exhaustion was used as the index of the forced swimming capacity. Therefore, the result of the prolonged swimming time to exhaustion in n-butanol fraction treatment groups indicated that n-butanol fraction significantly improved the forced swimming capacity (Christiaan and Li, 1998).

LPO is a well-established biomarker of oxidative stress. It has been reported that strenuous exercise produces an increase in LPO in target tissues and blood (Packer, 1997). The results of the present study also provides the evidence of a significant increase in LPO in liver, red quadriceps and blood by an acute exhaustive swimming exercise, as described previously (Vincent et al., 2002). The results also showed a significant decrease in LPO level of n-butanol and water fractions treatment sedentary and recovery groups. This may be due to the fact that the two fractions both have reduce the excessive oxidative stress and help to restore physical performance, especially in n-butanol fraction treatment.

SOD is an important antioxidant enzyme which serves to convert superoxide radicals ($O_2^{\cdot}$-) into H_2O_2 plus O_2, in the body. Treatment with n-butanol and water fractions, SOD activity increased in liver, red quadriceps and blood, probably as the antioxidative activity of *P. decora* Franch (Li et al., 2002). The previous literatures reported that SOD activity increased showed a response to an acute exercise test in trained subjects (Yagi, 1987; Urso and Clarkson, 2003). Hence, SOD activities of acute exhaustive swimming exercise groups were significantly higher than those of sedentary groups. The SOD activity was significantly decreased in n-butanol and water fractions treatment after an acute exhaustive exercise. This may be due to the oxidative stress decreased with the two fractions treatment and the antioxidant property of the fractions against oxygen free radicals as reported by others (Ozdag, 2010).

GPx is present in the cytosol and mitochondrial matrix which catalyzes the reduction of H_2O_2, lipid and nonlipid hydroperoxides (Bansal et al., 2005). GPx activity was significantly increased in n-butanol fraction treatment and exercise groups when compared to sedentary control group as other reports (Leeuwenburgh et al., 1997; Powers et al., 1994). The decrease in GPx activity in recovery mice further suggests that the two fractions have antioxidant activity in scavenging/detoxifying the endogenous metabolic peroxides induced by an acute exhaustive exercise. In addition, the increase in LPO and transient increase in SOD as well as GPx further evidenced that although both SOD or GPx activities and LPO levels are induced by forced swimming exercise, they have the ability to fluctuate independently of each other (Chen et al., 2005).

LDH is abundant in the cytosol of heart, skeletal muscle, and other tissue cells. The key role of LDH is involved in the path of lactate oxidation and clearance. The significant increase in LDH activity evidently indicate that an acute exhaustive swimming exercise causes a severe metabolic acidosis mainly characterized by increases in plasma lactate as other reports (Daniel and Donald, 2004). In our study, we found a significant decrease in LDH activity in recovery groups when compared to exercise groups. These results suggest the decrease in lactate levels in exercise mice after 24 h recovery in the body. Further, treatment with n-butanol fraction indicates that this fraction of *P. decora* Franch may have better effect on lactate clearance.

Hb concentrations exhibit a great deal of variation in terms of absolute affinities for oxygen. Further, Hb concentration and haemoglobin-O_2 affinity is two traits involved in blood O_2 transport (Rezende et al., 2006). Hence, the decrease in Hb may be due to the increase in muscle blood flow in acute exhaustive swimming exercise. The results obtained in the present study show that diminished convective circulatory O_2 transport would be offset by improved diffusive transport of O_2 at the lungs and muscles after an acute exhaustive exercise. It is a possible mechanism to preserve O_2 delivery. The significant increase in Hb after 24 h recovery indicates a greater O_2 delivery to convective circulatory and a more improvement in endurance capacity (Calbert et al., 2002). Treatment with the two fractions may reverse the decreased Hb changes in exercise mice and enhance the increased Hb levels. These may further confirm the modified effect of *P. decora* Franch on Hb concentration changes in an acute exhaustive exercise.

Muscle glycogen and blood-borne glucose normally serve as major fuels for muscular activity (Hespel and Richter, 1990). Accordingly, muscular glycogen and blood-glucose contents represent the major fuels mobilized to support muscle energy demands during exercise (Pinho et al., 2006). It should be noted that blood glucose, glycogen in liver and red quadriceps was reduced in exercised when compared to sedentary control mice. This decreased glucose and glycogen was associated with the increased energy expenditure in exercise mice as the reported literature (Burneiko et al., 2006). We observed that blood and glycogen contents in n-butanol and water fraction treated mice were markedly higher in recovery mice than in exercise mice, especially in n-butanol fraction treatment. These facts suggest that *P. decora* may act as an energy source and promote energy recovery in the body.

Additionally, the results of the comparison between the n-butanol treatment groups and the corresponding water fraction treatment groups evidently suggest that n-butanol fraction is more pronounced in ameliorating the oxidative stress induced by an acute exhaustive swimming exercise. This may be due to the different components in the two fractions from *P. decora* Franch root. Generally speaking, n-Butanol fraction is rich in iridoid glycosides, phenylpropanoid glycosides (PPGs) etc.; however, the water fraction is rich in polysaccharides, soluble tannin,

mannitol (Chen, 1993; Wang et al., 1996). The antioxidant activity of the iridoid glycosides and PPGs from other medicinal plants has been evidenced as the reported literatures (Kwak et al., 2009; Nhiem et al., 2009; Ahmad et al., 2009; Jensen et al., 2010). Thus, photochemical and pharmacological studies, performed to isolate and to characterize chemically the compounds found in the two fractions are underway to further elucidate the mechanism involved in the antioxidant effect during intense or eccentric exercise.

In short, the two fractions may both protect mice against oxidative stress induced by an acute exhaustive exercise. The n-butanol fraction is more effective to attenuate the excessive oxidative stress induced by an acute exhaustive swimming exercise than water fraction from *P. decora* Franch. This fraction may significantly improve the forced swimming capacity. As physical exercise is a double-edged sword, further studies are required to elucidate the two fractions-exercise interaction effect on moderate or regular exercise *in vivo*.

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