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Full Length Research Paper

Effects of *n*-butanol and water fractions from *Pedicularis decora* Franch on oxidative stress in mice induced by a single bout of swimming exercise

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This study investigated the effect of water and *n*-butanol fractions from *Pedicularis decora* Franch roots on oxidative stress induced by a single bout of swimming exercise. ICR mice were treated orally with water and *n*-butanol fractions for three weeks. Lipid peroxidation (LPO) levels, transaminases activities significantly decreased and superoxide dismutases (SOD), glutathione peroxidase (GPx) activities, haemoglobin (Hb) and glycogen concentrations significantly increased in sedentary mice. The results also showed that the water and *n*-butanol fractions protected mice from a single bout swimming exercise induced oxidative stress through a decrease in LPO levels, SOD, GPx, LDH, transaminases activities and an increase in Hb, glycogen and glucose concentrations. Taken together, the two fractions from *P. decora* ameliorated the oxidative stress induced by a single bout of swimming. The *n*-butanol fraction is more effective than the water fraction.

Key words: *Pedicularis decora* Franch, *n*-butanol fraction, water fraction, oxidative stress, a single bout of swimming exercise.

INTRODUCTION

Conflicting results about the effects of exercise and training on physical performance have been reported in different exercise (Nakao et al., 2000; Burneiko et al., 2004). Simultaneously, physical exercise associated with oxidative stress depends on the type and intensity of exercise. Some studies show that moderate and regular exercise or training has been proved to give protection against oxidative stress. In contrast, other reports show that acute, eccentric and forced exercise or training generates excessive oxidative stress. Hence, excessive free radicals may further attack lipid membranes, proteins and DNA. The consequence is brought to cell damage (Bailey et al., 2001; Selamoglu et al., 2000). Studies have been shown that short-term swimming exercise (Aydin et al., 2009) or a 1000 m race at maximum velocity (White et al., 2001) can induce excessive oxidative stress. Some reports suggest that intense physical exercise (Fatouros et al., 2004) or exhausting exercise (Khanna et al., 1999; Oztasan et al., 2004) may increase free radicals especially reactive oxygen species (ROS) production exceeding the capacity of antioxidant defenses.

Therefore, it is necessary to prevent excessive oxidative stress in these exercise or training. A counter-part system to scavenge ROS has been developed in the body, such as Cu, Zn- and Mn-superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and other antioxidative enzymes (Wei and Lee, 2002; Rodriguez et al., 2004). However, these endogenous antioxidants are not enough to eliminate the oxidative damage. Increasing evidence indicates that administration of exogenous antioxidants may reduce the excessive oxidative stress during exercise (Gochman et al., 2007; Tsakiris et al., 2006; Bonina et al., 2005). For this reason, supplementing exogenous antioxidants play an important role in

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scavenging excessive ROS.

In exogenous antioxidants, some traditional medicine has antioxidative effect and can scavenge ROS. Medicinal plants still present a large source of natural antioxidants because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs (Srinivasan et al., 2007; Zanatta et al., 2007). Many species of *Pedicularis* are used traditionally for a wide variety of ethnomedical properties for the treatment of general debility, collapse, exhaustion, invigorate the circulation of blood, and aid digestion, full of vitality (Jiangsu College of New Medicine, 1975). *Pedicularis decora* Franch is an important species of *Pedicularis* Linn. and a Chinese folk herbal medicine. Chemical investigations of the plant have shown the presence of many minerals, a lot of polysaccharide, oligo-saccharide, monosaccharide such as mannitol, flavonoids (Zheng et al., 1993) and alkaloid (Hultin and Torssell, 1965) etc. Its characteristic constitutes are iridoid glycosides (Li et al., 1999), phenylpropanoid glycosides (PPGs) (Wang et al., 1996, 1997). Iridoid glycosides have been reported to possess antiviral (Akkol et al., 2007) and anti - inflammatory (Yuan et al., 2003) function. PPGs extracted from other medicinal plants have been shown to have antibiotic activities, to inhibit the mouse liver microsome (Wang et al., 1996), to inhibit the growth of tumor cells and repairing DNA damage (Li et al., 1999) and to scavenge free radicals (Zheng et al., 1993).

Previous investigations on *P. decora* Franch root presented that the crude extract could attenuate the oxidative stress in alloxan induced mice, in eccentric exercise animals (Yang et al., 2002; Li et al., 2002; Tian et al., 2002). In view of the pharmaceutical uses of *P. decora* Franch described previously, it was proposed to screen its successive extracts' effects on the oxidative stress in exercise or training.

MATERIALS AND METHODS

Animals

ICR mice (the Experimental Animal Center of Xi'an Jiaotong University, Shaanxi Province, China), weighting 20 to 22 g were used in this study. Animals were housed in cage fitted with stainless-steel wire-mesh bottoms in a temperature-controlled (22 ± 2℃) room with a 12:12 h light dark cycle, humidity (50 to 60%). The animals were fed with lab chow and tap water ad libitum. They were handled for at least 7 days prior to experiment. Mice were randomly assigned to sedentary (S) or exercise (E) groups. Sedentary groups include sedentary control (SC), sedentary water fraction (SW) and sedentary *n*-butanol fraction (SB) group. Exercise groups contain exercise control (EC), exercise water fraction (EW) and exercise *n*-butanol fraction (EB). SC and EC groups were given the saline alone (9 g/L, 0.1 ml per day p.o.) for 3 weeks. SW and EW groups received water fraction. SB and EB groups were treated with *n*-butanol fraction. The two plant fractions were all given at dose level of 15 g/kg b.w. per day p.o. for 3 weeks. All animals received humane care, in compliance with the animal ethics guidelines of Institutional Animal Ethics Committee.

Chemicals

All the chemicals were analytical grade and chemicals required biochemical assays were provided locally.

Preparation of plant extract

P. decora Franch root was collected in December 2008, at Taibai Mountain, Shaanxi province, China, and authenticated through Institute of Botany, Chinese Academy of Sciences, Beijing, China. A voucher specimen of the root is deposited in the School of Life Science and Technology, Xi'an Jiaotong University, China.

The root of *P. decora* Franch was dried at room temperature and crushed into a coarse powder. The powder was extracted three times with 95% EtOH to yield EtOH extract. After removal of EtOH, the EtOH extract was dissolved in *n*-BuOH – H_2O (1:1). The solvent was completely removed by rotary vacuum evaporator. The fractions were preserved in a refrigerator for further use (Chen, 1993).

Animal training program

In the present study, swimming was chosen as a single bout intense exercise training since swimming is a natural behavior of rodents (Kramer et al., 1993). The swimming exercise was performed for 2 h in a glass tank (77×38×39 cm) filled with water (35℃) to a height of 31 cm after 21 days pretreated with the different fractions of *P. decora* Franch root or saline. Mice were made to swim in groups of ten (Ravi et al., 2004). No deaths occurred during or after swimming in any of the exercise groups. Sedentary groups of mice were confined to cage activity and handled only during changing of cages.

Tissue and blood sample preparation

After a single bout of swimming exercise, the mice were salvaged and dried with gauze. Mice were sacrificed killed by decapitation. Blood was rapidly collected and serum was separated by centri-fugation. Liver, red quadriceps and heart of each group mice was immediately collected. Tissues were homogenized in 50 mM phosphate buffer solution (PH 7.4) using a tissue Homogenizer at 4℃. The supernatant was collected after centrifuged at 1,000 g for 5 min and stored at -70℃ for further analyses. The content of protein in the homogenate was determined by the method of Lowry et al. (1951).

Lipid peroxidation (LPO) assay

LPO was assayed according to the method of Wright et al. (1981). The reaction mixture, in a total volume of 1.0 ml, contained 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml of tissue homogenate (10%, w/v), 0.2 ml ascorbic acid (100 mM), 0.02 ml ferric chloride (100 mM), and was incubated at 37℃ 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 of 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. At the end, the tubes were shifted to an ice-bath and centrifuged at 2500× g for 10 min. The amount of malonaldehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm against a reagent blank.

Superoxide dismutase (SOD), glutathione peroxidase (GPx) assay and lactic dehydrogenase (LDH)

SOD activity was determined by monitoring the auto-oxidation of

pyrogallol (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 (Hafeman et al., 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.

Evaluation of haemoglobin (Hb)

The Hb concentration of hemolysates was determined spectrophotometrically using the potassium ferricyanide and potassium cyanide (Drabkin's reagent). The Hb solution was dissolved in Drabkin's reagent to a concentration of 0.5 to 1.0 mg/ml and the A540 nm read against an appropriate blank solution after 5 min (Winterbourn 1990).

Evaluation of glucose and glycogen levels

Glycogen contents were determined as previously described by Lo et al. (1970). Briefly, liver and red quadriceps samples were collected immediately, cut, weighed, and boiled for 30 min in 1 ml of 30% KOH saturated with $NaSO_4$. Once tissues were completely digested, glycogen was precipitated with 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, absorbance was determined at a wavelength of 490 nm. The concentrations of serum glucose were determined by the glucose oxidase method (De Sousa et al., 2004).

Evaluation of aspartate and alanine transaminases (AST and ALT) and alkaline phosphatase (ALP) activities

AST and ALT were determined by the method of Wooten, (1964). The method is based on the ability of the enzymes to form pyruvate, which reacts with 2, 4-dinitrophenylhydrazine in hydrochloric acid.

The hydrazone thus formed turns into an orange complex in alkaline medium, which was measured at 540 nm. ALP was measured according to King (1965). Disodium phenyl phosphate was catalyzed by ALP (at pH10) and the phenol was liberated. Then reaction with 4-amino antipyrine (4-AAP), ferripotassium cyanide and the absorbance measurement at 500 nm was used for quantization.

Statistical analysis

All data were expressed as mean ± S.D. One-way analysis of variance (ANOVA) followed by Student's *t*-test were used to identify significance in different groups. Probability values $P \leq 0.05$ were considered significantly.

RESULTS

Effect of the n-butanol and water fractions of *P. decora* Franch on LPO levels

LPO levels of mice liver, red quadriceps, heart and blood in different groups were shown in Figure 1. LPO was significantly decreased in *n*-butanol and water fractions treatment groups except heart LPO in SW group when compared to SC group. However, LPO was significantly increased after a single bout of swimming exercise as compared to group SC. It was observed that LPO significantly decreased in EW and EB groups when compared to EC group.

Effect of the n-butanol and water fractions of *P. decora* Franch on SOD activities

SOD activity was significantly increased in *n*-butanol fraction of *P. decora* Franch root treatment group in red quadriceps, heart and blood when compared to SC group (Figures 2B to D). However, treatment water fraction did not significantly alter the SOD activity versus SC group. Comparisons of data obtained in the exercise groups revealed that a single bout swimming exercise markedly increased the SOD activities. It is worth noting that SOD activities were significantly decreased in the two fractions administration groups when compared to EC group (Figure 2).

Effect of the n-butanol and water fractions of *P. decora* Franch on GPx activities

As shown in Figure 3, GPx activity was significantly increased in SW and SB group when compared to group SC, but a small increase in blood and red quadriceps in SW group in Figures 3B and D. Additionally, there was a significant elevation in liver, red quadriceps, heart and blood GPx activity in exercise groups as compared to SC group. However, there was a significant reduction in the activity of GPx in the two fractions treated exercise groups, but not in red quadriceps in Figure 3B.

Effect of the n-butanol and water fractions of *P. decora* Franch on glycogen, glucose concentrations and LDH activities

As shown in Table 1, glycogen concentrations were significantly increased in *n*-butanol and water fractions treatment groups when compared with SC group. However, glycogen concentrations were significantly decreased in swimming exercise groups. But no significant difference was observed in liver glycogen in *n*-butanol fraction administration group and red quadriceps

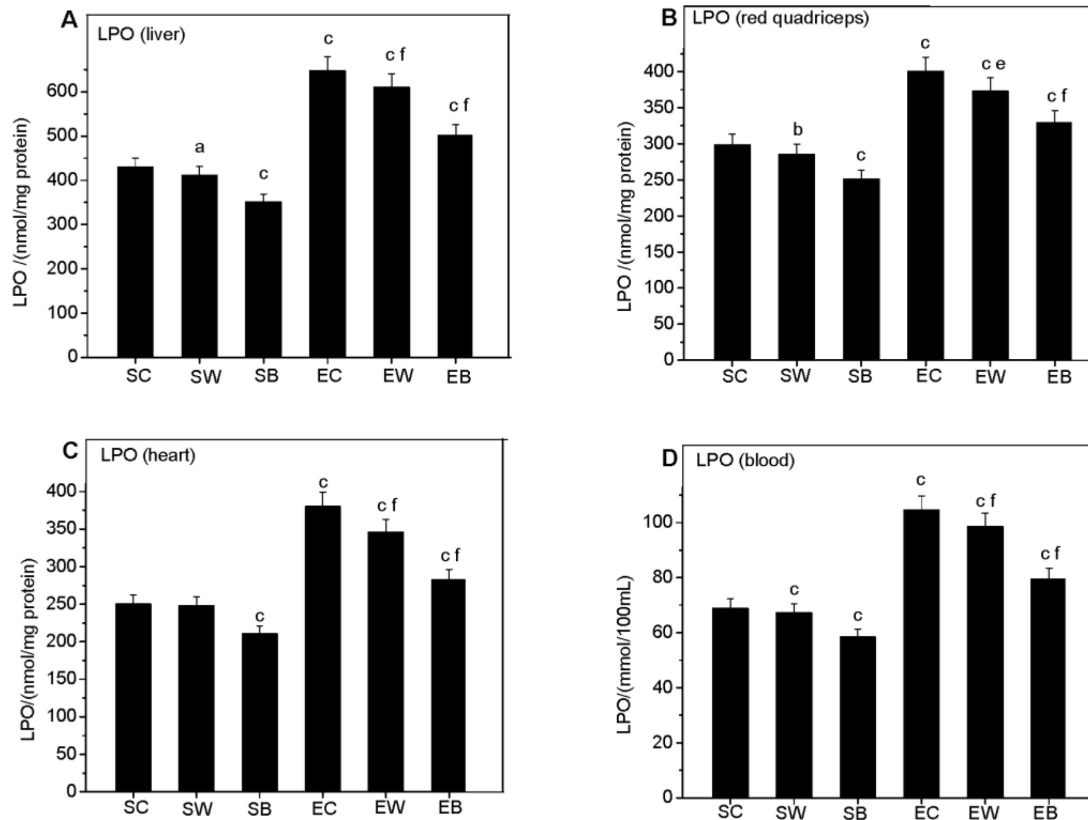

Figure 1. Effects of *n*-butanol and water fractions from *P. decora* Franch roots on LPO levels in liver, red quadriceps, heart and blood in sedentary and swimming groups' mice. All the values are expressed as mean ± S.D. for groups of ten animals each. [a] $P < 0.05$, when compared to group SC. [b] $P < 0.01$, 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 TC. [e] $P < 0.01$, for groups EW and EB when compared with group EC. [f] $P < 0.001$, for groups TWS and TBS when compared with group EC.

glycogen in water fraction administration group, a light increase in EB group as compared to the SC group. Additionally, treatment with the two fractions did not cause a significant difference in the blood glucose levels among sedentary groups. It was observed that glucose levels significantly decreased in EC and EW groups when compared to SC group, but not in EB group. A slight increase in blood glucose levels of the two fractions treated exercise groups was observed when compared to EC group in the experiment. Also, treatment of the two fractions did not cause a significant change for LDH activity among sedentary groups. A significant increase was observed in exercise groups except EB group. LDH activities were significantly decreased in EB group when compared to EC group.

Effect of the n-butanol and water fractions of *P. decora* Franch on Hb levels and AST, AKP, ALT activities

Table 2 summarized the activities of AST, ALT, ALP and Hb levels in sedentary and swimming exercise animals. The activities of blood AST, ALT and ALP were significantly decreased in SB group and ALP in SW group from the SC group, but not in SW group for the AST, ALT activities. However, there was a significant increase in exercise groups. The activities of AST, ALT, ALP were significantly decreased in the two fractions treated exercise groups when compared to EC group.

The levels of Hb in SB group were significantly higher and a slightly higher in SW group than the SC group. Hb levels were significantly decreased in EW group and not in EB group. However, a significant increase was observed in the two fractions treated groups when compared to EC group.

Comparison between the n-butanol and water fractions of *P. decora* Franch treatment groups

As shown in Table 3, *n*-butanol fraction treatment groups showed a significant lower ($P<0.001$) LPO levels in sedentary and swimming exercise mice. It was observed that SOD activity was significantly higher ($P<0.001$) in SB group and significantly lower ($P<0.05$, $P<0.01$, $P<0.001$,

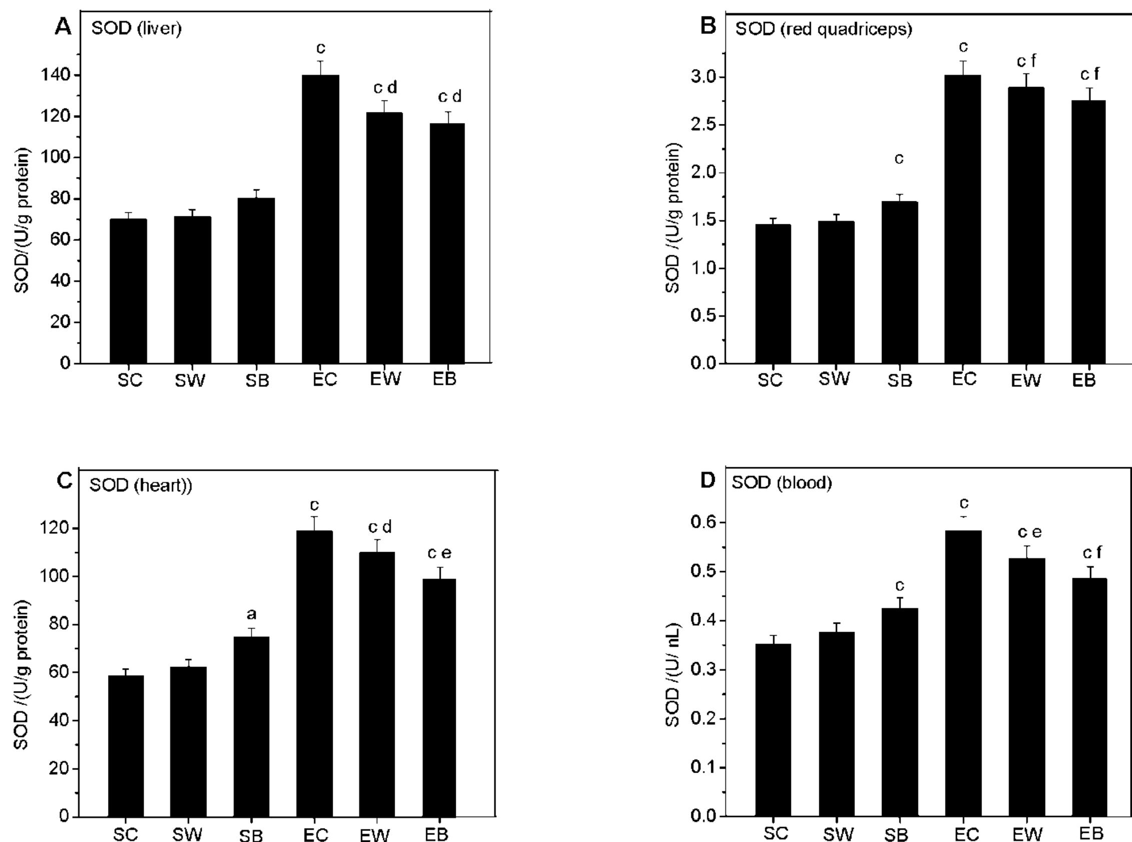

Figure 2. Effects of *n*-butanol and water fractions from *P. decora* Franch roots on SOD activities in mice liver, red quadriceps, heart, blood in sedentary and swimming groups. All the values are expressed as mean ± S.D. for groups of ten animals each. [a] $P < 0.05$, when compared to group SC. [b] $P < 0.01$, 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 TC. [f] $P < 0.001$, for groups EW and EB when compared with group EC.

respectively) in EB group except in liver tissue. As similar to SOD activity, treatment with *n*-butanol fraction caused a significant effect (P<0.01, P<0.001) on the activities of GPx but not in exercise groups for liver tissue. The change of Hb levels was significant difference (P<0.01) in exercise groups but not in sedentary groups for the two fractions treatment. In addition, a marked decrease (P<0.05, P<0.01, P<0.001, respectively) in the activities of AST, ALT and ALP was observed in *n*-butanol fraction treatment groups. However, the changes of glycogen, glucose concentrations and LDH activities had no significant difference between *n*-butanol and water fractions treatment animals.

DISCUSSION

It is well known that strenuous exercise is characterized by an increase in oxygen consumption by the whole body and is associated with a rise of the production of oxidative stress (Tsakiris et al., 2006). This may further cause a series of physiological and biochemical changes that occur during exercise (Metin et al., 2002). Additionally, antioxidant supplements are marketed and used by athletes as a means to counteract the excessive oxidative stress induced by eccentric exercise. As previously described, the crude extract of *P. decora* could reduce the harmful effects of oxidative stress in eccentric exercise mice. In the present study, the effect of the *n*-butanol and water fractions from *P. decora* Franch on oxidative stress induced by a single bout of swimming exercise was further investigated in ICR mice.

LPO, an important process in cellular damage, which is mediated through the free radical metabolites, affects the antioxidants and reduces the antioxidant status in the cell (Bray and Taylor, 1993). Our results showed a decreased level of LPO in the two fractions treatment mice. The results indicate that the two fractions may enhance the antioxidative activity in the organism. It has been reported that strenuous or intense exercise results in an increase in the marker of LPO in the body. Therefore, the significantly increased LPO levels in mice liver, blood cell,

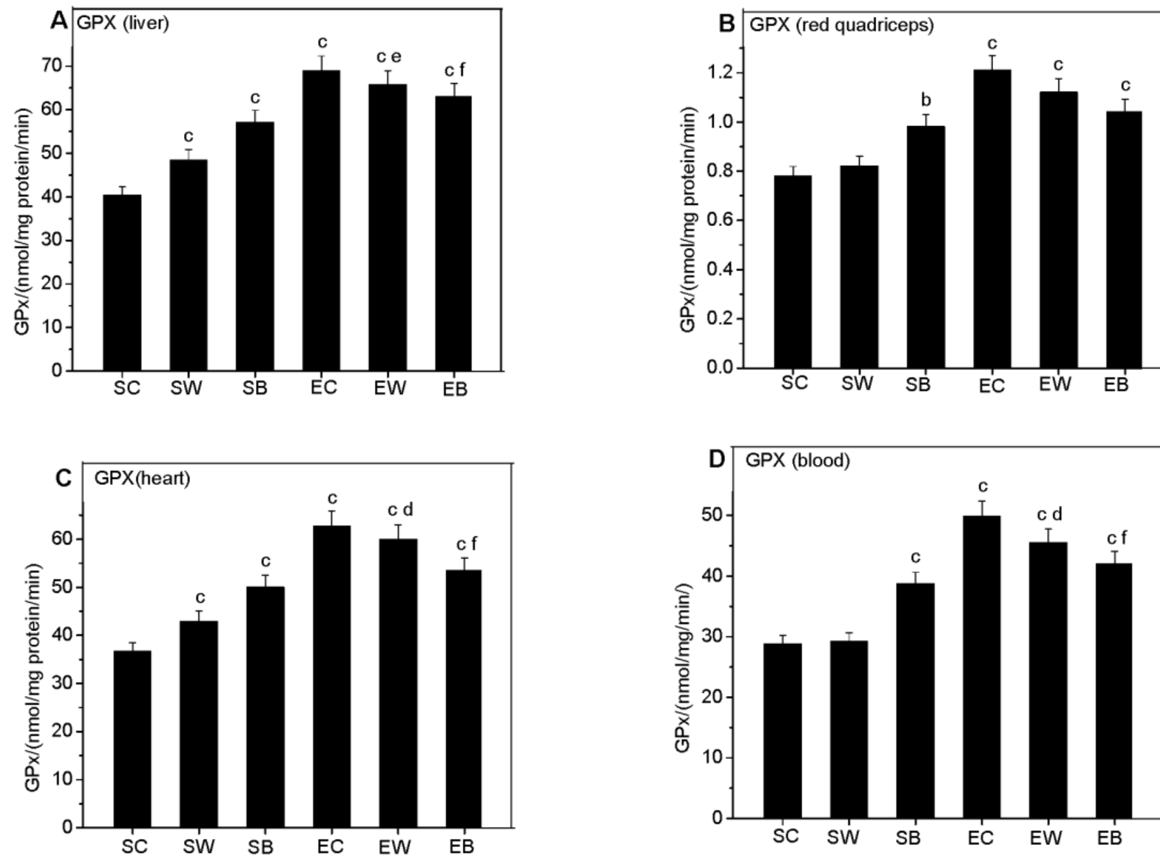

Figure 3. Effects of *n*-butanol and water fractions on GPx activities in mice liver, red quadriceps, heart, blood in sedentary and swimming groups. All the values are expressed as mean ± S.D. for groups of ten animals each. [a] $P < 0.05$, when compared to group SC. [b] $P < 0.01$, when compared to 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.

red quadriceps and heart, induced by an acute intense swimming exercise, are compatible with other reports (Avery et al., 2003; Packer, 1997). Generally, radicals such as superoxide anion, hydroxyl radical and H_2O_2, produced in acute swimming exercise, increased excessive oxidative stress. Prior treatment with the two fractions considerably decreased LPO levels in target tissues and blood, this may be due to the scavenging of free radicals for the two extracts.

Administration with the two fractions, we found, SOD and GPx activities significantly increased between sedentary conditions. The results may be due to the well-known antioxidant action of *P. decora* Franch (Yang et al., 2002). Acute or strenuous exercise including an acute swimming exercise is known to increase oxidative stress production.

An increase in oxidative stress production causes the organism to maintain its own antioxidant enzymes system more actively (Metin et al., 2003). Therefore, some studies reported that the activities of GPx, glutathione reductase (GR) and SOD increased transiently in exercise conditions (Parise et al., 2005; Stoppa et al., 2006). In the present study, the increase in the activity of SOD and GPx was noticed in blood, liver, heart and red quadriceps after a single bout of swimming exercise. These results were in agreement with the previous reports.

It is 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). The activities of SOD and GPx significantly reduced in EW and EB groups, though these values not returned to sedentary controls values. This indicates that the two fractions may both counteract the excessive oxidative stress induced by a single bout of swimming exercise. Energy intake in relation to requirements and expenditure is known to be an important determinant of lipid profile (Schrauwen and Westerterp, 2000).

The results showed that the two fractions of *P. decora* Fanch could increase glycogen concentrations in mice liver and red quadriceps. After a single bout swimming

Table 1. Effects of n-butanol and water fractions from *P. decora* Franch roots on mice glycogen and blood glucose levels in sedentary and swimming groups.

Groups	Glycogen (mg/g)		Glucose (mg/100 ml)	LDH (U/100 ml)
	Liver	Red quadriceps		
SC	5.85±2.41	1.14±0.59	83.5±15.3	113.7±17.8
SW	6.82±2.15	1.98±1.02[a]	81.2±16.7	115.2±14.7
SB	8.43±2.43[a]	2.05±0.10[c]	80.2±15.3	116.1±16.9
EC	2.73±1.04[b]	0.54±0.11[b]	63.2±9.7[b]	142.6±19.1[b]
EW	3.90±0.92[a,d]	1.41±0.79[e]	70.4±11.8[a]	130.2±16.3[a]
EB	4.58±1.46[e]	1.58±0.82[f]	73.5±12.6	120.5±15.6[d]

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.

Table 2. Effect of *n*-butanol and water fractions from *P. decora* Franch roots on mice blood Hb levels, AST, AKP and ALT activities in sedentary and swimming groups.

Groups	Hb (g/L)	AST (IU/˙L)	ALP (IU/˙L)	ALT (IU/˙L)
SC	128.8±9.4	78.3±10.8	22.7±0.93	10.45±0.82
SW	131.8±9.0	76.2±11.2	20.82±0.84[c]	10.32±0.75
SB	140.5±10.2[a]	65.7±9.8[a]	17.99±0.45[c]	9.01±0.84[b]
EC	100.9±7.8[a]	121.4±21.4[c]	38.44±1.01[c]	17.43±1.21[c]
EW	110.5±8.4[c,d]	110.0±19.3 [c]	34.23±0.93[c,f]	15.81±1.02[c,e]
EB	125.9±9.2[f]	90.8±15.4[a,e]	28.72±1.03[c,f]	13.70±0.91[c,f]

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.

exercise, glycogen concentrations in mice liver and red quadriceps were reduced when compared to sedentary mice, and this was associated with the increased energy expenditure. This finding is in agreement with that previously reported by Regina et al. (Burneiko et al., 2006). Additionally, *n*-butanol and water fractions could counter the decreased glycogen induced by a single bout swimming exercise.

Glycogen deposits are important for maintaining physical performance during exercise. These results suggest that components of the two fractions may increase the deposits of glycogen and the physical performance. Blood glucose levels were not affected in the sedentary group, including the two fractions treated mice.

However, blood glucose decreased in swimming exercise groups. The finding is consistent with other reports (Mayumi et al., 2006). In the two fractions treatment mice, glucose level was higher as compared to the exercise control group. Glycogen and glucose measurements can reflect energy state, these facts suggest that the two fractions from *P. decora* Franch may act as an energy source similar to the traditional crops (Abbasi et al., 2010; Hussain et al., 1990).

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. Our results indicate that a single bout swimming exercise causes a severe metabolic acidosis mainly characterized by increases in plasma lactate as other reports (Daniel and Donald, 2004). The decrease in LDH activities was corresponded with the decrease in lactate levels in the two fractions treated exercise mice in the body.

Haemoglobins (Hbs) exhibit a great deal of variation in terms of absolute affinities for oxygen (Raffaele et al., 1996). In our study, the higher Hb valuables were observed in SW and SB groups when compared with SC group, the results demonstrate that the two fractions may result in an elevation of affinity for O_2 and CO_2 delivery ability (Metin et al., 2003). Decrease in the Hb levels in response to the acute swimming exercise is associated mainly to oxidative stress, the results partialy suggest that “sports anaemia” is associated with intensive physical exercise. However, these values were within physiological ranges and cannot be accepted as a complete ‘sports anaemia’ (Calbet et al., 2002). Hence, Hb levels significantly increased in EW and EB groups indicated that the two fractions had the potential to resist the production of ‘sports anaemia’ during intensive

Table 3. Comparison between the two fractions treatment groups.

Parameters	Sedentary groups	Exercise groups
LPO	P<0.001	P<0.001
GPx	P<0.001	P<0.01, P<0.001, NS (in liver)
SOD	P<0.001, NS (in liver)	P<0.05, P<0.01, P<0.001, NS (in liver)
Hb	NS	P<0.01
AST	P<0.05	P<0.05
ALT	P<0.01	P<0.001
ALP	P<0.001	P<0.001
LDH	NS	NS
Glycogen	NS	NS
Glucose	NS	NS

*P values were considered when *n*-butanol fraction treatment group compared with the corresponding. Water fraction treatment group. NS: denotes no significant difference.

physical exercise.

The liver is regarded as one of the central metabolic organs, regulating and maintaining homeostasis. ALP and the serum transaminases of AST, ALT, show functional activity of liver. As the agent caused hepatotoxic effects, there was an increase in the activities of these enzymes, which was a generalized effect due to the toxic agent mainly affecting the liver functions (Bansal et al., 2005). In the present study, the decreases in AST, ALT and ALP levels were detected in SW and SB groups. Additionally, it was also observed that the liver function enzymes, which are the biochemical indicators of hepatic injury, remained substantially decreased in EW and EB groups, though still high when compared to SC group. The results show that the acute bout of swimming exercise causes hepatic damage as other strenuous exercise (Selamoglu et al., 2000; Fatouros et al., 2004). The findings indicate that the two fractions from *P. decora* Franch root, to a certain extent, possess hepatoprotective effect as previous reports (Yang et al., 2002).

Generally, *n*-BuOH fraction is rich of iridoid glycosides, PPGs etc., however, the water fraction is rich of polysaccharides, soluble tannin, mannitol (Chen, 1993; Wang et al., 1996). Based on the previous description about the function of chemical constitutes, the parameters assayed in the present study evidently showed that *n*-butanol fraction had more effect on antioxidative and hepaprotective activity. But, photochemical and pharmacological studies, performed to isolate and to characterize chemically the compounds found in the two fractions are still needed to further elucidate the mechanism involved in the antioxidant effect during exercise.

Conclusion

In conclusion, our results clearly demonstrated that both the two fractions of *P. decora* Franch had attenuated the oxidative stress and hepatic injury induced by a single bout of swimming exercise. Further, the *n*-butanol fraction was more potent than the water fraction. Generally, physical exercise is a double-edged sword: when practiced strenuously it causes oxidative stress and cell damage, in this case antioxidants should be given. But when practiced in moderation, it increases the expression of antioxidant enzymes and thus should be considered as an antioxidant. The interaction of the two fractions of *P. decora* Franch and the moderate or regular exercise will be necessary in further studies.

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REFERENCES

Abbasi FM, Ahmad H, Perveen F, Inamullah, Sajid M, Brar DS (2010). Assesment of genomic relationship between *Oryza sativa* and *Oryza australiensis*. Afr. J. Biotechnol., 9: 1312-1316.

Akkol EK, Tatli II, Akdemir ZS (2007). Antinociceptive and anti-inflammatory effects of saponin and iridoid glycosides from *Verbascum pterocalycinum var. mutense Hub.-Mor.* Nat. Hist. C, 62: 813-820.

Avery NG, Kaiser JL, Sharman MJ, Scheett TP, Barnes DM, Gómez AL, Kraemer WJ, Volek JS (2003). Effects of vitamin E supplementation on recovery from repeated bouts of resistance exercise. J. Strength Cond. Res., 17: 801-809.

Aydin C, Sonat SK, Sahin Sk, Cangul IT, Ozkaya G (2009). Long term dietary restriction ameliorates swimming exercise-induced oxidative stress in brain and lung of middle-aged rat. Indian J. Exp. Biol., 47: 24–31.

Bailey DM, Davies B, Young IS (2001). Intermittent hypoxic training: implications for lipid peroxidation induced by acute normoxic exercise in active men. Clin. Sci., 101: 465-471.

Bansal AK, Bansal M, Soni G, Bhatnagar D (2005). Protective role of Vitamin E pre-treatment on *N*-nitrosodiethylamine induced oxidative stress in rat liver. Chem. Biol., 156: 101-111.

Bass A, Brdicka PE, Eyer P, Hofer S, Pette D (1969). Metabolic differentiation of distinct muscle types at the level of enzymatic organization. Eur. J. Biochem., 10: 198-206.

Bonina FP, Puglia C, Cimino F, Trombetta D, Tringali G, Roccazzello AM, Insirello E, Rapisarda P, Saija A (2005). Oxidative stress in handball players: effect of supplementation with a red orange extract. Nutr. Res., 25: 917-924.
Bray TM, Taylor CG (1993). Tissue glutathione, nutrition and oxidative stress. Can. J. Physiol. Pharmacol., 71: 746-751.
Burneiko RC, Diniz YS, Faine LA, Galhardi CM, Padovani CR, Novelli EL, Cicogna AC (2004). Impact of training program on lipid profile and cardiac health. Biol. Res., 37: 53-59.
Burneiko RC, Diniz YS, Galhardi CM, Rodrigues HG, Ebaid GM, Faine LA, Padovani CR, Cicogna AC, Novelli EL (2006). Interaction of hypercaloric diet and physical exercise on lipid profile, oxidative stress and antioxidant defenses. Food Chem. Toxicol., 44: 1167-1172.
Calbet JA, Råegran G, Boushel R, Sødergaard H, Saltin B, Wagner PD (2002). Effect of blood haemoglobin concentration on V_{O2}max and cardiovascular function in lowlanders acclimatised to 5260 m. J. Physiol., 2: 715-728.
Chen Q (1993). The Chinese Medicine Pharmacology Method. People's Medical Publishing House, Beijing, P. 93.
Chen Y, Kong LD, Xia X, Kung HF, Zhang L (2005). Behavioral and biochemical studies of total furocoumarins from seeds of *Psoralea corylifolia* in the forced swimming test in mice. J. Ethnopharmacol., 96: 451-459.
Daniel EW, Donald CJ (2004). Effects of swimming on metabolic recovery from anoxia in the painted turtle. J. Exp. Biol., 207: 2705-2713.
De SE, Zanatta L, Seifriz I, Creczynski PTB, Pizzolatti MG, Szpoganicz B, Silva FR (2004). Hypoglycemic effect and antioxidant potential of kaempferol-3, 7-O-(α)-dirhamnoside from *Bauhinia forficate* leaves. J. Nat. Prod., 67: 829-832.
Fatouros IG, Jamurtas AZ, Viliotou V, Pouliopoulou S, Fotinakis P, Taxildaris K, Deliconstantinos G (2004). Oxidative stress responses in older men during endurance training and detraining. Med. Sci. Sports Exer., 36: 2065-2072.
Gochman E, Reznick AZ, Avizohar O, Ben AA, Levy Y (2007). Exhaustive exercise modifies oxidative stress in smoking subjects. Am. J. Med. Sci., 333: 1-6.
Hafeman DG, Sunde RA, Hoekstra WG (1974). Effect of dietary selenium and erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 104: 580-587.
Hultin E, Torssell K (1965). Alkaloid-screening of Swedish plants. Phytochem., 4: 425-436.
Hussain S, Ghaffar A, Aslam M (1990). Biological control of Macrophomina phaseolina charcoal rots of sunflower and mung bean. Egypt J. Phytopathol., 130: 157-160.
Jiangsu College of New medicine (1975). A Dictionary of the Traditional Chinese Medicines. Shanghai Sciences and Technology Press, Shanghai, pp. 286-287.
Khanna S, Atalay M, Laaksonen DE, Gul M, Roy S, Sen CK (1999). Alpha-lipoic acid supplementation: tissue glutathione homeostasis at rest and after exercise. J. Appl. Physiol., 86: 1191-1196.
King J (1965). The phosphohydrolases-acid and alkaline phosphatases, in: Practical Clinical Enzymology. D Van Nostrand Co. Ltd., London, pp. 191-208.
Kramer K, Dijkstra H, Bast A (1993). Control of physical exercise of rats in a swimming basin. Physiol. Behav., 53: 271-276.
Li C, Gu D, Tao B (1999). Iridoid glycosides from *Pedicula dicora* Franch. J. Trad. Chinese Med., 24: 41-44.
Li F, Tian J, Yang J (2002). Physical performance enhanced by *Pedicularis dicora* on exercised rats. J. Shaanxi Nor. Uni., 30: 89-91.
Lo S, Russell JC, Taylor AW (1970). Determination of glycogen in small tissue samples. J. Appl. Physiol., 28: 234-236.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem., 193: 265-275.
Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.
Mayumi I, Tomoyuki K, Jiro T, Kazunaga Y (2006). Effects of astaxanthin supplementation on exercise-Induced fatigue in mice. Biol. Pharm. Bull., 29: 2106-2110.
Metin G, Atukeren P, Alturfan AA, Gülyaşar T, Kaya M, Gümüştaş MK (2003). Lipid peroxidation, erythrocyte superoxide dismutase activity and trace metals in young male footballers. Yousei Med. J., 44: 979-986.
Metin G, Atukeren P, Gümüştaş MK, Belce A, Kayserilioglu A (2002). The effect of vitamine E treatment on oxidative stress generated in trained rats. Tohoku J. Exp. Med., 198: 47-53.
Nakao C, Ookawara T, Kizaki T, Ohishi S, Miyazaki H, Haga S, Sato Y, Ji LL, Ohno H (2000). Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissues. J. Appl. Physiol., 88: 649-654.
Oztasan N, Taysi S, Gumustekin K, Altinkaynak K, Aktas O, Timur H, Siktar E, Keles S, Akar S, Akcay F, Dane S, Gul M (2004). Endurance training attenuates exercise-induced oxidative stress in erythrocytes in rat. J. Appl. Physiol., 91: 622-627.
Packer L (1997). Oxidants, antioxidant nutrients and the athlete. J. Sports Sci., 15: 353-363.
Parise G, Phillips SM, Kaczor JJ, Tarnopolsky MA (2005). Antioxidant enzyme activity is up-regulated after unilateral resistanc eexercise training in older adults. Free Radic. Biol. Med., 39: 289-295.
Raffaele P, Gabriella A, Amalia L, Antonio G, Alessandro D, Bruno G (1996). Diving behaviour and haemoglobin function: The primary structure of the α- and β-chains of the sea turtle (Caretta) and its functional implications. Biochem. J., 316: 959-965.
Ravi KT, Subramanyam MV, Asha DS (2004). Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: Relationship to swim intensity and duration. Comp. Biochem. Phys., Part B 137: 187-196.
Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ (2004). Regulation of antioxidant enzymes: A significant role for melatonin. J. Pineal Res., 36: 1-9.
Schrauwen P, Westerterp KR (2000). The role of high-fat diets and physical activity in the regulation of body weight. Br. J. Nutr., 84: 417-427.
Selamoglu S, Turgay F, Kayatekin BM, Gonenc S, Yslegen C (2000). Aerobic and anaerobic training effect on the antioxidant enzymes of the blood. Acta Physiol. Hung., 87: 267-273.
Srinivasan R, Chandrasekar MJ, Nanjan MJ, Suresh B (2007). Antioxidant activity of *Caesalpinia digyna* root. J. Ethnopharmacol., 113: 284-291.
Stoppa GR, Cesquini M, Roman EA, Ogo SH, Torsoni MA (2006). Aminoguanidine prevented impairment of blood antioxidant system in insulin-dependent diabetic rats. Life Sci., 78: 1352-1361.
Tian J, Yang J, Li F, Yao Y (2002). Effects of *Pedicularis dicora* on exhaustive swimming exercise mice. J. Shaanxi Med., 31: 375-377.
Tsakiris S, Reclos GJ, Parthimos T, Tsakiris T, Parthimos N, Schulpis KH (2006). α-Tocopherol supplementation restores the reduction of erythrocyte glucose-6-phosphate dehydrogenase activity induced by forced training. Pharmacol. Res., 54: 373-379.
Wang C, Jia Z, Li G (1997). Phenylpropanoid and Irioid glycosides from *Pedicularis torta*. Phytochem., 45: 159-166.
Wang P, Kung J, Zheng R, Yung Z, Lu J, Guo J, Jia Z (1996). Scavenging Effects of Phenylpropanoid Glycosides from *Pedicularis* on Superoxide Anion and Hydroxyl Radical by the Spin Trapping Method. Biochem. Pharmacol., 51: 687-691.
Wei YH, Lee HC (2002). Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. Exp. Biol. Med., (Maywood), 227: 671-682.
White A, Estrada M, Walker K, Wisnia P, Filgueira G, Valdés F, Araneda O, Behn C, Martínez R (2001). Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 128: 99-104.
Winterbourn CC (1990). Oxidative reactions of hemoglobin. Methods Enzymol., 186: 265-272.
Wooten ID (1964). Microanalysis in Medical Biochemistry. J and A Churchill Ltd., London, pp. 101-103.
Wright JR, Colby HD, Miles PR (1981). Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. Arch. Biochem. Biophys., 206: 296-304.
Yang J, Gao M, Li F (2002). Effects of *Pedicularis dicora* fractions on Anti-oxidation Injury in Mice. J. Chin. Med. Mat., 25: 37-39.

Yuan CS, Zhang Q, Xie WD, Yang XP, Jia ZJ (2003). Iridoids from *Pedicularis kansuensis forma albiflora*. Pharm., 58: 428-430.

Zanatta L, Sousa E, Cazarolli LH, Junior AC, Pizzolatti MG, Szpoganicz B, Silva FR (2007). Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycemia in alloxan-diabetic rats. J. Ethnopharmacol., 109: 151-155.

Zheng RL, Wang PF, Li J, Liu ZM, Jia ZJ (1993). In inhibition of the autoxidation of linoleic acid by phenylpropanoid glycosides from *Pedicularis* in micelles. Chem. Phys. Lipids, 65: 151-154.