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Full Length Research Paper

Compounds of *Purslane* extracts and effects of anti-kinetic fatigue

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***Purslane*, a famous Chinese medicinal herb, has a long history of use as a traditional remedy for many diseases. In this study, compounds of *Purslane* extracts (PE) are analyzed according to thin layer chromatography (TLC) and anti-kinetic fatigue effects was evaluated by forced swimming exercise of male mice. The results suggest that the main compounds *Purslane* extracts included organic acid, flavonoids, alkaloids, monoterpene glycoside, catecholamines, saponin, polysaccharide. *Purslane* extracts had significant anti-kinetic fatigue effects on mice, which enhanced the maximum swimming capacity of mice by increasing fat utilization, and by delaying the accumulation of plasma lactate and ammonia.**

Key words: *Purslane* extracts, compounds, anti- kinetic fatigue.

INTRODUCTION

Kinetic fatigue refers to that due to over-exercise, the body cannot maintain physiologically its specific level or cannot maintain the predetermined exercise intensity, manifested as mental and physical fatigue (Li and Wei, 2005; Wang et al., 2006; Li et al., 2000). Over-intensity of exercise will lead to kinetic fatigue and affect sport skills brought into play. Therefore, to prevent and release kinetic fatigue is the hot topic in the researches on improving exercise quality. The exercise capacity can be improved by supplementing energetic substance, releasing metabolic production and administrating tonics, but these bring harms to the body even though retarding the fatigue (Li and Wei, 2005). In addition, some of the drugs are forbidden by International Olympic Committee. During seeking for the safe and effective anti-kinetic fatigue methods, the specialty of Chinese herbal medicine has drawn the attentions of scholars in the world.

Purslane (*Portulaca oleracea L.*) (Chinese name Ma-Chi-Xian) is a grassy plant with small yellow flowers and stems sometimes flushed red or purple, which grows widely in different areas of the world including the north of China (Zhao et al., 2007; Sun et al., 2006; Li et al., 2009). It is widely used in China not only as an edible plant, but also as a traditional Chinese herbal medicine for alleviating pain and swelling. It has the abilities of anti-bacteria, anti-virus, anti-antherasis, anti-caducity, anti-diabetes and enhancing immunity (Yang et al., 2007; Ou et al., 2007; Lu et al., 2007). However, there has been little research on the effects of anti-kinetic fatigue, and its compounds have not been clarified. In this study, the compounds of *Purslane* extracts (PE) are analyzed and the anti-kinetic fatigue effect are investigated through forced swimming exercise of male mice.

MATERIALS AND METHODS

Plant materials

Purslane was collected in Zhejiang Province in July. Identification of plant was verified by associate Professor Mei Li, Hangzhou Normal University. Fresh and intact *purslane* dried in the shade was chosen as experimental material.

Preparation of *Purslane* extracts and characterization

The shade dried whole plants (500 g) were powdered, and the powder was extracted with distilled water (1 g/10 ml) with constant stirring for 4 h and then filtered through a filter paper. Residue was

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again extracted as above with water (Tang et al., 2006; Cui et al., 2007; Hou et al., 2008). The filtrates from the extractions were combined and dried in a rotary evaporator (RE52, Kylin-Bell Lab Instruments Co., Jiangshu, China) under reduced pressure at 55pa, and then freeze-dried in a lyophilizer. The yield of the water extract was approximately 20% of the plant powder. (Since the heat sensitivity of the extract with reference to bio-activity was not known, the extraction was carried out at low temperature without using rigorous extraction procedures).

The extract was examined by thin layer chromatography (TLC) analysis to identify the main compounds (GF254, Qingdao Haiyang Chemical Co., Shandong, China). The *Purslane* solution was dotted on the TLC plates, and n-butanol-acetic acid: H_2O (19:1) was used as the solvent system, then the indicators were sprayed on the plates, respectively (Tu, 2000; Xin et al., 2008). The plates were heated at 105°C for 10 min in an oven. Nine kinds of indicator system were used to identify the compounds of *Purslane*.

Experimental animals

Kunming male mice (6-8 week) weighing 18-22 g were obtained from Animal Department of Zhejiang Institute of Traditional Medical and Pharmaceutical Sciences (Zhejiang, China). These mice housed for 1 week in a room maintained at (25±1)°C with 60% relative humidity and provided with free access to laboratory standard diet and water. The room lights were on for 12 h/day starting at 7:00. Mice were treated according to the ethical guidelines of the Animal Center, Hangzhou Normal University. The experimental protocol was approved by the Animal Studies Committee of Hangzhou Normal University.

Experimental protocol

Male mice were divided into four groups. The *Purslane* extracts were administered for 28 days.

Group I: Control mice administered distilled water daily for 4 weeks.
Group II: Mice administered low dose group *Purslane* extracts (100 mg/kg) daily for 4 weeks.
Group III: Mice administered middle dose group *Purslane* extracts (200 mg/kg) daily for 4 weeks.
Group IV: Mice administered high dose group *Purslane* extracts (400 mg/kg) daily for 4 weeks.

The mice in the treatment groups were orally administered *Purslane* extracts in a volume of 200 uL via stomach intubations, while the control group received the same volume of distilled water to keep all the animals at the same type of treatment condition as PE treatment groups.

Forced swimming exercise and blood collection

Forced swimming exercise was conduced with mice supporting constant loads (with lead fish sinkers attached to the tail) corresponding to 10% of their body weight (Wang and Xu, 2005; Gong et al., 2008; Li et al., 2008). The animals were considered fatigued when they were unable to rise to the surface for 5 seconds (Ikeuchi et al., 2006; Ikeuchi et al., 2009). The mice performed forced swimming exercise tests once weekly for 3 weeks. Forced swimming was carried out in acrylic plastic pool (90 × 45 × 45) filled with water to a depth of 35 cm (Matsumoto et al., 1996; Kamakura et al., 2001). The temperature of the water was maintained at 34±1°C. To avoid the influence of circadian variations in physical activity, swimming exercise was done from 11:00 to 17:00, a period during which minimal Variation of endurance capacity has been confirmed in rats (Matsumoto et al., 1996).

After a period of 4 weeks, the mice were made to swim for 20 min supporting loads corresponding to 5% of their body weight (Ikeuchi et al., 2006). After swimming, each mouse was quickly taken out, blotted using tissue paper and sacrificed under chloroform anaesthesia. The blood was collected by decapitation in heparinized, chilled tubes and quickly placed under ice cold condition. Plasma was separated by centrifugation at 3000 rpm for 10 min in cold centrifuge and kept in deep freezer at -30 °C until being used. The levels of plasma triglyceride (TG), glucose, lactate and ammonia were analyzed with commercial kits (Jiancheng Chemical Co., Jiangshu, China).

Statistical analysis

The results are expressed as mean±S.D. and all statistical comparisons were made by means of one-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test. The data were analyzed with Graph Pad Prism 4.0 v for Windows (Graph Pad Software, San Diego, CA, USA). The difference showing a Plevel of 0.05 or lower was considered to be statistically significant.

RESULTS

Components of *Purslane* extracts in the TLC assay

Results of TLC were shown in Table 1. There were seven main compounds in *purslane* extracts, including organic acid, flavonoids, alkaloids, monoterpene glycoside, catecholamines, saponin, polysaccharide.

Effect of *Purslane* extracts on forced swimming capacity

Forced swimming times of mice were shown in Figure 1. Group III and Group IV were significantly prolonged compared with that in the Group I from the first week onward ($P<0.05$). However, Group II did not significantly increase forced swimming time throughout the exercise period ($p > 0.05$).

Effect of *Purslane* extracts plasma biochemical parameters

Plasma biochemical parameters were shown in Table 2. Plasma TG, lactate and ammonia levels were lower in all treatment groups compared to the Group I ($P < 0.05$). Plasma glucose levels were higher in the Group III and Group IV compared to the Group I ($P < 0.05$).

DISCUSSION

Many reports have indicated that Chinese herbal medicine can provide energy immediately during exercise and are helpful on kinetic fatigue (Wu and Gao, 2003; Hou et al., 2006; Wang et al., 2008; Wang et al., 2009).

Table 1. Compounds of *purslane* extracts by TLC analysis.

Indicator	Examined Components	Ratio of Flow (Rf)	Color
Iodine / Potassic iodide	Alkaloide	0.2543	Brown
Ferric trichloride / Water	Hydroxybenzene	0.2231	Purple
Acetic anhydride / Sulfuric acid	Terpene ansteroid	0.2562	Prunosus
Phosphomolybdic Acid / Ethanol	Saponin	0.2447	Dark blue
Phenol / Sulfuric acid	Polysaccharide	0.2783	Brown
10%KOH	Anthraquinone	-	-
10% NaOH	Flavone	0.2418	Yellow
Sulfuric acid / Ethanol	Lignin	-	-
Bromophenol Blue / Ethanol	Organic acid	0.2198	Dark yellow

"-"means without any spot on the plates.

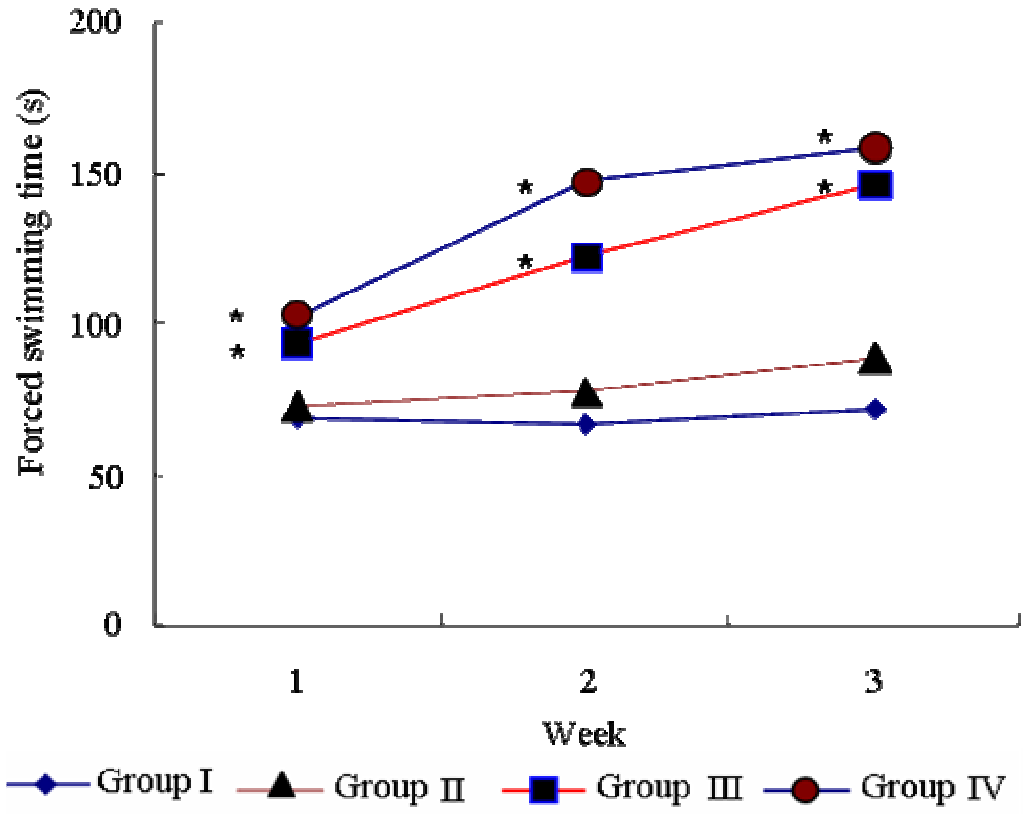

Figure 1. Effect of *Purslane* extracts on forced swimming capacity in mice (mean ± SD, n=8) *P <0.05 as compared with Group I (control Group).

Table 2. Effect of *Purslane* extracts plasma biochemical parameters (mean ± SD, n=8).

Group	TG (mmol/L)	Glucose (mmol/L)	Lactate(mmol/L)	Ammonia (ug/mL)
I	1.68±0.06	4.02±0.43	10.47±1.45	9.24±1.33
II	1.32±0.03*	4.31±0.32	9.94±1.23*	8.12±0.76*
III	1.27±0.06*	4.87±0.28*	9.71±1.82*	7.65±1.08*
IV	1.21±0.04*	4.94±0.51*	9.78±1.57*	7.61±1.27*

*P < 0.05 as compared with Group I (control Group).

However, there is no information in details concerning the anti-kinetic fatigue effect of Purslane.

In the present study, we used TLC assay to identify the main compounds of *Purslane* extracts, which included organic acid, flavonoids, alkaloids, monoterpene glycolside, catecholamines, saponin, polysaccharide. According to certain documents, polysaccharide and flavone are the usual anti-kinetic fatigue compounds in herbs (Tang et al., 2004; Shi et al., 2004; Jiang and Gao, 2005). Further work on isolation and purification of each compound of *Purslane* will be done to identify compounds effective on anti-kinetic fatigue.

Forced swimming exercise is commonly used in anti-kinetic fatigue and endurance tests (Deyama et al., 2001; Kim et al., 2002). Our results showed that different doses of *Purslane* extracts especially 200 and 400 mg/kg doses could significantly lengthen the swimming time, which indicated that *Purslane* extracts could elevate the exercise tolerance of mice. In order to clarify its mechanism, blood biochemical parameters were measured in the forced swimming-treated mice (Jung et al., 2004). The swimming exercise was known to induce blood biochemical changes (Moriura et al., 1996).

Generally, it was interpreted that the elevation of TG and FFA and the decrease of glucose showed a consumption of energy and that the increase of lactate presented a fatigued condition during prolonged exercise (Jung et al., 2004). In the present study, Plasma glucose level was significantly higher in the Group III and Group IV and Plasma TG levels decreased significantly in all treatment groups compared to the Group I, which indicated the *Purslane* extracts could increase fat utilization of mice during swimming. This would be an advantage during prolonged exercise, since a larger proportionate utilization of fat allowed glycogen sparing and therefore delays fatigue (Walberg et al., 1983).

The levels of plasma lactate and ammonia, metabolites known to cause to fatigue, decreased significantly in all treatment groups compared to the Group I. It was reported that blood lactate and ammonia accumulated during exercise (Mutch and Banister, 1983; Jung et al., 2004). Our results showed that *Purslane* extracts administered for the experimental period made mice resistant to the physical fatigue by delaying the accumulation of plasma lactate and ammonia during the forced swimming.

Conclusions

Our results suggested that *Purslane* extracts had signifycant anti-kinetic fatigue effects on mice, which enhanced the maximum swimming capacity of mice by increasing fat utilization, and by delaying the accumulation of plasma lactate and ammonia.

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