



Ethnopharmacological communication

Anti-fatigue activity of the water-soluble polysaccharides isolated from *Panax ginseng* C. A. Meyer

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ABSTRACT

Aim of the study: *Panax ginseng* C. A. Meyer (ginseng) is a well-known Chinese herb often used in Asian countries for physical strength development. Ginseng polysaccharides are its active component and have a lot of pharmaceutical activities. However, anti-fatigue activity of ginseng polysaccharides has not yet been tested. The current study was designed to evaluate the anti-fatigue activity of ginseng polysaccharides (WGP) in an animal test for fatigue and compare the activities between the neutral (WGPN) and acidic (WGPA) portion in an attempt to determine whether the medicinal uses are supported by pharmacological effects.

Materials and methods: WGP, WGPN and WGPA were orally administrated to mice once daily for 15 days. Anti-fatigue activity was assessed using the forced swim test (FST) and serum biochemical parameters were determined by autoanalyzer and commercially available kits.

Results: While all compounds were found to reduce immobility in the FST, the effect of WGPA was demonstrated in lower doses compared with WGP and WGPN. Moreover, the FST-induced reduction in glucose (GLU) and glutathione peroxidase (GPx) and increase in creatine phosphokinase (CK), lactic dehydrogenase (LDH) and malondialdehyde (MDA) levels, all indicators of fatigue, were inhibited by the corresponding doses of WGP, WGPN and WGPA.

Conclusions: Ginseng polysaccharides have anti-fatigue activity, also reflected in the effects on the physiological markers for fatigue. The acidic polysaccharide is more potent than the neutral polysaccharide.

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1. Introduction

Panax ginseng C. A. Meyer (ginseng) (Family Araliaceae) contains more than 15% of water-soluble polysaccharides (WGP). These polysaccharides are its active components and contain neutral (WGPN) and acidic (WGPA) portions. The neutral portion is a mixture of glucans (starch-like polysaccharides) and arabinogalactans. The acidic portion contains arabinogalactans, type-I rhamnogalacturonan-rich pectins and homogalacturonan-rich pectins (Zhang et al., 2009). It has been demonstrated that ginseng polysaccharides have diverse pharmaceutical effects including anti-tumor, antioxidant and hypoglycemic activities (Konno et al., 1985; Shin et al., 2004; Luo and Fang, 2008). In Chinese medicine, ginseng has been traditionally used for the development of physical strength, especially in patients who suffered from severe fatigue (Saito et al., 1974). However, little information about the anti-fatigue effects of ginseng polysaccharides is currently known.

Therefore, the present study was designed to evaluate the anti-fatigue activities of ginseng polysaccharides, as well as its neutral and acidic portions, in an animal model for fatigue, the forced swim test (FST). The effects of these compounds on biochemical markers for fatigue were also assessed. Specifically, the levels of glucose (GLU), triglyceride (TG), lactic dehydrogenase (LDH), creatine phosphokinase (CK), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were evaluated. Energy use leads to GLU level reduction and to a compensatory increase of fat mobilization (Jung et al., 2004). Moreover, fatigue results in the release of reactive oxygen species (ROS) which cause lipid peroxidation of membrane structure. MDA, an oxidative degradation product of cell membrane lipids, is generally considered as an indicator of lipid peroxidation (Alessio and Goldfarb, 1988). In fatigue conditions, MDA level is increased and is accompanied with a decrease in levels of the antioxidant enzymes GPx and SOD (Lenaz, 1998). These conditions are also marked by the release of LDH and CK into the serum, serving as an indirect index of the damage of membrane structure (Passarella et al., 2008).

It was hypothesized that (1) the FST-induced reduction in immobility demonstrated in mice will be inhibited following treatment with WGP, WGPN and WGPA and (2) the FST-induced decrease in

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GLU, GPx and SOD and increase in TG, MDA, LDH and CK levels will be restored following treatment with WGP, WGPn and WGPA.

2. Materials and methods

2.1. Plant material and extract

The roots of *Panax ginseng* C.A. Meyer were collected from Changbai Mountain, Jilin, China, and were identified by botanist Prof. Y. Zhao (School of Life Sciences, Northeast Normal University, China). The voucher specimen has been deposited at the School of Life Sciences, Northeast Normal University, China (No. 20081001). The water-soluble polysaccharides (WGP), neutral (WGPn) and acidic (WGPA) portions were prepared following the procedure established in our lab (Zhang et al., 2009). Briefly, WGP was isolated by water extraction at 100 °C, deproteinized by Sevag-reagent and precipitated by ethanol. WGP was further separated into WGPn and WGPA on a DEAE-Cellulose column eluted with distilled water and 0.5 M NaCl, respectively.

2.2. Experimental animals

Male ICR mice, 11–12 weeks old, were procured from Pharmacology Experimental Center of Jilin University, China. The mice were housed at a room temperature of 23 ± 1 °C with a 12 h light:12 h dark cycle (lights on from 6:00 am to 6:00 pm). Food and water were available ad libitum. The experiments were carried out according to the institutional regulations and national criteria for animal experimentation. The Institution Animal Ethics Committee reviewed the entire animal protocol prior to conducting the experiments.

2.3. Forced swim test

The forced swim test (FST) was carried out as described in the literature (Porsolt et al., 1978). Briefly, ICR mice were placed individually into a glass cylinder (height: 25 cm, diameter: 10 cm) containing 10 cm of water at 23–25 °C for a 6 min session. At the end of the session, mice were removed from the water, dried with a paper towel, and placed back in their home cage. Water in the container was changed after each session. The duration of immobility was manually recorded by a trained experimenter, blind to the experimental conditions, during the last 4 min of the testing period and immobility was defined when mice ceased struggling and were floating motionless in the water, making only those movements necessary to keep its head above water.

2.4. Experimental design

Mice were divided into the following experimental groups, with 8 mice in each group. Group 1: untreated control (UTC)-mice unexposed to the FST and treated with saline; Group 2: untreated swimming (UTS)-mice exposed to the FST and treated with saline; Groups 3–13: treated swimming (WGP, 50, 100 and 200 mg/kg, WGPA, 40, 100, 160 and 200 mg/kg and WGPn, 40, 100, 160 and 200 mg/kg)-mice exposed to FST and treated with the corresponding polysaccharide portions. Saline/polysaccharides were administered orally (8:00 am) to mice for 15 days and the FST was conducted on the last day, 1 h after compound administration.

2.5. Serum analysis

Mice were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). After anesthetization, blood was withdrawn from its heart and serum was prepared by centrifugation at 3000 rpm at 4 °C for 10 min. Levels of GLU, TG,

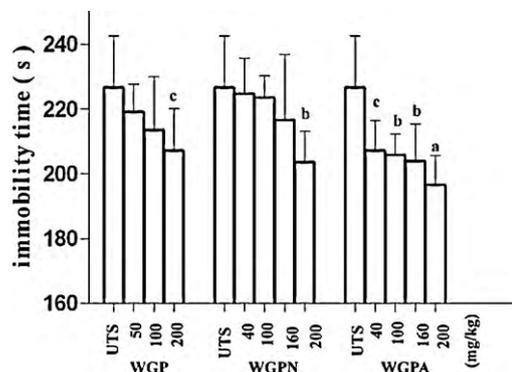


Fig. 1. Effects of ginseng polysaccharides in the forced swim test. Values are expressed as mean \pm S.D.; $n=8$ in each group, a, b and c represent significant difference from UTS mice. ^a $P<0.001$, ^b $P<0.01$, ^c $P<0.05$. UTS = mice exposed to the FST and treated with saline.

LDH and CK were determined using an autoanalyzer (Hitachi 7060, Hitachi, Japan). Levels of MDA, SOD and GPx were determined using commercially available kits from the Nanjing Jiancheng Bio-company (Nanjing, China).

2.6. Statistical analysis

All values were expressed as mean \pm S.D. Data was analyzed using one-way ANOVA with main factor of treatment (UTC/UTS/WGP/WGPA/WGPn) followed by Dunnett's post hoc comparisons. Significant was assumed at $P<0.05$.

3. Results

3.1. Effects of ginseng polysaccharides in the forced swim test

As expected, in comparison with the saline-treated mice, all three polysaccharides reduced immobility times in the FST. However, while the effects of WGP and WGPn were demonstrated at 200 mg/kg, WGPA reduced immobility at 40 mg/kg [$F(11, 84)=5.17$, $P<0.0001$, see Fig. 1 for results of post hoc comparisons].

3.2. Effects of ginseng polysaccharides on serum biochemical parameters

As shown in Table 1, exposure to the forced swim test led to an increase in LDH, CK and MDA levels and to a decrease in GLU and GPx levels in serum in comparison with the UTC group. All these effects were blocked by 200 mg/kg of the total polysaccharide mixture WGP and most of them (LDH, MDA and GPx) were blocked by 200 mg/kg of the neutral polysaccharide extract WGPn. Importantly, all of these effects were blocked by the acidic polysaccharide extract WGPA, but in lower doses compared with the other two compounds (CK and MDA, 100 mg/kg, LDH and GPx, 160 mg/kg).

4. Discussion and conclusions

Ginseng has several pharmaceutical benefits including anti-tumor, antioxidant and hypoglycemic properties (Konno et al., 1985; Shin et al., 2004; Luo and Fang, 2008). One of its traditional uses in Asian countries is to fight fatigue (Saito et al., 1974; Banerjee and Izquierdo, 1982). However, the specific effects of its important active components, which include water-soluble ginseng polysaccharides (WGP), neutral (WGPn) and acidic (WGPA) portions, have not yet been fully assessed.

The present study demonstrates an anti-fatigue activity of ginseng polysaccharides in the FST, a valid animal model for screening

Table 1
Effects of ginseng polysaccharides on serum biochemical parameters after the forced swim test.

Group	Dose (mg/kg)	GLU (mmol/L)	TG (mmol/L)	CK (U/L)	LDH (U/L)	MDA (nmol/mL)	SOD (U/mL)	GPx (U)
UTC	–	9.12 ± 2.18	1.97 ± 0.15	830.60 ± 145.23	828.50 ± 130.97	12.34 ± 1.61	71.43 ± 20.43	788.90 ± 71.83
UTS	–	7.39 ± 2.09 ^c	2.17 ± 0.39	1107.17 ± 201.41 ^c	1054.83 ± 107.79 ^c	15.47 ± 2.49 ^c	56.84 ± 17.80	575.15 ± 174.25 ^c
WGP	50	7.57 ± 1.01	1.89 ± 0.33	1100.33 ± 220.82	999.17 ± 78.45	13.23 ± 1.13	72.12 ± 20.17	595.14 ± 162.76
	100	7.73 ± 0.94	1.78 ± 0.37	1071.33 ± 177.08	946.83 ± 67.55	13.11 ± 2.18	75.43 ± 17.89	616.38 ± 204.62
	200	9.66 ± 1.78 ^b	1.64 ± 0.31 ^b	841.00 ± 95.49 ^c	843.67 ± 107.39 ^c	10.28 ± 1.88 ^a	86.43 ± 21.23 ^c	800.63 ± 184.02 ^c
WGPN	40	8.11 ± 0.80	1.94 ± 0.09	1088.83 ± 117.13	1068.68 ± 139.74	13.48 ± 1.71	68.63 ± 21.39	595.14 ± 162.76
	100	8.16 ± 1.62	1.86 ± 0.44	1033.17 ± 297.96	959.00 ± 253.43	13.61 ± 1.62	64.88 ± 19.88	642.13 ± 106.02
	160	8.76 ± 1.83	1.82 ± 0.51	1087.50 ± 135.57	904.67 ± 170.00	13.23 ± 2.33	67.75 ± 20.66	704.38 ± 105.41
	200	8.86 ± 0.98	1.79 ± 0.11	1016.67 ± 153.13	884.83 ± 75.52 ^c	12.60 ± 2.62 ^c	66.51 ± 17.03	811.88 ± 152.34 ^c
WGPA	40	8.26 ± 0.53	1.83 ± 0.30	1002.00 ± 121.48	979.83 ± 72.89	13.96 ± 1.43	55.55 ± 18.60	581.40 ± 183.60
	100	8.39 ± 0.79	1.84 ± 0.15	833.61 ± 127.47 ^c	861.76 ± 139.68	12.59 ± 2.55 ^c	65.16 ± 18.00	630.90 ± 145.71
	160	9.04 ± 1.41	1.77 ± 0.23	814.36 ± 136.36 ^b	849.17 ± 235.92 ^c	11.63 ± 1.09 ^b	82.87 ± 18.54	787.15 ± 99.14 ^c
	200	9.57 ± 1.33 ^b	1.64 ± 0.17 ^b	789.83 ± 146.30 ^b	789.17 ± 117.05 ^b	10.96 ± 1.36 ^a	91.03 ± 24.76 ^c	802.49 ± 95.73 ^c

Values are expressed as mean ± S.D.; n = 8 in each group, a, b and c represent significant difference from UTS mice, ^aP < 0.001, ^bP < 0.01, ^cP < 0.05. GLU = glucose, TG = triglyceride, LDH = lactic dehydrogenase, CK = creatine phosphokinase, MDA = malondialdehyde, SOD = superoxide dismutase, GPx = glutathione peroxidase, UTC = mice unexposed to the FST and treated with saline, UTS = mice exposed to the FST and treated with saline.

anti-fatigue agents (Kim et al., 2001; Koo et al., 2004) and on several biochemical markers for fatigue. Specifically, it is shown that the increase in immobility times following exposure to the FST is significantly attenuated in mice treated with WGP, WGPN and WGPA at 200, 200 and 40 mg/kg, respectively. Moreover, the effects of ginseng polysaccharides in the FST are accompanied by an attenuation of the FST-induced effects on the physiological markers relevant for fatigue. Specifically, all polysaccharides restored the levels of lactic dehydrogenase (LDH), malondialdehyde (MDA) and glutathione peroxidase (GPx) to baseline. The results suggested that the anti-fatigue effect of ginseng polysaccharides occurred probably through protection of corpuscular membrane by preventing lipid oxidation via modifying several enzyme activities. These findings are consistent with Yu et al. (2006), which have demonstrated similar effects of polysaccharides from the *Euphorbia kansui* (Euphorbiaceae) on MDA and GPx levels.

Another possible explanation for the anti-fatigue effect seen following treatment with ginseng polysaccharides could involve triglyceride (TG) (or fat) mobilization during exercise, as indicated by the decrease in TG level and the simultaneous increase in glucose (GLU) level. Such an effect might become advantageous during prolonged exercise, since better utilization of TG allows the sparing of glycogen and GLU and therefore delays fatigue (Jung et al., 2004). Further experiments are needed in order to identify the mechanism through which ginseng polysaccharide might affect fat mobilization.

Importantly, the pharmacological profile of the three polysaccharides was demonstrated in different doses, with the acidic polysaccharide extract, WGPA, yielding an effect in lower doses compared with WGP and WGPN. The favorite potency of WGPA in the FST might indicate that it has heightened ability to protect the corpuscular membrane by preventing lipid oxidation. This suggestion is supported by the finding that WGPA inhibit FST-induced increase in MDA levels and reduction in SOD and GPx levels in lower doses compared with the other two extracts.

Taken together, our results show for the first time that ginseng polysaccharides possess an anti-fatigue activity. Moreover, it is demonstrated that the acidic polysaccharide have higher potency to induce an anti-fatigue activity compared with the neutral polysaccharide. Although a few biochemical mechanisms are suggested as modulating these effects, further research is needed

in order to expose the nature of polysaccharides effects on fatigue and specifically on fat mobilization and cytoprotection.

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