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Influence of Tartary Buckwheat Extracts Supplementation on Oxidative Stress Induced by Acute Exhaustive Exercise in Rats

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Abstract: Tartary Buckwheat Extracts (TBE) has been reported to be a potential antioxidant that may affect health and exercise performance. The objective of this study was to examine the effects of TBE supplementation on oxidative stress induced by acute exhaustive exercise in rats. Male Sprague-Dawley rats were randomly divided into four groups, each consisting of ten rats. The first group designated as control group was administered with distilled water by gavage every day for 28 days. The other three groups designated as TBE supplementation groups were administered with TBE of 60, 120 and 240 mg kg⁻¹ body weight (b.wt.), respectively, by gavage every day for 28 days. After acute exhaustive exercise, the antioxidant enzyme activities and malondialdehyde (MDA) levels were determined. The data showed that TBE at the dose 60, 120 and 240 mg kg⁻¹ b.wt. increased Super Oxide Dismutase (SOD) activities in liver tissue of rats significantly by 28.13, 34.65 and 45.95%, respectively, increased catalase (CAT) activities significantly by 29.49, 41.63 and 59.30%, respectively and decreased MDA levels significantly by 31.21, 51.12 and 45.68%, respectively. TBE at the dose 120 and 240 mg kg⁻¹ b.wt. increased glutathione peroxidase (GPx) activities significantly by 21.33 and 28.22%, respectively. TBE at the dose 240 mg kg⁻¹ b.wt. increased Glutathione Reductase (GR) activities significantly by 31.58%. These results suggested that TBE supplementation has a protective effect on oxidative stress induced by acute exhaustive exercise.

Key words: Influence, Tartary buckwheat extracts, oxidative stress, acute exhaustive exercise, rats

INTRODUCTION

Buckwheat is an herbaceous plant that belongs to the Polygonaceae family and it is an important staple food consumed in East Asia and parts of Europe, e.g., China, Japan, Korea, Russia and Poland (Liu *et al.*, 2008). There are two main buckwheat species used for food around the world, i.e., common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* Gaertn.). Common buckwheat is widely grown on all continents with a long history. It was first recorded in a Chinese ancient book of the fifth century BC named Shennong Book that buckwheat was grown. Tartary buckwheat is grown only in parts of Asia, Europe and North America, mainly in and Loess Plateau and the Yunnan-Guizhou Plateau in China. Cultivation area of Tartary buckwheat is one third of common buckwheat (Jin and Wei, 2011).

Many studies have reported that buckwheat has many important biological activities, including antioxidant, antihypertensive, antibacterial, antiviral, hypolipidemic

and hypoglycemic, etc. (Fabjan *et al.*, 2003; Tomotake *et al.*, 2006; Yao *et al.*, 2008; Ushida *et al.*, 2008) which may be due to its high flavonoids content and the content in Tartary buckwheat is higher than that in common buckwheat (Cao *et al.*, 2008). The mainly flavonoids in Tartary buckwheat is rutin and minor flavonoids include quercetin, kaempferol and kaempferol-3-rutinoside (Gu, 1999; Li *et al.*, 2001). Our previous studies also have shown that Tartary Buckwheat Extracts (TBE) has the anti-fatigue property and increasing the activities of glutathione peroxidase (GPx) and Super Oxide Dismutase (SOD) in mice (Jin and Wei, 2011) but the effects of TBE on exercise-induced oxidative stress haven't been studied. So, the objective of this study was to examine the effects of TBE supplementation on oxidative stress induced by acute exhaustive in rats.

MATERIALS AND METHODS

Plant material: The dry grains of Tartary buckwheat were purchased in June 2011 from Zhejiang Agricultural

Institution (Hangzhou, PR China). The grains was grinded into powder (180 micrometer) using a grinding machine (LH-08B, Jishou Zhongcheng Pharmaceutical Machinery Factory, Hunan, China) and stored in dry condition until being used.

Chemicals and reagents: Assay kits for determination of Super Oxide Dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT) and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). Assay kits for determination of glutathione reductase (GR) were purchased from Beyotime Institute of Biotechnology (Nantong, China). All other chemicals used were analytical grade.

Preparation of Tartary buckwheat extracts: Tartary Buckwheat Extracts (TBE) was prepared according to previous reports (Cao *et al.*, 2008). In brief, 10 g of Tartary buckwheat powder were extracted by 200 mL ethanol-water (70:30, v/v) for 40 min by an ultrasonic generator (KQ2200E, Kunshan Ultrasonic Instrument Co. Ltd., Jiangsu, China). The supernatant and the residues were separated by vacuum-filtration. The residues were extracted again with the above described method. The first and second extraction solutions were mixed and the solvent was evaporated in the case of vacuum with the rotatory evaporator and with temperature controlled at 40°C and the residues were frozen-dried and stored at 4°C until being used. The contents of flavonoids were measured by means of UV-Vis spectrophotometry with chromogenic system of NaNO₂-Al(NO₃)₃-NaOH (Zhang *et al.*, 2010).

Experimental animals: Male Sprague-Dawley rats (body weight 250±20 g) used for experiments were purchased from the Experimental Animal Center of Zhejiang Province (SPF grade, Certificate No. 20081476). The animals were housed under diurnal lighting conditions (12/12 h) and allowed free access to food and water. Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Chinese National Institutes of Health.

Exercise programs: Animals were allowed to get accustomed to their surroundings for 1 week before the experiments started. After that, the rats were randomly divided into four groups, ten in each. The first group designated as control group was administered with distilled water by gavage once a day for 4 weeks. The second, third and fourth group designated as TBE supplementation groups were administered with TBE of 60, 120 and 240 mg kg⁻¹ b.wt., respectively, by gavage once a day for 4 weeks.

During the 4 weeks, the rats underwent an exercise program on a Quinton rodent treadmill. During the first 3

weeks the exercise was performed at 8 m min⁻¹ and at 0% grade for 10 min day⁻¹, 3 days week⁻¹. After 3 weeks the exercise intensity was gradually increased to 25 m min⁻¹ and 5 % grade. On the last day of experiment, the rats and performed an acute exhaustive exercise at the above mentioned speeds and grades. Exhaustion was defined as the inability of a rat to right itself when being laid on its side. No rat ceased exercise because of foot or any other type of injury (Bejma and Ji, 1999; Gomez-Cabrera *et al.*, 2005).

Tissue processing and homogenate preparations: After acute exhaustive exercise, the rats were anaesthetized with pentobarbital sodium (5 mg 100 g⁻¹ b.wt. i.p.), then the liver tissue were quickly removed, freeze-clamped immediately and stored at -70°C until being used. The tissue samples were homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The latter was next subjected to high-speed centrifugation at 15000 xg for 30 min at 4°C (Smolka *et al.*, 2000; Li, 2007). The supernatant was decanted and assayed for SOD, GR, GPx, CAT and MDA. The antioxidant enzyme activities and MDA levels were determined using commercial diagnostic kits following the manufacturer's instructions.

Statistical analysis: Statistical analysis was carried out using ANOVA followed by post-hoc Turkey test (SPSS 15.0 for Windows). The criterion of significance was set at p<0.05. All results are given as Mean±SD.

RESULTS

Effects of Tartary buckwheat extracts on SOD activities in liver tissues of rats: As shown in Fig. 1, after exhaustive exercise, the SOD activities in liver tissue of

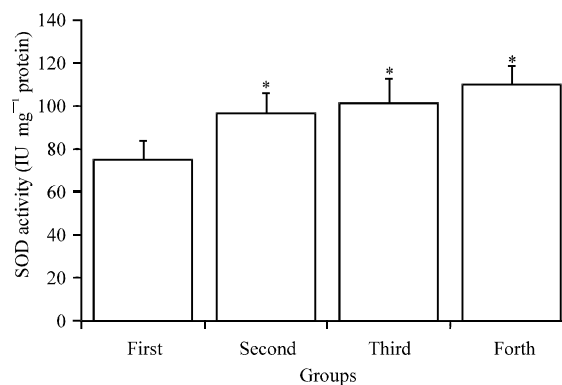


Fig. 1: Effects of Tartary buckwheat extracts on SOD activities in liver tissues of rats, Values are Mean±SD, *p<0.05 when compared with first group (control group)

the second, third and fourth group was 96.43 ± 9.48 , 101.34 ± 11.36 and 109.84 ± 8.76 IU mg^{-1} protein), respectively and were significantly ($p < 0.05$) higher than that of the first group (75.26 ± 8.59 IU mg^{-1} protein). The increase ratios were 28.13, 34.64 and 45.95%, respectively.

Effects of Tartary buckwheat extracts on GPx activities in liver tissues of rats: As shown in Fig. 2, after exhaustive exercise, the GPx activities in liver tissue of the second, third and fourth group was 312.94 ± 36.89 , 361.78 ± 38.49 and 382.33 ± 51.41 IU mg^{-1} protein), respectively. The GPx activities of third and fourth groups were significantly ($p < 0.05$) higher than that of the first group (298.19 ± 46.37 IU mg^{-1} protein) and the increase ratios were 21.33 and 28.22%, respectively.

Effects of Tartary buckwheat extracts on GR activities in liver tissues of rats: As shown in Fig. 3, after exhaustive exercise, the GR activities in liver tissue of the second,

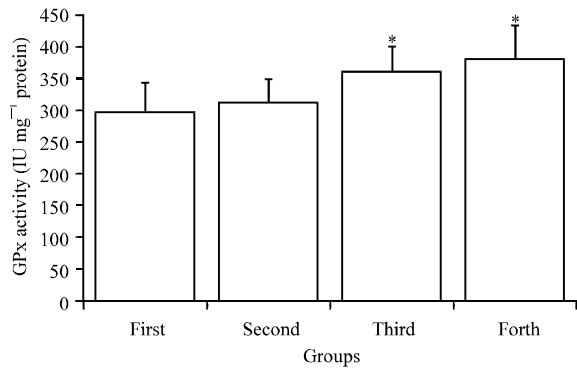


Fig. 2: Effects of Tartary buckwheat extracts on GPx activities in liver tissues of rats, Values are Mean±SD, * $p < 0.05$ when compared with first group (control group)

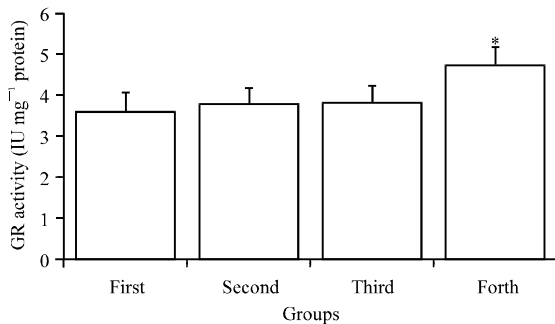


Fig. 3: Effects of Tartary buckwheat extracts on GR activities in liver tissues of rats, Values are Mean±SD, * $p < 0.05$ when compared with first group (control group)

third and fourth group was 3.79 ± 0.39 , 3.82 ± 0.41 and fourth group were significantly ($p < 0.05$) higher than that (4.75 ± 0.43 IU mg^{-1} protein), respectively. The GR activities of the first group (3.61 ± 0.47 IU mg^{-1} protein) and the increase ratio was 31.58 %.

Effects of Tartary buckwheat extracts on CAT activities in liver tissues of rats: As shown in Fig. 4, after exhaustive exercise, the CAT activities in liver tissue of the second, third and fourth group was 47.25 ± 6.33 , 51.68 ± 5.01 and 58.13 ± 6.28 U mg^{-1} protein), respectively and were significantly ($p < 0.05$) higher than that of the first group (36.49 ± 5.23 U mg^{-1} protein). The increase ratios were 29.49, 41.63 and 59.30 %, respectively.

Effects of Tartary buckwheat extracts on MDA levels in liver tissues of rats: As shown in Fig. 5, after exhaustive

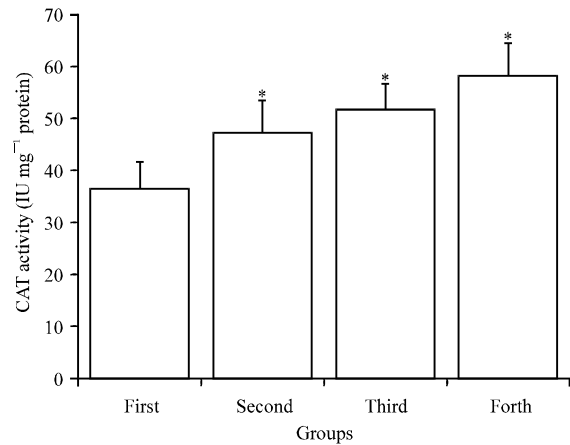


Fig. 4: Effects of Tartary buckwheat extracts on CAT activities in liver tissues of rats, Values are Mean±SD, * $p < 0.05$ when compared with first group (control group)

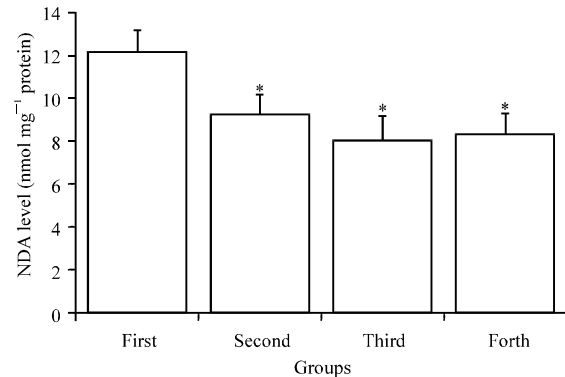


Fig. 5: Effects of Tartary buckwheat extracts on MDA levels in liver tissues of rats, Values are Mean±SD, * $p < 0.05$ when compared with first group (control group)

exercise, the MDA levels in liver tissue of the second, third and fourth group was 9.26 ± 0.94 , 8.04 ± 1.16 and 8.34 ± 0.97 nmol mg^{-1} protein), respectively and were significantly ($p < 0.05$) lower than that of the first group (12.15 ± 1.03 nmol mg^{-1} protein). The decrease ratios were 31.21, 51.12 and 45.68%, respectively.

DISCUSSION

There is strong evidence that exhaustive physical exercise is closely related to accelerated generation of Reactive Oxygen Species (ROS) that leads to oxidative stress (Aguilo *et al.*, 2005; Rosa *et al.*, 2008), which can induce adverse effects on health and well being. It has been reported that specific sources of ROS during exercise include leakage of electrons from the mitochondrial electron transport chain, xanthine oxidase reaction, haemoglobin oxidation and activated neutrophils (Powers and Jackson, 2008; Di Giacomo *et al.*, 2009). The ROS has been reported to induce damage in all cellular macromolecules, such as lipids, proteins and DNA (Miyazaki *et al.*, 2001). It has been suggested that exercise-induced oxidative stress may be closely related to muscle fatigue, muscle damage and a decrease in physical performance (Kerksick and Willoughby, 2005). Antioxidants are substances that help reduce the severity of oxidative stress either by forming a less active radical or by quenching the reaction. The literature suggests that exogenous antioxidants, primarily obtained as nutrients or nutritional supplements, may prevent muscle damage because they are able to detoxify some peroxides by scavenging ROS produced during exercise (Atalay *et al.*, 2006; Davis *et al.*, 2009; Shan *et al.*, 2011). TBE has been reported to be a potential antioxidant that may affect health and exercise performance. Therefore, TBE might reduce exercise induced oxidative stress.

It is recognized that SOD, CAT, GPx and GR are considered as the first line of defense by the antioxidant enzyme system against ROS generated during exhaustive exercise (Huang *et al.*, 2009). SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide. CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water. GPx, responsible for enzymatic defense against hydrogen peroxide, is strictly linked with the concentration of GSH because it catalyses the reaction between glutathione and hydrogen peroxide, leading to the formation of glutathione disulphide. Glutathione Reductase (GR) do not act on ROS directly but they enable the GPx to function (Fig. 6) (Weydert and Cullen, 2010; Al-Othman *et al.*, 2011). In the current study, the data showed that high dose of TBE supplementation groups (240 mg kg^{-1}) significantly increased the SOD, GR, CAT and GPx activities after exhaustive exercise. These results showed that TBE played the role of antioxidant to prevent oxidative stress in liver tissue of rats. This might be due to scavenging of free radicals generated by As-exposure and breakage of radical chain reaction. Future studies will be performed to confirm this assumption.

Lipid Peroxidation (LPO) refers to the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semistable peroxides, which, in turn, damage the enzymes, nucleic acids, membranes and proteins (Niki, 2009; Arukwe and Mortensen, 2011). MDA is the most abundant product of LPO, which is a common consequence of oxidative stress (Devaraj *et al.*, 2008). Some reports showed that exhaustive exercise caused oxidative damage as significantly increased LPO in the muscle, liver and kidneys (Sen *et al.*, 1994; Selman *et al.*,

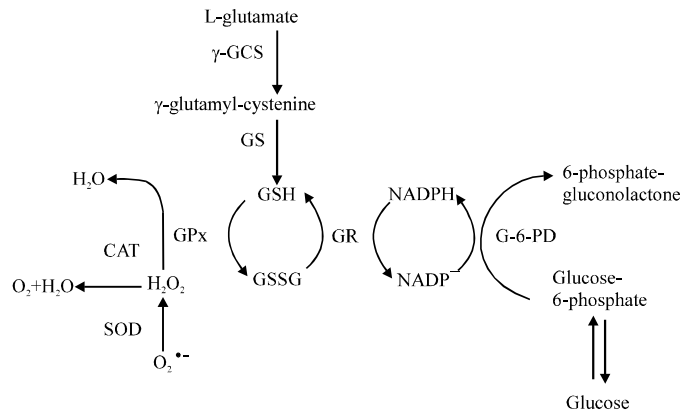


Fig. 6: Antioxidant enzyme schematic

2002; Vollaard *et al.*, 2005; Huang *et al.*, 2009). In the current study, the data showed that TBE supplementation groups significantly decreased the MDA levels after exhaustive exercise. These results indicated that TBE could reduce LPO and protected liver tissue from ROS-mediated oxidative damage after exhaustive exercise.

CONCLUSION

The present study provided evidence that TBE supplementation has a protective effect on oxidative stress induced by acute exhaustive exercise in rats. To our knowledge, for the first time, we demonstrated that administration of TBE profoundly increased the SOD, GR, CAT and GPx activities and decreased the MDA levels in liver tissue of rats after exhaustive exercise. These results revealed that TBE played the role of antioxidant to prevent oxidative stress and ROS-mediated oxidative damage in liver tissue of rats. Therefore, TBE could be used as an antioxidant supplement for competing athletes participating in exhaustive endurance events. However, related human studies are encouraged to prescribe the TBE as a nutraceutical supplement to athletes.

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