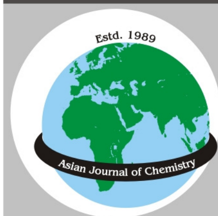
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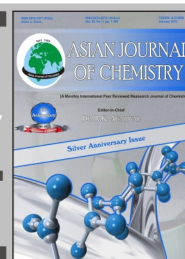

Antioxidant Activity of *Arctium lappa* L. and Its Effect on Biochemical Parameters in Exercised Rats

CHI-TING HORNG[1,2], MING-LING TSAI[3], CHAO-WEN HSUEH[4], SHUN-YUAN HSU[5], HUI-YUN WANG[2] and FU-AN CHEN[2,*]

[1]Department of Ophthalmology, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan
[2]Department of Pharmacy & Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung, Taiwan
[3]Department of ophthalmology, Tri-Service General Hospital, Taipei, Taiwan
[4]Department of Medicine, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan
[5]Department of Orthopedics, Kaohsiung Armed Force General Hospital, Kaohsiung, Taiwan

*Corresponding author: E-mail: fachen@mail.tajen.edu.tw

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The root of the medicinal plant locally known as burdock (*Arctium lappa* L.) has long been cultivated as a popular vegetable across Taiwan for human consumption and traditional medicine. The present study is to investigate the antioxidant activity of *Arctium lappa* and its effect on biochemical parameters in exercised rats. The extract of root of burdock was prepared and then subjected to analysis of polyphenols and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. Sprague-Dawley rats were divided into five groups including control (fed with drinking water) and three groups of different doses of burdock extracts (BE). After eight weeks, an exhaustive exercise test on a treadmill and the measurement of biochemical parameters related to fatigue were carried out. The results revealed that burdock extract was able to extend significantly the endurance time of treadmill running to exhaustion, as well as decreasing the blood lactate and serum urea nitrogen contents with comparison to that of control group. Malondialdehyde level of rats in burdock extract treated groups were significantly decreased, while superoxide dilmutase (SOD) were significantly increased compared with that of control group. The results shows that *Arctium lappa* is capable of ameliorating biochemical parameters related to fatigue in the exercised rat model that could be associated with the antioxidant polyphenols with free radical scavenging activities.

Key Words: *Arctium lappa*, Burdock, Antioxidant, Biochemical parameters, Antifatigue.

INTRODUCTION

With stressful workloads being commonplace, people often feel fatigue in modern life. The definition of fatigue is a failure to maintain the required or expected force or power output[1]. In other word, fatigue is defined as the failure of the body to function at a certain level so that organs cannot maintain their regular pace due to excessive activity and the body cannot make use of operating muscle resulting in a declined ability to act. Until now, different types of hypothesis on the subject of human fatigue were proposed due to its complex phenomenon and multiple factors involvement[2]. The delay of the occurrence of fatigue and quick recovery from fatigue are the current focuses of medical chemistry studies. Since the available therapies for fatigue in modern medicine are very limited, potential alternatives from traditional medicine are worth investigating[3]. Traditional/herbal products become a source of popular nutritional supplements used by athletes for reducing fatigue in Singapore[4]. Furthermore, the potential side effects of most chemical and biological agents help to improve body strength and delay occurrence of fatigue should be concerned[5]. As a result, how to look for supplement with effective ameliorating activity on biochemical parameters related to fatigue becomes more important to human.

Burdock (*Arctium lappa* L.) has long been cultivated as a vegetable in Taiwan for human consumption[6]. Health drinks made from this plant have become more popular in Taiwan in recent years. Scientific literature has also indicated that burdock possesses various pharmacological properties including antibacterial activity[7], desmutagenic activity[8], antioxidant ability[9-12], hepatoprotective effect[13,14], gastroprotective efficiency[15,16] and antiinflammatory activity[17], among which the hepatoprotective efficacy, antiinflammatory activity and antioxidant activity are associated with the free radical scavenging activity. But, there is only little information available concerning the relationship between biochemical aspects of anti-fatigue and the medicinal plant, burdock. The present study is to investigate the antioxidant activities of *Arctium lappa* and its effect on biochemical parameters related to laboratory rats involved in the physical exercise experiments.

EXPERIMENTAL

Several samples of roots from the plant, *Arctium lappa* L. (burdock) was obtained from the Gueilai Community Developmental Institute in Pingtung County, southern Taiwan. Thiobarbituric acid (TBA) and anthrone were purchased form Sigma Chemicals (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) and *n*-butanol were obtained from Merck (Darmstadt, Germany) and J.T. Baker (NJ, USA), respectively. Commercial diagnostic kits used to determine blood lactate and serum urea nitrogen (SUN) were purchased from Roche Diagnostics (Basel, Switzerland). Superoxide dismutase (SOD) kit was the product from Randox Laboratories (Antrim, UK). All other chemicals were of analytical reagent grade.

Preparation of burdock aqueous extract: Five hundred grams of root of burdock were added to 2000 mL of distilled water and then refluxed for 3 h in a reflux extraction apparatus (Angu, Kaoshiung, Taiwan). After that, the aqueous extract solution was filtered using filter paper and filter funnel. The filtered extract was further lyophilized to obtain aqueous extract of burdock by a freeze dryer (Panchun, Taipei, Taiwan). The obtained burdock extract was stored in an electronic dry cabinet (Komry, Taipei, Taiwan) for following study.

Determination of total polyphenols in burdock extract: Total polyphenols in burdock extract were measured spectrophotometrically using the Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction[18,19]. To 0.2 mL of diluted aqueous acetone sample, 1 mL of Folin-Ciocalteu reagent (diluted 10 times with water) was added. After that, 0.8 mL of 7.5 % Na_2CO_3 was added and mixed thoroughly. After 0.5 h of standing, the absorbance was measured at 765 nm (Hitachi, Tokyo, Japan). The amount of total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions and expressed as mg gallic acid/g burdock extract. All measurements were done in triplicate.

DPPH free radical scavenging activity of burdock extract: The free radical scavenging activity of burdock extract was evaluated using DPPH free radical-scavenging assay as described previously[20]. A stock solution (1 mg/mL) of each extract was prepared and diluted with methanol into various concentrations. An aliquot of 50 µL of each dilution was transferred into a 96-well microplate (NUNC, Roskilde, Denmark). A working solution of DPPH (250 µM) in methanol was freshly prepared and then an aliquot of 150 µL was added to each well. After incubation for 0.5 h, the DPPH scavenging percentage was measured at 490 nm on an ELISA reader (ThermoLabsystems, Cheshire, UK). Each dilution was performed at least in triplicate.

Experimental rat groupings: Thirty two male Sprague-Dawley (SD) rats, bought from BioLASCO Taiwan Co. Ltd., were enrolled in this experiment. Rats were maintained under standard laboratory conditions (12 h light/dark cycle, temperature (22 ± 2 ºC). Rats (263.40 ± 6.07 g) were randomly divided into four groups: Control (fed with distilled water) and the other three different doses of burdock extract including LBE (low dose, 190 mg/kg), MBE (middle dose, 380 mg/kg), HBE (high dose, 570 mg/kg) with gavage feeding. The change of body weight is documented within the 8 week period. This study was approved by the appropriate animal care and use committees (approval #IACUC-98-09, Tajen University, Taiwan).

Exercise measurement on treadmill: The whole experimental period is 8 weeks. All the experimental rats were trained on the treadmill (rat/mice treadmill T306, Singa, Taipei, Taiwan) for twelve days before exhaustive exercise according to the exercise program showed in Table-1 based on the method described previously with modification[21]. Motivation was provided by an electric shock zone at the rear of each compartment. On the day of the exhaustive exercise, rats were required to run to exhaustion on the treadmill at a final speed of 24 mph. The point of exhaustion was determined when the rat was unable to right itself when placed on its back. Blood samples were collected from orbital puncture before exercise and after exhaustion by a microcapillary tube with the rat anesthetized. These blood sample were for serum urea nitrogen and lactate test. After exhaustive exercise, the rats were sacrificed by CO_2 inhalation. The blood from the hepatic veins of scarified rats was collected for evaluation of SOD and malodialdehyde (MDA) levels. After blood was taken, part of the liver in rats was used for hepatic glycogen test. The liver and kidney of the rats were cleaned with normal saline repeatedly, dried with fuel filtered paper and soaked in 10 % neutral formalin solution. The fixed liver and kidney were embedded in paraffin wax and processed in a paraffin tissue processing machine (Leica, Nussloch, Germany). Sections were made at a thickness of 5 µm and stained with hematoxylin and eosin (H & E) for histopathology assessment.

TABLE-1
EXERCISE PROGRAM OF RATS ON THE TREADMILL

Gradient (º)	Speed (mph)	Enduring time (min)	Training days
0	8	15	2
2	12	25	2
3	15	30	2
5	18	40	2
6	21	50	2
7	24	60	2

Biochemical parameters: Biochemical parameters related to fatigue including blood lactate, serum urea nitrogen and hepatic glycogen were evaluated. Then blood lactate and serum urea nitrogen contents were tested according to the recommended procedures provided by the commercial diagnostic kit. The hepatic glycogen concentration was determined as described previously with modification[22]. 0.45 g of liver was homogenized with 2 mL 30 % KOH. The mixture was boiling for 20 min at 100 ºC. After boiling, a homogeneous solution of 200 µL was poured in each tube and then absolute ethanol 1 mL was added. The upper layer was easily removed after 4000 rpm centrifuged for 10 min. The residual sediment was added with 0.5 mL distilled water and finally, 1 mL anthrone reagent was used. After mixing 10 min, the absorbance was read at 620 nm by a ELISA reader (ThermoLabsystem, Cheshire, UK) and converted into the level of hepatic glycogen. SOD contents in blood were tested according to the recommended procedures provided by the commercial diagnostic kit. In case of MDA analysis, a modified thiobartituric acid

reactive species (TBARS) assay was use to measure lipid peroxide as described by Ohkawa *et al*., with modification[23]. Malondialdehyde, produced by the oxidant of polyunsaturated fatty acids, reacts with two molecules of TBA. At first, we used the above solution and shake to mix well. Then the solution was bathed at 37 ºC water for 1 h. Each tube was given 500 µL 0.1 N HCl and 200 µL 9.8 % SDS. Then 900 µL pure water was poured in and mixed well and 2 mL 0.6 % TBA was mixed at 95 ºC hot water bath for 1 h. Lately the solution was then cooled to room temperature for about 5-10 min. The *n*-butanol was added at the amount of 5 mL and mixed well by centrifugation at speed of 3000 rpm for 25 min at the temperature of 25 ºC. The upper clean solution was taken and loaded 200 µL/well. The layer of *n*-butanol yielding a pink red chromogen with an absorbance maximum at 532 nm measured by the ELISA reader (Thermo Labsystem, Cheshire, UK). We analyzed the changes of TBARS in each group after exhaustive running.

Statistics: All the experimental values are presented in the means ± standard deviation (SD). Statistical comparisons were made by one-way ANOVA and subsequently applying Duncan test was performed using a SPSS statistic software, version 10.0 (Illinois, USA). Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Total polyphenols and the free radical scavenging activity of burdock extract: In preparation of burdock extract, 29.7 % of recovery was obtained and then subjected to analysis of total polyphenols and the DPPH free radical scavenging activity. The content of total polyphenols in burdock extract was determined to be about 48.4 ± 5.6 mg/g (mg gallic acid/g burdock extract). The free radical scavenging activity of burdock extract was evaluated using a DPPH free radical scavenging activity. As shown in Table-2, burdock extract was able to scavenge significantly DPPH radical with concentration-dependant manner.

Effects of burdock extract on endurance time: As shown in Fig. 1, the MBE (middle-dose) and HBE (high-dose) groups increased significantly the endurance time to exhaustion compared with that of the control group ($p < 0.05$). However, endurance time in LBE (low-dose) group showed no significant difference compared with that of the control group ($p > 0.05$). The exhaustive test on the treadmill has been used widely for the measurement of the antifatigue properties of herbal medicine[3]. Our data showed that administration of burdock extract could evidently extend endurance time to exhaustion of rats, which indicated that burdock extract had antifatigue activity and could enhance the exercise tolerance.

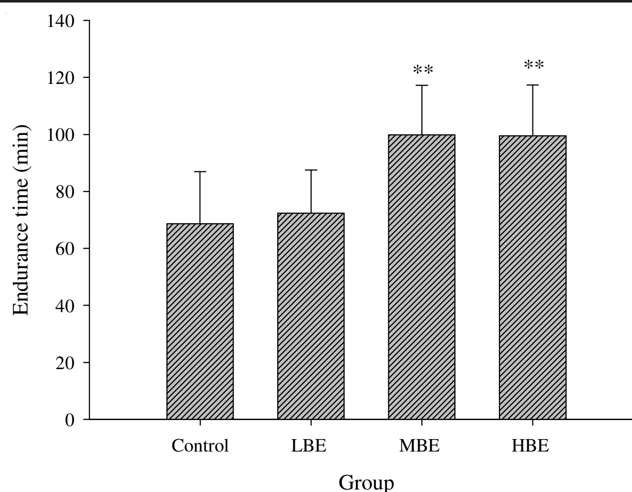

Fig. 1. Effect of burdock extract on endurance time to exhaustion of rats. Values represent the means ± SD (n = 8). ** $p < 0.01$ as compared with control. LBE: low dose (190 mg/kg); MBE: middle dose (380 mg/kg); HBE: high dose (570 mg/kg)

Effects of burdock extract on biochemical parameters: As shown in Table-3, burdock extract treated group may slightly increase the hepatic glycogen contents compared with that of the control group after exhaustion, but no significant difference. After exhaustion, serum urea nitrogen (SUN) contents of all burdock extract treated groups were significantly lower than that of the control group ($p < 0.05$) (Table-3). Urea is synthesized by hepatocytes from ammonia generated by catabolism of amino acids. Serum urea nitrogen arises from the catabolism of amino acids. The accumulation of ammonia is linked to physical fatigue[24]. Therefore, serum urea nitrogen is an important biochemical blood parameter related to fatigue.

TABLE-2
FREE RADICAL SCAVENGING ACTIVITY OF BE

BE concentration (ppm)	Control	125	250	500	1000
DPPH (%) scavenging[a]	0.8 ± 0.3	5.6 ± 0.7*	11.1 ± 0.9*	19.6 ± 1.2*	37.6 ± 1.8*

[a]The free radical scavenging activity was evaluated as the DPPH scavenging percentage based on the reduction of the absorbance at 490 nm in the presence of BE for 0.5 h. *There was a significant difference between the % DPPH scavenging of the test group and the control according to Duncan test ($p < 0.05$). Data are presented as the mean ± standard deviation (n = 3).

TABLE-3
EFFECTS OF BE ON BLOOD LACTATE, SERUM UREA NITROGEN AND HEPATIC GLYCOGEN OF RATS (n = 8)

Group	Treatment (mg/kg)	Hepatic glycogen (mg/g)	Blood lactate (mmol/L)			SUN (mg/dL)	
		Exhaustion	Before exercise	Exhaustion	0.5 h after exhaustion	Before exercise	Exhaustion
Control	–	0.23 ± 0.09	2.10 ± 0.28	2.78 ± 0.53 [a]	2.73 ± 0.69	20.40 ± 1.13	27.20 ± 2.05
LBE	190	0.29 ± 0.14	2.10 ± 0.22	2.18 ± 1.35	2.15 ± 0.43*	19.30 ± 1.97	22.60 ± 2.99**
MBE	380	0.32 ± 0.04	2.55 ± 0.79	2.65 ± 0.71	1.60 ± 0.22**	19.10 ± 1.51	23.00 ± 2.43**
HBE	570	0.29 ± 0.07	2.20 ± 0.31	2.70 ± 0.83	1.68 ± 0.35**	18.63 ± 1.31	23.30 ± 1.44**

* $p < 0.05$ and ** $p < 0.01$ as compared with control; [a] $p < 0.05$ as compared before exercise and after exhaustion. Values represent the means ± SD. SUN: serum urea nitrogen. LBE: low dose (190 mg/kg); MBE: middle dose (380 mg/kg); HBE: high dose (570 mg/kg).

In this study, the data indicate that burdock extract possess the ability to lower the formation of serum urea nitrogen after exhaustive exercise. The levels of blood lactate of rats before exercise and after exhaustion were shown as Table-3. The results revealed a significant increase of blood lactate found in control group before exercise and after exhaustion. In case of burdock extract treated group, there were no significant difference of blood lactate before exercise and after exhaustion. After exhaustive exercise for 0.5 h, all burdock extract treated groups with significantly decrease of blood lactate contents were found when compare with that of the control group ($p < 0.05$). Blood lactate is the glycolysis product of carbohydrate under anaerobic conditions[25]. The accumulation of lactate can interfere with nerve impulses and muscle contraction resulting in fatigue[26]. Decrease of blood lactate formation and rapid removal of lactate is thus beneficial to relieving fatigue. Our data indicate that burdock extract may effectively reduce the increased lactate level and remove the lactate in the blood, which is beneficial to postpone the appearance of physical fatigue.

After exhaustive exercise, malondialdehyde (MDA) levels of rats in the all burdock extract treated groups were significantly decreased compared with control group ($p < 0.05$) as shown in Table-4. MDA, the end product of lipid peroxidation, is a good marker of free radical radical-mediated damage and oxidative stress[27]. Most studies show that endurance exercise causes an increase in MDA[3]. Lou *et al.*, had revealed that the burdock extracts possess the inhibitory activity of lipid peroxidation apparently and the level of MDA may decrease[12]. The findings are in agreement with our results. In this experiment, the MDA level decreased apparently in the all burdock extract treated groups indicating that burdock extract had the effective antioxidants preventing from the lipid peroxidation damage. In case of antioxidant enzyme, SOD levels of rats in the all burdock extract treated groups revealed significant elevation when compared with that of the control group ($p < 0.05$) after exhaustive exercise (Table-4). SOD is very important in protecting against oxygen free radical damage that results in direct peroxidative damage to cellular components. SOD reduces superoxide to hydrogen peroxide and glutathione peroxidase reduces hydrogen peroxide from the SOD reaction to water[3]. Exhaustive exercise can increases the degree of lipid peroxidation and reduces the antioxidant activity[28]. The results indicate that burdock extract were able to up-regulate antioxidant enzyme activities to protect against oxidative stress induced by exhaustive exercise. The mechanism of antifatigue of burdock is still unclear. Free radical production during exercise contributes to fatigue and antioxidant treatment might be a valuable therapeutic approach[29]. It has been reported free radical scavenging activity of burdock was associated with its phenolic compounds[11,30]. Polyphenols in plant extracts with free radical scavenging activity may play important role to combat fatigue[29,31]. The results suggested that the free radical scavenging activity and polyphenols of burdock extract could be involved in ameliorating biochemical parameters related to fatigue.

In addition, it is well known that arginine and aspartate play a leading role in the urea cycle and these amino acids may help with fatigue reduction. It has been reported that arginine possess protective effects against exhaustive exercise-induced oxidative stress in young rat tissues[32]. The increased MDA levels of these tissues significantly decreased in exercised rats supplemented with arginine[32]. It is interesting that rats fed with any doses of burdock extract all showed decreased serum urea nitrogen and MDA levels after exhaustive exercises. The burdock extracts were known to have rich amino acids such as arginine and aspirate and they may enter the urea cycle to decrease ammonia formation. At the same time, the muscle will make use of branched-chain amino acid for energy when the muscle glycogen level is depleted[33]. The arginine and aspartate in burdock extract were analyzed to be about 453.5 and 685.4 mg/100 g, respectively. Burdock extract with arginine and aspartate could be another factor involved in the biological function of exercise-induced fatigue activity. In case of safety evaluation, there are no apparent abnormal histopathology changes in the liver and kidney of rates fed with burdock extract for 8 weeks (Chen, unpublished data) indicating that burdock is safe as functional food.

TABLE-4
EFFECTS OF BE ON SUPEROXIDE DILMUTASE AND MALONDIALDEHYDE LEVELS (n = 8)

Group	SOD (U/mg Pro)	MDA (nmol/mg Pro)
Control	34.35 ± 3.10	0.45 ± 0.08
LBE	57.21 ± 6.72*	0.32 ± 0.11*
MBE	92.91 ± 8.51**	0.34 ± 0.08*
HBE	87.53 ± 0.74**	0.32 ± 0.10*

*$p < 0.05$ and **$p < 0.01$ as compared with control. Values represent the means ± SD. MDA: malondialdehyde; SOD: superoxide dilmutase. LBE: low dose (190 mg/kg); MBE: middle dose (380 mg/kg); HBE: high dose (570 mg/kg).

Conclusion

We have demonstrated that burdock extract could extend the endurance time to exhaustion of the rats besides increasing the blood SOD levels and decreasing the blood lactate, serum urea nitrogen contents and MDA levels. The overall results indicate that burdock is a safe functional food with ameliorating biochemical parameters related to fatigue. Burdock extract with antifatigue activity may be associated with its polyphenols and free radical scavenging activity, arginine and aspartate. Further studies on the mechanisms of action are currently under investigation.

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