The effects of Rhodiola Rosea extract on endurance exercise performance in rats

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Abstract: The purpose of this study is to discuss the effects of anti-fatigue of Rhodiola rosea extracts. 40 eight-week old Sprague-Dawley (SD) rats were divided into 5 groups: control (fed with drinking water), P-control (fed with essence of chicken), three groups of different doses of Rhodiola rosea extract including low dosage (190mg/kg), mid-dosage (380mg/kg) and high dosage (570mg/kg). After feeding for 8 weeks, the rats ran on a running machine one by one at the speed of 22-24 m/min until exhaustive exercise-induce fatigue occurred and then their biochemical parameters in blood were collected and measured. At the end of this study, these rats were all scarified. Their livers and kidneys were excised for series of examination including serologic tests and histological evaluation. Finally, our results indicated that 3 groups that consumed Rhodiola rosea extracts are found to experience a greater increase in body weight than that of the control group. Moreover, the groups fed with Rhodiola rosea extract may help to significantly reduce the lactic acid concentration in blood, mild increase the liver glycogen to enhance the endurance abilities of the rats. Besides, we found that the Rhodiola rosea extract may possess the anti-oxidant activities and reduce the production of lipid peroxide which may benefit for recovery from the physiological fatigue. Furthermore, SD rats with consumption of Rhodiola rosea extract did not result in liver and kidney dysfunction. In short, Rhodiola rosea extract is proved to be a safe and effective food for promoting recovery from fatigue by mainly the antioxidant ability. Therefore, we suggested that everybody could reinforce the endurance ability and physical fitness performance by regular intake of Rhodiola rosea extract supplementation.
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In Republic of China Army, the soldiers were requested to test the physical fitness including 2-minutes sit-up (muscular endurance), 2-minutes push-up (muscular endurance) and 3000 meters run (cardiorespiratory endurance)〔1〕. Moreover, the military pilots in ROCF should perform the weight training for emergent acceleration stress or high-acceleration force exposure〔2〕We exposed to the vigorous acceleration condition in daily real-life activities every day〔3〕. Indeed, regular exercise for solider and other peoples is known to help and alleviate hypertension, cardiovascular diseases, diabetes, stroke, hyperlipidemia, and even cancers〔4,5〕. However, most of the peoples must sit and work on the computer, and therefore, several associated injuries and diseases may occur during long-term worked in front of the computers or video. Moreover, the overload makes the time for regular exercise is impossible in modern time. Therefore, if scheduled exercise was impossible, to relieve the fatigue from works or exercises may also prolong the working time and endurance the physical vigorous stress. It is reported that endurance training increases the capacity to sustain exercise possibly by altering muscle energy metabolism and contractile properties〔6〕.

When struggled with the military duties, how to endurance the fatigue from the various exercise became very important. In general, with stressful workloads being commonplace, people often feel fatigue in modern life. At first, we should be to know the "mean" of fatigue. Gibson et al. stated that fatigue is a failure to maintain the required or expected force or power output. Fatigue is a self-protective mechanism against the damage of contractile machinery of muscle. Moreover, the development of muscular fatigue during exercise is a common phenomenon, and several forms depend on the precise type of exercise performed〔6〕. 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, many researchers revealed different type of hypothesis about fatigue. In general, there are many factors of fatigue in mental, metabolic and physiological consideration. For example, Maclaren et al. reported that fatigue should be classified into central fatigue and peripheral fatigue 〔7〕. Firstly, central fatigue occurs as a result of the failure at any one link in the chain which from the brain to the formation of actin-myosin cross bridges within the muscles〔8〕. Exercise is associated with an increase in ammonia production by the skeletal muscles, and the extra-muscular action of ammonia is directed mainly toward the central nervous system, particular the brain. Afterwards, the central fatigue developed. As for the peripheral fatigue, it occurs at three possible sites: the neuromuscular junction and muscle cell membrane (excitation), the calcium release mechanism (activation), and the sliding filaments (contractile processes)〔9〕. There are also some clues about the formation of fatigue. Simonson proposed another accumulation hypothesis. He found that the accumulation of a number of metabolites, namely hydrogen (H^+), ammonia (NH_3), and inorganic phosphate, which have been shown to result in an impairment of force generation by muscle fiber and interfere with the function of enzymes in glycolysis 〔10〕.

The answers about how to improve our physical fitness performance are to diminish the muscle fatigue. Therefore, we proposed that the delay of the occurrence of fatigue and quick recovery from of fatigue are the current focuses of medical studies. In order to delay fatigue initiated after various exercise, military training, or laboring work as well as improve the endurance and muscle strength needed by human bodies, the oral supplements are often used. In China, the associated histories may be traced back to 4,000 years ago by our ancestors. Now, many nutritional supplements are used daily for the special sportsmen. For example, there are many healthy nutritional supplement used by the university athletes in Singapore 〔11〕. In many literatures, most chemical and biological agents was found in the animals, plants, or minerals which could improve the body strength, delay the occurrence of any types of fatigue, and relieve the fatigue associated symptoms (e.g.: painful sensation, shortness of breath, muscle spasm). However, remarkable side effects from the supplements are also observed in clinic. Besides, some anti-fatigue agents contain compounds and substances which could damage our body. For example, ephedrine is sometime dangerous for us and illegal in some countries. As a result, how to look for the safer and a more effective anti-fatigue foods or agents supplementation becomes more important to endurance physical fitness performance in human 〔12〕.

The roots of *Hodiola Rosea* is a herb that grows in mountainous regions of North America, Europe and Asia and has been used in traditional folk medicine for centuries as a treatment for fatigue and mood disorders〔16,17,18〕. In Taiwan, it is known as an enhancing strength power substance and made with powder in oral capsule and in drink which is very popular several years ago. Furthermore, *Hodiola Rosea* is considered as the medicine to treat the high mountain sickness. Therefore, it is a very common over-the-counter drug in Taiwan. Booker et al. also found that in the UK, *Hodiola Rosea* belonged to a traditional herbal medical product which is used in treatment of stress-fatigue fatigue, exhaustion and anxiety in clinics〔25〕. In addition, series of studies showed that *Hodiola Rosea* has been shown to possess both antioxidant and anti-inflammatory properties 〔12,20〕, improve cognitive function and reduce the mental fatigue〔21,22〕, and further decreased educe biological markers of physiological and psychological stress〔21,23〕. Besides, Kang et al. also had the same report. They considered that *Hodiola Rosea extract* effectively against fatigue caused by strenuous exercise〔26〕. Moreover, *Hodiola Rosea* may protect hypoxia-induced pancreatic cell. We believed that it is a stronger antioxidant which may scavenge all the free radical-induced oxidative activities in all human body including prevent from various cancers〔26〕. Lee and co-workers reported that rhodiola crenulate extract would inhibit the hepatic gluconegenesis through activating AMPK pathway which may be helpful for the management of type II diabetes 〔27〕. In the diabetic rats studies, Cheng and his colleagues found that the ethanol extract of rhodiola should increase the myocardial output and enhance the physical performance〔28〕. Besides, Lee et al. demonstrated that rhodiola water extract can lower the blood pressure for the purpose of treatment of hypertension and the β-endorphin play an important role〔29〕. Recent, most of the peoples were costumed to sit for work or for enjoyment for a long time. Many research revealed that the advantages of excellent physical performance may relieve the associated chronic low back pain, muscle fatigue, muscles spasm of lower extremities, and sarcopenia〔30,31,32〕.

In this study, our completed all the experiments in medical laboratory center in Kaohsiung Armed Forced General Hospital (Kaohsiung, Taiwan, ROC). Furthermore, we would focus on enhancing fitness performance and evaluate the anti-fatigue effects of Rhodiola rosea extract as nutritional supplement use in the amelioration of exercise-induced fatigue in rats.

Material and Methods

2.1 Materials:

The root of Rhodiola rosea was purchased in Kaohsiung City, southern Taiwan. At first, we took 250g Burdock dregs and put them in a round flask. Then the dregs from Rhodiola rosea were extracted with 1000 ml pure water (vehicle only). After this procedure, a Büchner funnel was used for filtering until the fluid concentration reached the paste form. It was then frozen and dried to acquire the Rhodiola rosea next. Secondary, the chicken of essence is widely used, particularly in Chinese communities, as a traditional remedy for several aliments. Some researchers believed that it may reinforce the whole body and enhance their muscle power〔34〕. In our study, the rats in one group were fed with the chicken of essence labeled as positive control to further evaluate the anti-fatigue effects of Rhodiola rosea extract.

2.2 Lab animals:

The animals are total 40 eight-week old male Sprague-Dawley (SD) rats with a body weight between 250~270 gram (g) which were all from the Taipei Animal Laboratory Center since July 2016. At first, they were housed in a temperature and light controlled room (22±2℃; the cycle of 12 hours light / 12 dark). All 40 SD rats were divided into 5 groups (8 rats in each group) according to different diets including control group (fed with water), P-Control (fed with Brand's Essence of Chicken Drink, a famous health food in Taiwan) and three different Rhodiola rosea concentration groups including low dose of Rhodiola rosea extract (LBE), middle dose of Rhodiola rosea extract (MBE), high dose of Rhodiola rosea extract (HBE) were fed with extract of the dosage of 190mg/kg, 380mg/kg, and 570mg/kg respectively with garage feeding every day. The total experimental period is 8 weeks (2 months) and the supply of routine feedings and drinking water were also given. The changes of body weight and the condition of food intake every day were documented. All the experimental rats started to receive the endurance performance (strenuous running) at the beginning of the 8^{th} week. During this week, we collected the serum BUN and lactate levels before and after violent running. After one week of exhaustive exercise (the end of 8^{th} week), the rats were sacrificed by CO_2 inhalation. Then series of various examinations were performed.

The periocular blood from each rat was obtained by micro-capillary tubes after conducted with ethyl-ether anesthesia 30-40 minutes at the beginning of 8th week. These blood sample were for BUN and lactate test. The blood from the hepatic veins of scarified rats at the ending of the 8^{th} week were collected for evaluation of SOD, GPx, CAT, GSH, ABTS and TBARS test. After the blood was taken, part of the livers in rats was excised for liver glycogen test. Finally, the livers and kidneys of 40 SD rats were fixed in 10 % neutral formalin solution for one week. Subsequently, the prepared tissues were dehydrated in 75 % ethanol solution. The cross-section (5 μm) of various specimens was stained with hematoxylin and eosin (H & E) for photomicroscopic assessment.

2.3 Measurement of the effects of anti-fatigue by run to exhaustion

Total SD rats are divided into experimental and control groups. They were fed with Rhodiola rosea extract at different dosage (experimental groups) and pure water (control groups) one week before the experiment. After feeding for 30 minutes, each SD rat was adjusted to daily treadmill training program for one week (from 7^{th} to 8^{th} week).

The modified method was proposed by Bedford et al who designed a treadmill test and standardized to secure the VO_2max of rats assigned to various tests〔35〕. These measurements may be repeated and reliable for many different experiments. In our study, we made use of the running machine for every rat to evaluate their endurance performance after feeding. Thirty to sixty minutes after the feeding, each rat was arranged on the running machine. The designed running pace was gradually increased (from 8m/min to 22-24m/min), and one electric bar was installed at the posterior region of the machine. If the rats refused to run and stop exercising, the bar may stimulate the body of rats to go on running because the rats felt pain. All recordings started from the beginning of running and ended until exhaustion was all demonstrated. When the rats became tired but not exhaustion, they may be pushed to run again by the electronic bar. If the running rats touched several times of the bar and the rats still failed to continue running, they were considered to exhaustion and terminated this one study. Finally, we recorded the exhaustive time of each rat in 5 groups for further evaluation later.

2.4 Determination of blood urea nitrogen (BUN) before and after exhaustive exercise in each group

At first rats were conducted with ethyl-ether anesthesia 30 minutes and we collected the blood from the peri-ocular region. After the rats regained consciousness, they were arranged to run on the running machine for strenuous exercise. Until the exhaustive SD rats were observed, we used the same method to collect the blood sample again. At first we added urase into the serum and mixed for 30 minutes. Then ammonia was added as a color reagent. By the colorimetric method, the red-color compound would be detected and then the absorbance ratio was measured at 660 nm. Finally, the results were converted into the level of BUN. We will compare the changes in the blood before and after exhaustion.

2.5 Determination of blood lactate before and after exhaustive exercise in each group

The blood samples were taken from the periocular region by minor puncture before and after exhaustive exercise. Lactate in the blood was determined by serologic test and color metric method. Lactate oxidase was first added and followed by 4-aminoantipyrine and 1,7-dihydroxy- naphthalene. Lately, we observed the red compounds produced by peroxidase and the absorbance ratio was measured colorimetrically at 540 nm. All the results were converted into a concentration level of lactic acid, and the data were recorded in detail. Moreover, we obtained the serum lactate before and after exhaustion in each group for further analysis later.

2.6 Determination of liver glycogen after feeding for 8 weeks in each group after exhaustive exercise

Livers were removed after scarified, followed cleaning with normal saline solution and drying with fuel filtered paper by us. 0.45g of liver was homogenized with 2ml 30% KOH. Afterward, the mixture was boiling for 20 minutes at 100℃. After heating, a homogeneous solution of 200μl was poured in each tube and then 1 ml absolute ethanol was added. The upper layer was easily removed after 4000rpm centrifuged for 10 minutes. At the time, we found that residual sediment was added with 0.5mL D.D.W and 1ml anthrone reagent was used finally. After mixing for 10 min carefully, the absorbance was read at 620 nm by a spectrophotometer and converted into the levels of liver glycogen. We compared the liver glycogen level after feeding in 40 SD rats after exhaustive running.

2.7 Determination of superoxide dismutase activity after feeding for 8 weeks in each group after exhaustive exercise

Firstly, the blood was gained from the hepatic veins as quickly as possible after killing the rats. The agent SD 125 (Randox Laboratories, Antrim, UK) was purchased from the market. We collected the upper layer of sample and then diluted with 50 μL of solution. Secondarily, the SOD standard solution with different bioactivity levels, 1.7 mL reactive solution （50 M xanthine, 25MI.N.T.）and 250 μL enzyme solution,（80 U/L xanthine oxidase）were prepared. After full mixture, 1 mL was poured and incubated. Under 37℃ and wave length 505 nm, the absorbance was measured. We found that the lower absorbance level presents the higher inhibiting activity and higher bioactivity of SOD. The measured value was then introduced to a standard curve and was timed with a dilution ratio. Finally, blood hemoglobin was quantified to calculate specific activity of SOD（U/g Hb）. The change of serum SOD level after various feeding in each group was recorded.

2.8 Determination of glutathione peroxidase (GPx) activity after 8-week-peroid feeding in each group after exhaustive exercise

At the ending of 8th week, the rats were scarified after exhaustive running. We collected the blood sample from the hepatic vein of SD rats. The agent RS 504 （Randox Laboratories, Antrim, UK）was purchased from the market. Blood sample was collected and added with a buffer diluted to 2% and mixed well for 37℃ (water bath) and heated for 3 minutes. The experimental group and contrast group were added with 200μl 1.25 mM H_2O_2 and mixed well for 37℃ water bath for 3 minutes. Then 4mL metaphosphate was added to sediment protein for mixing and centrifugated under 3000 rpm at 4℃ for 10 minutes. Afterwards, the upper clear fluid of 100μl was poured in 100μl 0.4 M Na_2HPO_4 and 50μl 0.4 mg/ml DTNB and put inside 37℃ oven for 3 minutes heating. At 422nm, absorbance was measured and in contrast with GPX, a standard curve was introduced to calculate the specific activity of GP_X (U/mL) in each extract solution. At last, the changes of serum GPX levels in each group after exhaustive running were determined with care.

2.9 Determination of catalase after feeding for 8 weeks in each group after exhaustive exercise

We got the blood from the hepatic veins of scarified rats. The changes of CAT bioactivity has been modified and measured according to the introduction of Beers et al 〔36〕. This is a simple, rapid, quantitative spectrophotometric method for following the action of catalase on hydrogen peroxide, based upon the measurement of the UV light absorption of peroxide, is suited to studies of catalase kinetics. At first, catalase (CAT) was taken in the original solution at the amount of 0.05 ml blood and PBS was added to increase to 3ml and the concentration of 200unit/ml. Then 200unit/ ml Catalase was diluted into 180, 160, 140, 120, 100, 80, 60, 40 and 20 unit/ ml and placed inside an ice bucket. Then, the diluted samples at the amount of 30 μL were taken to mix with 570 μL D.D. water and 300 μ reactive solution（59 mM H_2O_2 in 50 mM potassium phosphate, pH 7.0）. After mixing, 1 mL was poured and incubated. At the wave-length of 240 nm, the changes of absorbance within 3 minutes were recorded. The unit of CAT（1U）is defined as the decrease of 1 mole H_2O_2 per minute. The measured value was then introduced to the equation 〔(ΔA240nm/min) × 1000〕/ 43.6 and multiplied by the diluted ratio of the sample solution. Finally, the contained hemoglobin was quantified to calculate specific CAT activity（U/mg Protein）. Therefore, we could compare the changes of CAT level between each group and analyzed the results for further

explanation.

2.10 Determination of reduced glutathione activity after feeding for 8 weeks in each group after exhaustive exercise

The reduced glutathione concentration was analyzed by slightly modifying by the method of Tietze〔37〕. It is used to analyze the nanogram quantities of glutathione which is based on the catalytic action of GSH or GSSG in the reduction of Ellman reagent (DTNB) by a mixture of TPNH and yeast glutathione reductase. Firstly, the samples received equal volume 10% TCA (1:1) and after mixing, it was placed in micro-centrifuge tube under 4℃ and centrifuged 15 minutes at 3000 rpm. The upper clear solution about 40µl was taken and then added with 200µlEDTA and 10µl DTNB. After full mixing, it was put into 96well and recorded at 412 nm by ELISA.

2.11 Torlox equivalent anti-oxidant capacity in the blood after feeding for 8 weeks in each group after exhaustive exercise

First, blood sample were collected from the hepatic veins of rats in each group. The agent HG 980 (Randox Laboratories, Antrim, UK) was purchased from the market and modified by Salah and his co-workers 〔38〕 Afterwards, the ABTS (2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonate)) and $K_2S_2O_8$ (2:1) were mixed with PH 7.4 PBS; a sample was also added until 10 ml. Then, ABTS radical is green and can be monitored at 734 nm. Sample and ABTS (40ul:180ul) were placed in 96 well holes and each hole received a 40ul sample and then 180ul ABTS to measure and place in a shaded place for 6min to prevent from sunshine. Absorbance was measured at 734 nm. Finally, the differences between every group were analyzed carefully.

2.12 Determination of malondialdehyde level after feeding for 8 weeks in each group after exhaustive exercise

A modified thiobartituric acid reactive species (TBARS) assay was use to measure lipid peroxide proposed by Ohkawa and his colleagues〔39〕. Malondialdehyde (MDA), produced by the oxidant of polyunsaturated fatty acids, could react with two molecules of thiobarbituric acid (TBA). At first, we used the above solution and shake to mix well. Afterwards, the solution was bathed at 37 ℃ water for one hour. Each tube was given 500 µl 0.1N HCl and 200µl 9.8% SDS. Then 900µl pure water was poured in and mixed well and 2ml 0.6% TBA was mixed at 95℃ hot water bath for one hour. Lately the solution was then cooled to room temperature for about 5~10 minutes. The n-butyl alcohol was added at the amount of 5ml and mixed well by centrifugation at speed of 3000rpm for 25 minutes at 25℃. The upper clean solution was taken and loaded 200µl/well. The layer of n-butyl alcohol yields a pink red chromogen with an absorbance maximum at 532 nm which was measured. We analyzed the changes of TBARS in each group after exhaustive running.

2.13 Liver and kidney tissue section after feeding for 8 weeks in each group after exhaustive exercise

At the ending of our experiment, the livers and kidneys of SD rats were removed. The tissues were cleaned with normal saline repeatedly, dried with fuel filtered paper and soaked in neutral formalin. We observed the morphological changes of the livers and kidney of rats fed with various foods for 8-week-peroid.

Statistics:

All the experimental values are presented in the means± standard deviation (SD). Each group of lab animals is compared with One-way ANOVA internally. Then we used the Duncan method to compare significance between every group. If the difference were apparently, the *p* value may be defined as lower 0.05(P <0.05).

Results

3.1 The effects of Rhodiola rosea extract on body weight and the food intake rate in each group during eight-week period

The body weight of 8 SD rats in 4 groups (e.g.; Control, LBE, MBE, and HBE) showed increased weight after feeding for 8 weeks. However, the weight of P-Control group fed with essence of chicken remained no apparent change after the 8-week period (Fig.1). Meanwhile, the rate of dietary food in each group revealed no apparent significance (Fig. 2).

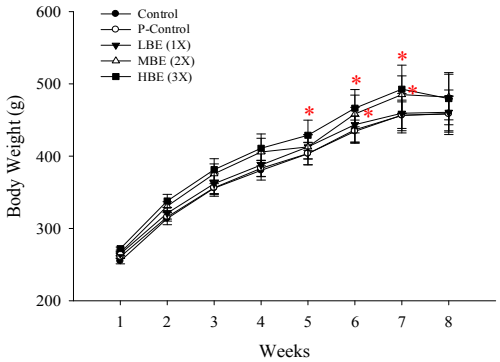

Fig.1. We compared the body weight gain of rats with various foods for 8 weeks among all group. We can clearly find out that the rate fed with water (control) and any dose of Rhodiola rosea extract showed remarkable change in body weight. However, the rats with essence of chicken remained unchanged. *: p<0.05 showing statistical significance when compared with the control group.

3.2 The influence of Rhodiola rosea extract on running time during exhaustive exercise

It revealed that the MBE and HBE group own the increased endurance performance by 30.9% and 25%, respectively, as well as significance (p<0.05) when compared with the control group (Fig. 3). It is interesting to find two special results that the endurance performance of rats fed with chicken essence is nearly the same as the MBE and HBE group. The other one is that the anti-fatigue effect of LBE group is not apparently significant when compared with the control group.

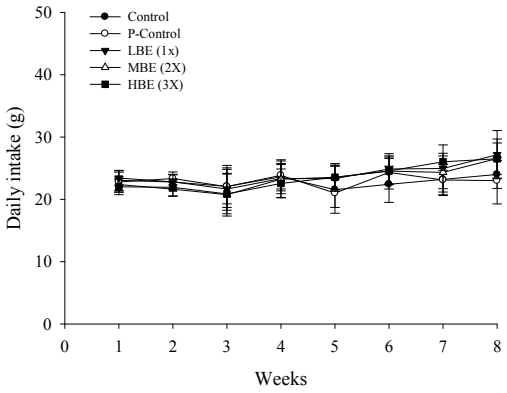

Fig.2 We analyzed the influence of food intake rate of SD rats with various foods for 8 weeks between all groups. There are no significantly abnormal findings between the 5 groups. *: p<0.05 showing statistic significance when compared with the control group.

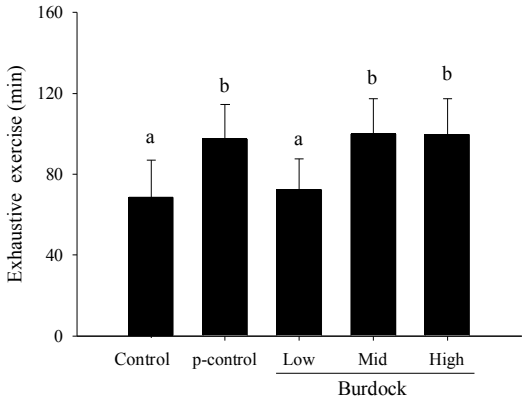

Fig.3 We compared the running time (endurance performance) of different groups rats with various foods for 8 weeks. It is clearly that the rats fed with Brank's chicken essence, middle and high dose of Rhodiola rosea extract possessed the excellent endurance performance (each group, n = 8) *: p <0.05 showing statistic significance when compared with the control group.

3.3 Influence of Rhodiola rosea extract on blood urea nitrogen level of rats before and after exhaustive exercise

We compared the level of BUN of rats with different concentration levels of Burdock extract before and after exhaustive exercise. It indicates a decrease of blood urea nitrogen of P-Control group (Chicken essence), and BE groups (any doses of Rhodiola rosea extract) when compared with Control group (p<0.05) (Table 1).

Table 1. We compared the blood urea nitrogen level of rats fed with various foods for 8 weeks before and after exhaustive exercise in each group

Group	Before Exercise	Exhaustion
Control	20.4 ± 1.13^{b}	27.2 ± 2.05^{b}
Positive Control	19.7 ± 2.17^{b}	22.2 ± 3.44^{a}
LBE	19.3 ± 1.97^{ab}	22.6 ± 2.99^{a}
MBE	19.1 ± 1.51^{ab}	23.01 ± 2.43^{a}
HBE	17.61 ± 1.32^{a}	23.36 ± 1.44^{a}

The control, positive control, LBE, MBE and HBE groups expressed as the means ± standard deviation. In each group, n = 8. One-way ANOVA is used for internal comparison and Duncan is used to compare significance between groups among which ^{a,b,ab} indicate significance.

Table 2. We compared the blood lactic acid level of rats fed with various foods for 8 weeks before and after exhaustive exercise

Group	Before Exercise	Exhaustion	Exhaustion after 30 min
Control	2.26 ± 0.28^{a}	2.78 ± 0.53^{a}	2.73 ± 0.69^{c}
Positive Control	2.47 ± 0.36^{a}	2.71 ± 1.07^{a}	2.12 ± 0.46^{b}
LBE	2.10 ± 0.22^{a}	2.18 ± 1.35^{a}	2.15 ± 0.43^{b}
MBE	2.55 ± 0.79^{a}	2.65 ± 0.71^{a}	1.60 ± 0.22^{a}
HBE	2.20 ± 0.31^{a}	2.70 ± 0.83^{a}	1.68 ± 0.35^{ab}

The control, positive control, LBE, MBE and HBE groups expressed as the means ± standard deviation. In each group, n = 8. One-way ANOVA is used for internal comparison and Duncan is used to compare significance between groups among which ^{a,b,ab} indicate significance.

3.4 Influence of Rhodiola rosea extract on serum lactic acid level of rats before and after exhaustive exercise

The changes of serum lactic acid of rats before and after exhaustive exercise are shown (Table 2). It indicates the inhibition of increasing serum lactic acid of P-Control and BE groups and after resting for 30 minutes, the reduction is more significant (p<0.05).

3.5 Change of liver glycogen of rats after exhaustive exercise with Rhodiola rosea extract feeding

The amounts of glycogen of the rats after exhaustive exercise showed mild increase in P-Control and all BE groups when compared with the control group. However, the change was statically insignificant (p > 0.05) (Fig. 4).

3.6 The anti-oxidative ability of the SOD in erythrocytes of rats after exhaustive exercise with Rhodiola rosea extract feeding

We could find that the anti-oxidative activity of the SOD in erythrocytes of rats fed with Rhodiola rosea extract and Chicken essence (P control group) is remarkable when compared with control group (p <0.05) (Fig. 5).

3.7 Influence of Rhodiola rosea extract on GAT, GSH, and GPX of red blood cells after exhaustive exercise

One of our results indicated that exhaustive exercise after Rhodiola rosea extract feeding may decrease the level of GAT, GSH, and GPX of red blood cells of SD rats. However, the changes are no statistical significance (Fig. 6,7,8).

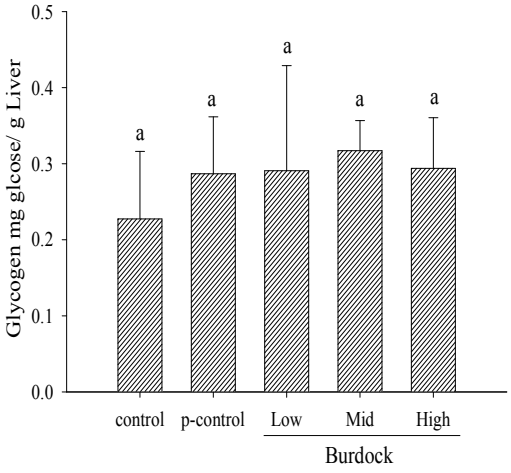

Fig.4. Change of liver glycogen of rats fed with various foods for 8 weeks after exhaustive exercise were compared (n = 8 in each group). There are no remarkable changes between all groups.

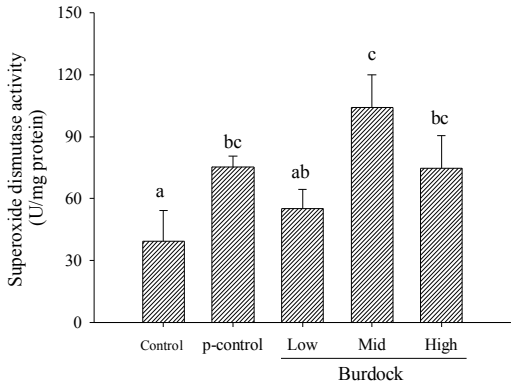

Fig.5. Change of the SOD level of SD rats fed with various foods for 8 weeks after exhaustive exercise were compared (One-way ANOVA is used for internal comparison and Duncan is used to compare significance between groups among which ^{a,b,ab} indicate significance.) We can find the increase the SOD level significantly on the rats with any dosage of Rhodiola rosea extract and Brand's chicken essence.

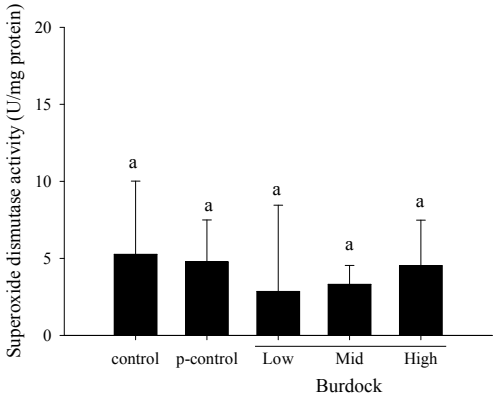

Fig.6. We evaluated the GAT level on red blood cells of SD rats fed with various foods for 8 weeks after exhaustive exercise. However, the changes have been no significance found between all groups.

3.8 Influence of Rhodiola rosea extract on peroxidase and total anti-oxidation level of rats after exhaustive exercise

TBARS presents the anti-oxidant ability to Lipid peroxidation and ABTS test accounts for the total antioxidant activity. We could find that the rats fed with burdock extract chicken essence all showed increasing level when compared with rats fed with water. The changes are apparent significantly (p<0.05) (Fig. 9,10).

3.9 Biopsy result of liver and kidney tissue

There are no apparently abnormal pathologic changes of livers and kidneys of SD rates fed with burdock extract and chicken essence under gross or microscope (Fig.7).

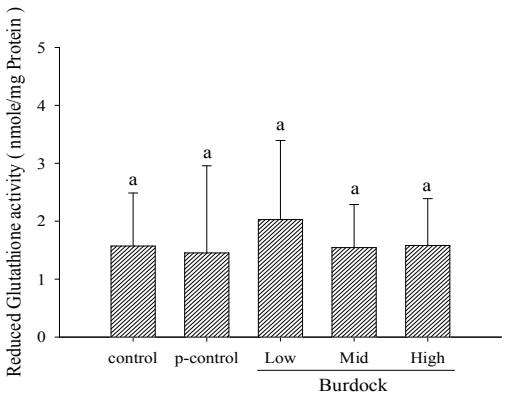

Fig.7. We investigated the GSH amount on red blood cells of SD rats fed with different foods for 8 weeks after exhaustive exercise. There were no apparent changes of reduced glutathione level between each group.

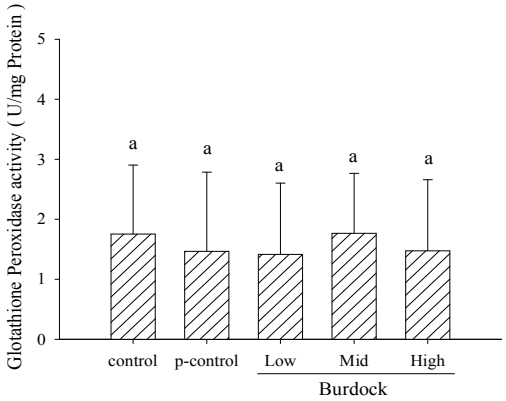

Fig.8. We compared the influence of GPx level on red blood cells of SD rats with different foods for 8 weeks after exhaustive exercise. (n = 8, in each group) We could not find out the apparent change of glutathione peroxidase level between each group.

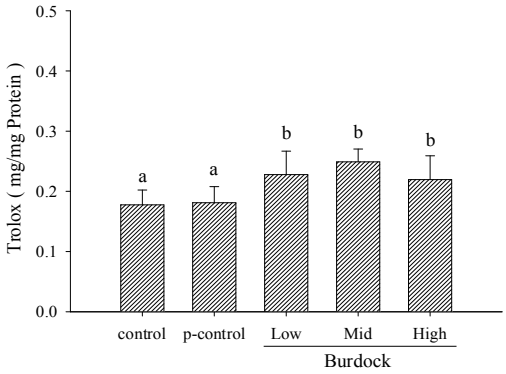

Figure 9: We compared the total anti-oxidation level of rats with various foods for 8 weeks after exhaustive exercise. (n=8, in each group). In terms of the amount Trolox, rats with any dosage of Rhodiola rosea extract all showed increase level when compared with rat with drinking water and chicken essence (p<0.05).

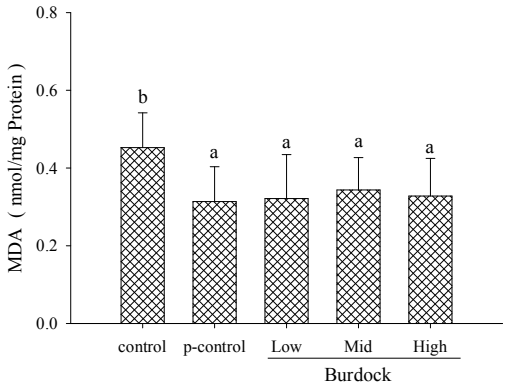

Figure 10: The lipid peroxidase level of SD rats fed with various foods for 8 weeks after strenuous exercise between each group were compared. In term of MDA level, we are interesting to discover that the rats fed with Brand's chicken essence and any dosage of Rhodiola rosea extract has the stronger and excellent anti-oxidative activities to the lipid peroxidase. (p<0.05).

Discussion

Components of physical fitness including cardiorespiratory fitness, muscular endurance, muscular strength flexibility, and body composition are important to overall health and performance of daily function activities. A complete picture of physical ability and health-related fitness is reflected in 5 domains: (1) cardiorespiratory f unction (2) muscle strength (3) muscular endurance (4) flexibility and (5) body composition〔34〕. No good physical fitness easily resulted in fatigue. It has been hypothesized that long work hours, long hours of physical or mental activity, insufficient break time between shifts, inadequate rest, excessive stress, or a combination of these factors may be associated with fatigue. Disrupted circadian rhythms have been found to be associated with changes in mental and physical performance〔13,14〕. Besides, a study showed that the fatigue may induce series of trouble problems in their performance in carrier. For example, Hughes and his co-workers surveyed 33000 registered nurses in 2011. They found that almost one-third participates in shift work. Fatigue is experienced by 19-29% of shit workers. It has been suggested that fatigue may contribute to medical error thereby causing negative health outcome〔15〕. The results were similar to the condition of fatigue induced-military errors in soldiers. For example, Wangner et al. reported that salidroside, an active principle of Rhodiola, improved mental ability. Moreover, in correction tests, the error rates were reduced by approximately 50%〔40〕. Furthermore, Darbinyan and his co-workers demonstrated that an increase mental work capacity

after intake Rhodiola rosea preparation. Besides, the subjects who take Rhodiola rosea had been on night duty for a considerably long time than those ones did not take〔41〕.

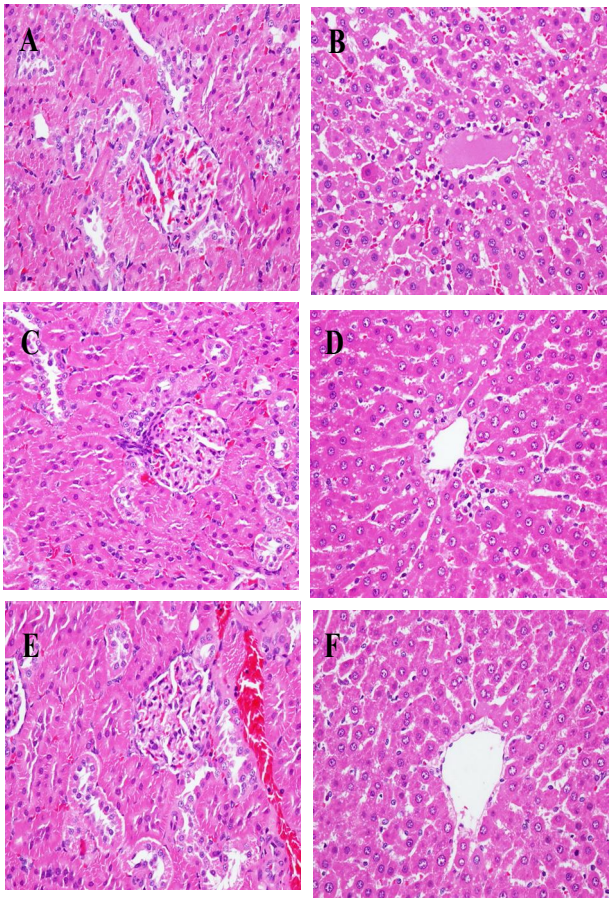

Figure 11: Pathology organization of rats supplemented with Rhodiola rosea extract after exhaustive exercise shown here with a 400 times enlarged structural diagram of HBE and P-Control groups after HE dying; W (Fig. A and B), L (Fig. C and D) and M (Fig. E and F).

Fatigue is commonly defined as a feeling of tiredness, lack of energy, emotional stability and motivation, or difficulty in concentration and memory. The clinical course is frequency aggravated by a variety of attending symptoms such as headache or muscle pain〔43〕. In general, the common applicable duration categories are: recent fatigue < 1 month; prolonged fatigue: 1-6 months; and chronic fatigue > 6 months〔44〕. In daily activities and exercises, push-up, sit-up, and climbing belong to the short-term activities and easily result in acute fatigue, however, 3000 meters run, marathon race, and long term sitting for work may be the types of chronic disease which may lead to chronic fatigue. Besides, the military training may induce the acute and prolonged fatigue for soldiers〔45,46,47〕. However, any types of daily work may use of specific muscles and function of the human organ. For example, push-up may make use of the upper extremities mainly. The endurance from abdomen and lower extremities strength is necessary for the repeated and rapid sit-up exercise. The excellent cardio-respiratory fitness is essential for marathon running, dancing, climbing mountain and swimming. As for the long-term sitting, the good function of abdomen and lower legs muscles is very important〔32,48,49,50〕.

The energy of acute and vigorous exercises is mainly from the blood and muscular sugar; however prolonged moderate and high intensity activities may cause significant muscle glycogen depletion. Because skeletal muscle glycogen is an important fuel for exercise performance at first such as Adenosine tri-phosphate (ATP) formation, many researcher have sought nutritional strategies to both maximize muscle glycogen storage prior to exercise and glycogen re-synthesis following exercise〔42〕. In normal human physiology, ATP is an immediate energy source available for the maintainace of muscle cell hemostasis and for contraction function. The onset of fatigue in high intensity exercise is related to phosphagens (such as ATP) depletion and the accumulation of metabolism of metabolic end products such as lactate, ammonia and H^+ ions〔51〕. The primary source of ATP is from glucose (so called "muscle glycogen"). Secondly, if the exercise prolonged, fatigue may occur. In this stage, lower blood glucose concentration will stimulate glycogenolysis with resulting depletion of hepatic glycogen. A number of studies which employed muscle biopsy techniques and subsequent histo-chemical analysis have found that muscle glycogen stores were depleted during sustained exercise at 65-75% of the maximum oxygen uptake (VO_2 max) and that this depletion was closely associated with fatigue〔52〕. Therefore, a high carbohydrate (CHO) diet was suggested to maintain the endurance exercise〔53〕. Moreover, Bergstrom et al. even suggested that after endurance performance, low or high carbohydrate contest was indicated for 3 days〔54〕. Rhodola rosea extract may prevent exercise-induced ATP decrease in mitochondria after exhaustive swimming〔71〕. Furthermore, it is surprised that Lee and his workers found the Rhodiola extract may enhance the re-formation of ATP during strenuous exercise. Furthermore, they also reported that Rhodioa extract plays an important role in hepatic glucose production. Therefore, it may also benefit for the relief of chronic fatigue from long-time activities〔42〕.

Feling et al. had estimated that conversion of amino acids to glucose provided 30% of the carbohydrate utilized by skeletal muscle during exercise of low intensity (30% VO_2 max) 〔55〕. In exercise intensities above 70% VO2max, the glycolysis activity is very high〔56〕. Initial muscle glycogen concentration was related to the ability tom perform prolong and heavy exercise. Hence, they strongly suggested the importance of carbonate-rich diet. They also concluded that carbohydrate were the most essential fuel during heavy muscle work. Moreover, the muscle glycogen concentration seems to the key factor for the increase in performance capacity for prolonged work which has practical applications in such situations as manual labor, military activity and athletics〔58〕. Glycogen is ever known as stored both in the liver and skeletal muscles. Liver glycogen is an important source of energy during intense exercise. However, after intensive exercise, excessive use of glycogen may inhibit gluconenogenesis and reduces the blood sugar levels which may induce fatigue and interrupt exercise performance〔57〕. Therefore, the levels of liver glycogen reflect the ability to resist to fatigue in some way. If liver glycogen can be decomposed as glucose, endurance performance will become stronger and so does anti-fatigue ability. Although the increase of liver glycogen was slight and statically insignificant in the three Rhodiola rosea extract group in our study, we still believed that the Rhodiola rosea may be benefit for the liver glycogen storage. This is because of the Rhodiola rosea extract was proved to possess abundant polysaccharides and carbon-hydrate in several literatures. Therefore, after feeding with the Rhodiola rosea extract for a period of time, we still believed that the liver glycogen may trigger gluconenogenesis and releases glucose to the blood in order to supply the insufficient sugar levels. Therefore, we demonstrated that the rats fed with Rhodiola rosea extracts may enhance the running time of the rats and endurance exercise in this experiment.

In many reports of endurance exercise, the concentration of blood lactate increase rapidly just before exhaustion, while blood glucose levels increase temporarily at the start of exercise, then gradually decrease to exhaustion. Excess production and accumulation of lactate in blood and muscle may induce acidosis. The muscle PH from 7.0 (at rest values) should reduce to approximately 6.3. Approximately 85% of the free H^+ formed during exercise induce acidosis is through lactic acid. Therefore, lactic acid inside the body also increases 〔59〕. The accumulation of lactic acid will interfere with nerve impulses and muscle contraction resulting in fatigue 〔42〕. In the same time after exhaustive running, a decrease in blood glucose level directly can also suppress the function of the central nervous system and lead to inability to exercise and fatigue. Thus, how to maintain glucose homestasis and lower lactate becomes an important factor in exercise activity〔61〕. In the UK and other countries, they are generally sold as “ food supplement” and therefore, provides they do not make any medical claim, they can legally be placed on the market without the need of either a medicines products license or traditional herbal medical product registration (THR). In Taiwan, the Rhodialo rosea was also considered as an over-the-counter drug and the people can easily buy for use in treatment various diseases (e.g.: stress-induced fatigue, exhaustion and anxiety).

Changes in blood lactic acid level before and after exhaustive exercise found in our study showed the remarkable inhibitory activity in lactic acid formation in P-Control (fed with the essence of chicken) and all burdock groups after exhaustive exercise. Lo and his colleagues also mentioned that the effect if essence of chicken may diminish the exercise and ammonia 〔62〕. In addition, the proposals of Abe was compatible with our findings that the burdock extract may maintain blood sugar levels and reduction of the lactic acid for recovery from endurance performance〔61〕. Why the burdock extract owns the ability ? It should be noted that rhodiola rosea extract had many different categories of bioactive constitutions including glycosides, flavones and phenolic acid〔62〕. Glycosides is similar glucose may directly supply the energy immediately and decrease the body fatigue. Moreover, flavones and phenoic acid are stronger antioxidants which may protect from the oxidative stress induced-fatigue〔63〕. Any stress (e.g. depression) and oxidative stress is considered a major possible precondition for the onset of fatigue by several articles〔64,65〕. Therefore, the characters of rhodiola rosea which is proven to scavenge the free radicals are able to reduce any type of fatigue and improve the physical fitness performance〔65,66〕.

There are over 200 species of Rodiola, each with distinctive pharmacology. The phenylethanol salidroside is common through the genus. Rhodiola, an herbal medicine used in Asian and Eastern European countries, is distributed in extremely altitude, chilly, hypoxic, and radioactive surrounding. Most of the reasons is that the extract of Rhodioia owns the stronger antioxidant ability and can scavenge free radical which may attack the cell membrane〔67〕. Rhodiola has been used for treating high altitude illness, depression, anxiety, anemia, fatigue, impotence, infection, cancer, headache, nervous system disorders, diabetes, improving mental

ability and quality of life of patients with short-term hypothyrodisim induced by hormone withdrawal. Moreover, It is also regarded as a tonic and stimulant and used to increase physical endurance, stress resistance, attention span, memory and work productivity 〔68,69〕. A recent study reported that oral administration of Rhodio extract was able to improve abnormal glucose and lipid metabolism as well as insulin sensitivity. Moreover, preliminary clinical trials showed a comparable efficacy in controlling blood glucose concentrations in type II diabetic patients. Based on the traditional application and the results of current studies, the Rhodiola extract might represent a promising anti-diabetic agent. To date, the detailed mechanisms of the control of blood glucose by Rhodiola are mostly unknown. Salidroside, a bioactive compound of the Rhodola species, was previously shown to enhance peripheral glucose utilization by promoting glucose uptake by both skeletal muscle cells and adipocytes〔70〕. In Russia, Rhodiola rosea has been used traditionally and former USSR that preparations based on Rhodiola rosea rhizome and the glycoside salidroside have gained an established position and use within the official medicine. Among 175 most important Tibetan drugs is mentioned in 10 formulations, of which 9 are indicated for lung disorders in 1986. After several years of studies, preparations based on Rhodola rosea became incorporated into the official medicine by 1969 and are described in the last official medicine USSR/Russian Pharmacopoeia in 1996. In the analysis of the components of Rhodiola rosea, salidroside has been pronounced and well-documented stimulant and adaptogenic action. Adaptogenic action is a pharmacological effect seen in clinical studies as an increased resistance to the harmful effects of various stressors. Recently, Rhodolia rosea was introduced into Taiwan and became the popular plant because of its several biological effects. After 8 weeks of feeding with Rhodolia extract, the body weight of rats increased significantly in the middle and high dosages group (MBE and HBE group). Besides, rats fed with any dose of rhodolia rosea extract are found to have a higher food intake rate than those in Control (drinking water) or P-Control (chicken essence) groups but no statistical significance is observed. Therefore, we can come into conclusion that rats fed with rhodolia rosea extract should contribute to good appetite and normal body weight development. In addition, there is no influence of the body weight gain and food intake rate of rats fed with chicken essence in our study.

Swimming and Running are aerobic sports that need to maintain endurance. The prolongation of running may reflect the endurance performance in some exhaustive trials. In this experiment, our results indicate the rats receiving middle and high dosage of rhodiola extract had longer persistent running time. This proves that rhodolia rosea extract effectively improves the aerobic exercise capability of rats and delays the occurrence of any stress and fatigue. The mechanisms of the anti-fatigue activity are complex. One hypothesis revealed that rhodolia extract contains many antioxidant substances which may prevent from the free radicals attacking the cells and decreased physical and mental fatigue. Physical exercise is characterized by an increase in O_2 uptake and consumption and induced stressors such as elevations of body temperature, the formation of reactive oxygen species (ROS), and a decrease in glycogen 〔72〕. Exhaustive exercise enhances xanthine oxidase activities of plasma and skeletal muscle, muscular myeloperoxidasive activity and malondialdehyde (MAD) concentrations of plasma and tissues 〔73〕. Vigorous exercise leads to oxidative stress may impair liver, kidney, skeletal muscle and other tissues by different degrees of ROS production. It has been speculated that increased antioxidant/oxidative damage-repairing enzyme activities, increased resistance to oxidative stress and lower levels of oxidative damage may protect oxidative stress-related cardiovascular, kidney, liver, and neuronal damage. However, the effects of Rhodiola rosea supplementation on exhaustive exercise-induced oxidative stress has been found〔74〕.

Exercise training increases the efficacy of antioxidant supplementation. There are many antioxidant enzymes which help to reduce oxidative damage during exercise; two which help to scavenge lipid hydroperoxides are glutathione peroxidase (which catalyses the breakdown of peroxides) and glutathione reductase. The level of these enzymes in blood increase following exercise 〔75〕. However, strenuous physical exercise induced oxidative stress contrarily which will produce a significant increase in the levels of free radicals and the concentration end-products of lipid peroxidation in different tissues following exhaustive exercise. There are several mechanisms by which reactive oxygen species may be produced during exercise. During high endurance exercise, free radicals may attack the cell membranes and bother the membrane function and lipid peroxidation proposed by Davies and his co-workers 〔75〕. It is widely accepted and experimentally proven that catabolic process can generate oxygen free radicals and other ROS. The majority of ROS is produced in the mitochondrial electron transport during exercise. In the same time, the change of antioxidant enzymes and substances including the reduction of GSH (γ-glutamylcysteinylglycerine) levels were noted 〔76〕. A particularly useful

antioxidant is gluthione, which is found both in reduced form (GSH) and oxidized form (GSSG). In general, there is agreement that exercise cause oxidation of blood gluthione. In normal condition, GSH is the most abundant source in the cell, and GSH reduce hydrogen and organic-peroxides via a reaction catalyzed by GSH pereoxidase (GP_X).

Moreover, the human body contains an elaborate antioxidant defense system with endogeneous antioxidants including glutathione, catalase (CAT), superoxide dismutase (SOD), and GP_X. During intense physical exercise, they may be overwhelmed by the higher production of ROS. The blood oxidized glutathione/glutathione ratio (GSSG/GSH) ratio increases when exercise is exhaustive〔77〕. Other experimental results indicate that exhaustive exercise results in the mild reduction of CAT, GP_X, and reduced GSH activity. Tauler et al. proposed that variously strenuous exercise may influence the different findings in antioxidant substances and enzymes〔78〕. They found that the maximal exercise test did not affect the activities of SOD, CAT, glutathione reductase but that of GPx decreased by about 4% in erythrocyte after the maximal exercise test in amateur sportsmen. On the other hand, the submaximal test resulted in more changes of enzymatic level in the amateur athletes. Superoxide dismutase activity increased by 25%, while catalase, GPx and glutathione reductase decreased by 12%, 14% and 16% respectively, after the prolonged submaximal exercise test. However, various outcomes of influence of physical exercise on different sportsmen have been reported. These differences could be due to the training status of the sportsmen, the intensity and duration of the exercise, and the environmental factor.

Many researcher found that at least 5 major ingredients (e.g.: stroysol, salidroside, rosin, rosarin, and rosavin) and Rhoddiola rosea extracts on xanthine and xanthine oxidase enhance O_2^-, H_2O_2 induced H_2O_2 by its several ingredients〔80〕. Besides, flavones and phenolic acid are all antioxidants which may improve the fatigue, and exhaustion and impact from oxidative stress and scavenging the free radicals. Therefore, we could relive the fatigue and enhance the physical fitness performance. In short, we could use the rhodiola extracts to decrease the attack from the free radicals and protect the cells in any organ (e.g.: heart, lung, and various muscle during or after exercise)〔79〕. Therefore, we could find the elevated the anti-oxidant titers and the associated damaged factors in the blood. Furthermore, the muscle strength and cardio-respiratory function will be enhanced, and the fatigue should be decreased, In addition, we observed that the kidneys and lives of rats fed with burdock and brand's chicken essence all revealed normal morphologic features under gross and light microscopy. It is well known that Rhodolia rosea extract possesses the hepato-protective activity due to the radical scavenging effect〔81〕. In addition, Panossion et al. found the caffeine acid owns the anti-flammatory effects in the kidneys〔82〕. Therefore, we suggest that the supplement of Rhodiola rosea extract is safe for sportsmen and human.

Conclusion

Recently, many people has focus on the health benefits of phytochemicals in various fruits, vegetables, cereals, and beverages through the antioxidant characters, not only from the contents of antioxidant nutrients such as β-carotene, vitamin C and vitamin E, but also other stronger antioxidant components. The oral supplementation becomes a trend for the public to promote their health〔83〕.

The results of our experiments conclusively demonstrated that rats fed with middle to high levels of Rhodiola extract for 8 weeks are able to possess prolong running time during vigorous exercise. After exhaustive exercise, the blood lactic acid and urea nitrogen is inhibited which would improve fatigue. Moreover, the total anti-oxidative capacity and avoiding the processes of lipid peroxidation is believed to be benefit for endurance performance. Thus, the anti-fatigue effects from of the Rhodiola rosea extracts should be expected.

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