Nutritional Sciences 6(3), August, 2003, pp. 135-140

Effects of Glucuronic Acid Derivative Isolated from Xylan on Antioxidative Defense System in Rat Red Gastrocnemius after Aerobic Exercise

Mi-Ji Kim and Soon-Jae Rhee[§]

Department of Food Science and Nutrition, Catholic University of Daegu, Gyungsan 712-702, Korea

The purpose of this study was to investigate the effects of glucuronic acid (isolated from xylan) on the antioxidative defense systems of red gastrocnemius in rats after aerobic exercise. The glucuronic acid was isolated from xylan. Male Sprague-Dawley rats weighing 150±10 g were randomly assigned to one normal group and three exercise training groups. The exercise training groups were classified as T (glucuronic acid-free diet), TU (250 mg glucuronic acid/kg bw) and 2TU (500 mg glucuronic acid /kg bw) according to the level of glucuronic acid supplementation. The rats in the normal group were confined to a cage for 4 weeks. The rats in the exercise training groups ran on a treadmill for 30 min/day, 5 days/week at a speed of 28 m/min (7% incline) for 4 weeks. Glutamate oxaloacetate transaminase (GOT) activity in the exercise training groups increased significantly compared with that of the normal group. That of the TU and 2TU groups decreased significantly compared with that of the T group. Xanthine oxidase (XOD) activity in the T group increased significantly to 74% compared with that of the normal group. That of the 2TU group decreased to 42% compared with that of the T group, thus recovering to a normal level. Superoxide dismutase (SOD) activity in the T group decreased to 32% compared with that of the normal group. That of the TU and 2TU groups increased to 28% and 34%, respectively, compared with that of the T group. Glutathione peroxidase (GSHpx) activity in the T group decreased to 16% compared with that of the normal group, but that of the TU group increased to 17% compared with that of the T group. Glutathiones transferase (GST) activity in the T group decreased to 11% compared with that of the normal group, but that of the TU and 2TU groups increased to 28% and 31%, respectively, compared with that of the T group. The contents of thiobarbituric acid reactive substances (TBARS) in the T group increased to 81% compared with that of the normal group, but the glucuronic supplementation groups recovered to the normal level. In conclusion, the effects of glucuronic acid on red gastrocnemius in rats engaged in exercise training would appear to be to reduce lipid peroxidation of tissue as an antioxidative defense mechanism.

Key words : glucuronic acid, aerobic exercise, red gastrocnemius, XOD, antioxidative defense system

INTRODUCTION

Aerobic exercise is known to reduce the probability of cardiovascular diseases due to the improvements it promotes in blood lipid composition and cardiovascular function. It has been reported that the probability of cardiovascular diseases in training groups that engage in aerobic exercise is relatively low compared to that of other groups that do not engage in aerobic exercise.[1] Nowadays, exercise is taken for granted as a way of preventing disease. But excessive exercise forcibly increases the generation of free radicals and oxidative stress by exhausting the electron in the process of electron transport in the cellular system.[2,3] Bredy et al.[4] reported that exhaustion due to exercise brought about increased lipid peroxide in groups that exercise on an irregular basis. Other studies[5-8] also reported that exhaustion in a short time accelerated the generation of free radicals through the consumption of the electron and increasing oxygen utilization through the electron transport system in mitochondria.[9] Meanwhile, Powers et al.[10] and Somani et al.[11] showed that regular exercise on a treadmill induced an increase in the activity of antioxidative enzymes such as superoxide dismutase.

On the other hand, free radicals such as superoxide radical and hydroxy radical in living bodies were protected by antioxidative defense enzymes such as superoxide dismutase, catalase and glutathione peroxidase. They were also protected by physiological antioxidative materials such as vitamin E and glutathione.

Under these circumstances, the development of pro-

Accepted : August 5, 2003
[§] To whom correspondence should be addressed.

tective materials that activate the antioxidative defense system after oxidative damage due to free radicals has been a hot issue. Research[12-15] has focused on the effect of antioxidative material on exercise with promising results. In particular, concern with the antioxidative effect of natural antioxidative materials has increased.[12-14]

Some studies have shown that the antioxidative effect of glucuronic acid extracted from xylan on the liver and soleus muscle increased liver glycogen contents in rats after aerobic exercise using a treadmil.[16] Others[17] examined the antioxidative effect of glucuronic acid on white gastrocnemius in rats after aerobic exercise. In this study, red gastrocnemius was used as experimental tissue. It requires more oxygen utilization during exercise and involves a more sensitive exchange of antioxidative defense enzyme activity in muscle tissue.

This study aims to show the changes in the antioxidative defense enzyme and oxidative damage in the red gastrocnemius after aerobic exercise with intubating glucuronic acid in order to examine the enforcing antioxidative effect of glucuronic acid.

MATERIALS AND METHODS

1. Preparation of glucuronic acid from Xylan

Glucuronic acid was extracted from oak (*Quercus mongolica*) wood chips through the process of chipping, filtrating using Amberllite IR 120 (Sigma Lot No 47H0428) and Amberllite IRA 67 (Sigma, Lot No 97H0002), evaporating and concentrating. In the series of processes described above, crude xylan was separated by adding ethanol of 4 times to a concentrated solution. After the extracted xylan was neutralized at the level of pH 5.5 with saturated $Ba(OH)_2$ solution, a deposit was removed. The neutralized solution was eluted by spraying distilled water into the column filled with negative ion exchange resins, Amberllite IR-120(H^+). By re-spraying an ammonia solution onto the ion exchange resins, glucuronic acid was separated from the ion resin. Then, after the removal of ammonia, xylan was extracted.

2. Experimental animals, diet and exercise training

Male Sprague-Dawley rats weighing 150 g were used for this study. The animals were housed individually in a stainless steel cage in a room with controlled temperature (20-23℃) and lighting (alternating 12h periods of light and dark). They were assigned randomly into one normal group and three exercise training groups. The exercise training groups were classified as T (glucuronic acid free diet), TU (250 mg glucuronic acid/kg bw) and 2TU (500 mg glucuronic acid/kg bw) according to the level of glucuronic acid supplementation before exercise training (Table 1). The experimental animals were freely fed a commercial non-purified diet made by Samyang Limited, of which the contents were as follows : 5.3% moisture, 24.6% protein, 5.4% fat, 54.7% carbohydrate, 3.5% fiber, 6.5% mineral. The glucuronic acid (25% glucuronic acid) was intubated to the mouth in the amount of 0.1 ml per 100 g of weight before exercise every day.

Exercise training was designed for rats with the regulation of speed, grade, time and frequency. The exercise training group (T, TU and 2TU) animals ran on a treadmill for 30 min/day, 5 days per week at a speed of 28 m/min (with 7% incline) for 4 weeks. The normal group was confined for 4 weeks. Table 2 describes the conditions of the exercise.

Table 1. Classification of experimental groups

Groups	Treadmill	Glucuronic acid (*250 mg glucuronic acid /kg bw*)
Normal[1]	-	-
T[2]	+	-
TU[3]	+	+
2TU[4]	+	+ +

[1]Normal : basal diet
[2]T : basal + training
[3]TU : basal + training + glucuronic acid (250 mg/kg bw)
[4]2TU : basal + training + 2×TU (500 mg/kg bw)

Table 2. Exercise training schedule of experimental rats.

	Duration(week)			
	1	2	3	4
Speed (m/min)	10	20	25	28
Grade (degree)	7	7	7	7
Time (min)	10	20	25	30
Frequency (days/week)	5	5	5	5

3. Preparation of serum and red gastrocnemius

On the last day of the growing weeks, the rats were intubated glucuronic acid 1 hour before exercise. Just after training, the rats were anesthetized with pentobarbital (made by Greencross Co, Korea). After blood was drawn from the artery of the abdominal region, the serum was obtained by centrifuging at 3000g for 10 minutes. Then, the red gastrocnemius was taken from the femoral region. The serum and red gastrocnemius were stored at -70℃ after being fast frozen.

4. Determination of white blood corpuscles (WBC) and red blood corpuscles (RBC)

The contents of WBC and RBC were measured using cell dyyn 1300 (Abbott Co. USA).

5. Measurement of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity

The activity of GOT and GPT in serum were measured using Asan kit made in compliance with the method of Reitman and Frankel.[18]

6. Measurement of xanthine oxidase (XOD) activity

The activity of XOD in red gastrocnemius was measured in compliance with the method of Stripe and Deracorte.[19]

7. Measurement of antioxidative defense enzyme activity

The activity of superoxide dismutase (SOD) in red gastrocnemius was measured in compliance with the methods of Marklund and Marklund.[20] The activity of glutathine peroxidase (GSHpx) in red gastrocnemius was measured according to the method of Lawrence and Burk.[21] That of glutathione-s-transferase (GST) in red gastrocnemius was measured according to the method of Habig.[22]

8. Determination of lipid peroxide in the red gastrocnemius

The contents of lipid peroxide in red gastrocnemius were measured in accordance with Satoh's method[23], which measures malondialdehyde produced by a reaction of thiobarbituric acid (TBA) and lipid peroxide.

9. Protein Determination.

The protein in the red gastrocnemius was measured in compliance with the method of Lowry et al[24] with bovine serum albumin as the standard solution.

10. Statistical analysis

Results were assessed by ANOVA and Tukey's Honestly Significant Difference test. Difference was considered significant at $p<0.05$.

RESULTS

1. The values of white blood corpuscles (WBC) and red blood corpuscles (RBC) in red gastrocnemius

An appreciation of the role of glucuronic acid in functional damage can be gained by observing the level changes of WBC and RBC in red gastrocnemius, as shown in Table 3. The contents of RBC in the exercise training groups were not significantly different from those of the normal group. However, that of WBC in the T group was slightly lower compared with that of the normal group. But, it was not significant.

Table 3. Effect of glucuronic acid on white blood corpuscles (WBC) and red blood corpuscles(RBC) values of red gastrocnemius in exercise training rats.

Group	WBC(1×10^3/ul)	RBC(1×10^6/ul)
Normal	7.75 ± 0.76^{NS}	6.95 ± 0.18^{NS}
T	6.64±0.61	6.52±0.15
TU	6.40±0.72	6.51±0.36
2TU	6.30±0.44	6.60±0.25

All values are mean±SE(n=10)
Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test.
Normal : basal diet
T : basal diet + training
TU : basal diet + training + glucuronic acid (250 mg/kg bw)
2TU : basal diet + training + 2 × glucuronic acid (500 mg/kg bw)

2. The activity of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in red gastrocnemius

The activity of GOT and GPT, which are indicators of damage in the liver, are shown in Fig 1. The levels of GOT activity in the exercise training groups increased significantly compared with those of the normal group. However, those of the Glucuronic acid supplementation groups decreased significantly compared with those of the T group ($P<0.05$). The levels of GPT activity did not differ significantly among the groups.

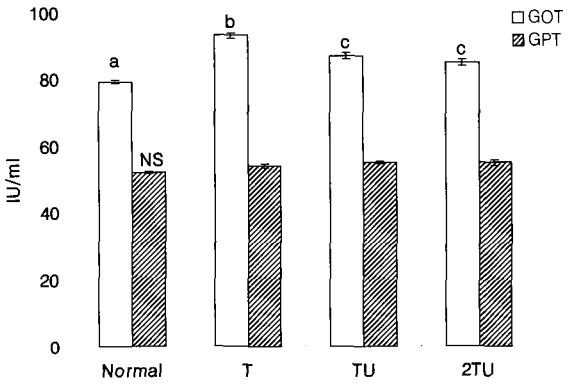

Fig 1. Effect of glucuronic acid on glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities of red gastrocnemius in exercise training rats.
All values are mean±SE (n=10)
Bars with different letters are significantly different at $P<0.05$ by Tukey's -HSD test.
Normal : basal diet
T : basal diet + training
TU : basal diet + training + glucuronic acid (250 mg/kg bw)
2TU : basal diet + training+ 2 × glucuronic acid (500 mg/kg bw)

3. The activity of xanthine oxidase (XOD) in red gastrocnemius

The activity of XOD, which is known as the enzyme that produces superoxide radical in the process of producing uric acid with xanthine as a substrate, in red gastrocnemius is shown in Fig 2. The level of XOD

activity in the T group increased to 74% compared with that of the normal group. That of the 2TU group decreased to 42% compared with that of the normal group. This is the same as the normal level.

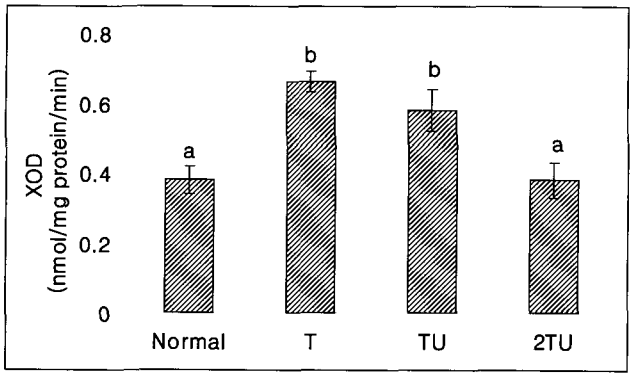

Fig 2. Effects of glucuronic acid on xanthine oxidase (XOD) activities of red gastrocnemius in exercise training rats.
All values are mean ±SE(n=10)
Bars with different letters are significantly different at P<0.05 by Tukey's -HSD test.
Normal : basal diet
T : basal diet + training
TU : basal diet + training + glucuronic acid (250 mg/kg bw)
2TU : basal diet + training + 2 × glucuronic acid (500 mg/kg bw)

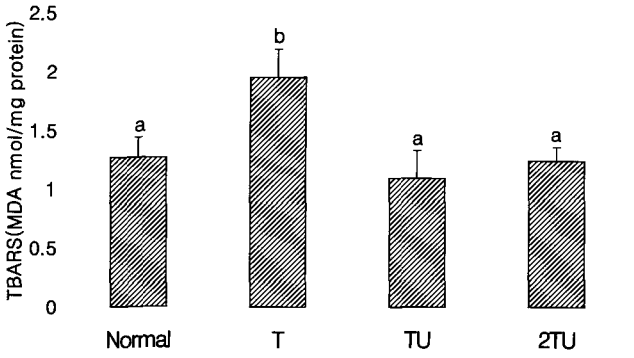

Fig 3. Effect of glucuronic acid on thiobarbituric acid reactive substance (TBARS) contents of red gastrocnemius in exercise training rats.
All values are mean ±SE(n=10)
Bars with different letters are significantly different at p<0.05 by Tukey's -HSD test.
Normal : basal diet
T : basal diet + training
TU : basal diet + training + glucuronic acid (250 mg/kg bw)
2TU : basal diet + training + 2 × glucuronic acid (500 mg/kg bw)

4. The activity of antioxidative defense enzyme in red gastrocnemius

The levels of superoxide dismutase (SOD) activity are shown in Table 4. The SOD is one of the antioxidative defense enzymes, which protects living cells from oxygen toxicity by deoxidizing superoxide radical to H_2O_2.

The activity of SOD in the T group decreased to 32% compared with that of the normal group, but those of the TU and 2TU groups with glucuronic acid supplementation increased to 28% and 34%, respectively, compared with that of the T group. The activity of GSHpx in the T group decreased to 16% compared with that of the normal group. The activity of GSHpx in the TU group increased to 17% compared with that of the T group. GST activity in red gastrocnemius of the T group decreased to 11% compared with that of the normal group. But, those of the TU and 2TU groups increased to 28% and 31%, respectively, compared with that of the T group.

Table 4. Effect of glucuronic acid on superoxide dismutase (SOD), glutathione peroxidase(GSHpx) and glutathione-s-transferase (GST) activities of red gastrocnemius in exercise training rats.

Group	SOD (unit/mg protein/min)	GSHpx (nmol NADPH/ min/mg protein)	GST (nmol DNCB/ min/mg protein)
Normal	1.35±0.14^a	60.74±1.77^a	85.6±5.54^a
T	1.02±0.11^b	50.71±4.46^b	75.9±6.75^b
TU	1.31±0.09^a	59.10±1.13^a	97.5±5.78^c
2TU	1.37±0.21^a	55.33±3.27^{ab}	99.5±6.54^c

All values are mean ±SE(n=10)
Values within a column with different superscripts are significantly different at p<0.05 by Tukey's test.
Normal : basal diet
T : basal diet + training
TU : basal diet + training + glucuronic acid (250 mg/kg bw)
2TU : basal diet + training + 2 × glucuronic acid (500 mg/kg bw)

5. Levels of lipid peroxide in red gastrocnemius.

The TBARS concentrations in red gastrocnemius as an index of lipid peroxidation increased to 81% in the T group compared with those of the normal group. But, those of the TU and 2TU groups with glucuronic acid supplementation decreased to 24% and 45%, respectively, compared with those of the T group. This shows that glucuronic acid can maintain the level of lipid peroxide at the normal level.

DISCUSSION

This study was conducted to observe the antioxidative effects of glucuronic acid after aerobic exercise. The study was carried out by having rats do aerobic exercise on a treadmill and then observing any change in both the free radical generation system and free radical scavenging system of red gastrocnemius, as well as, its related oxidative damage.

The study showed that WBC and RBC contents were not significantly different among the groups. GOT activity in the exercise training groups increased significantly compared with that of the normal group.

The levels in the groups supplied with glucuronic acid decreased slightly but meaningfully compared with that of the T group. The effect is similar to those mentioned in the reports of Parikh and Ramanathan[25], in which serum enzyme activity in the human body, such as GOT and GPT levels, increased significantly after exercise.

The activity of XOD, part of the free radical generation system[26], in the T group increased to 74% compared with that of the normal group. That of the 2TU group decreased to 42% compared with that of the T group. This was the same as the normal level. These results are similar to those cited in the reports of Kim and Rhee[17] and Laughlin et al[27], which showed that the level of XOD activity in white gastrocnemius and skeletal muscle increased after aerobic exercise.

SOD reduces superoxide radical to H_2O_2 when the generated H_2O_2 becomes unpoisoned by the action of GSHpx and catalase and, therefore, protects the living body from oxygen poison.[28,29] Ji LL[30] reported that the activity of SOD increased in the skeletal muscles, liver and heart tissue after exercise. But in this study, the exercise training group without glucuronic acid (T group) saw a decrease to 32% compared with that of the normal group. However, the T and 2TU groups increased to 28% and 34%, respectively, compared with that of the T group. These results are similar to those cited in the reports of Kim and Rhee[17], which showed that the level of SOD activity in white gastrocnemius decreased after aerobic exercise, but that of the glucuronic acid supplementation group recovered to the normal level.

GSHpx takes a catalytic reaction to generate not only H_2O and oxidized glutathione (GSSG) from H_2O_2 and reduced glutathione (GSH), but also alcohol (ROH) and H_2O from peroxide (ROOH).[31] In this study, GSHpx activity in the T group decreased to 16% compared with that of the normal group. That of the TU group increased to 17% compared with that of the T group. These results are similar to those cited in the reports of Kim and Rhee[17] and Choi[16], which showed that the level of GSHpx activity in the liver and white gastrocnemius increased after aerobic exercise when glucuronic acid was added.

On the other hand, GST is an enzyme taking a catalytic reaction that generates glutathione (R-S-G) by including a reduced glutathione in electron-affinitive substances among variant matters, carcinogen and endogenous poison. These results are similar to those cited in the reports of Kim and Rhee[17], which showed that the level of GST activity in white gastrocnemius after aerobic exercise increased when glucuronic acid was added.

Lipid peroxide contents as an index of lipid peroxidation in the T group without glucuronic acid increased significantly compared with that of the normal group. But the glucuronic acid supplementation group remained at the normal level. These results are similar to those cited in the reports of Allessio[32], which showed that lipid peroxide could be increased by boosting oxidative stress after exercise. In this study, glucuronic acid supplementation reduced lipid peroxide by suppressing free radical generation. The glucuronic acid caused the activation of the antioxidative defense enzyme in red gastrocnemius, which requires more oxygen utilization during exercise, and reduced oxidative damage.[17,33]

In conclusion, glucuronic acid supplementation reduced the increased concentration of lipid peroxide in the red gastrocnemius of rats undergoing exercise training by increasing the antioxidative defense system enzyme.

Literature Cited

1) Wood PD. Physical activity, diet, and health : Independent and interactive effects. *Med Sci Sports Exerc* 26 : 838-834, 1984

2) Durstine JL and WL Haskell. Effects of exercise training on plasma and lipoproteins. *Exerc Sports Sci Rev* 22 : 477-521, 1984

3) Haskell WL. The influence of exercise in the concentrations of triglyceride and cholesterol in human plasma. *Exerc Sports Sci Rev* 12 : 205-244, 1984

4) Bredy PS, Bredy LJ, Ullrey DE. Selenium, vitamin E and response to swimming stress in the rat. *J Nutr* 106 : 1103-1109, 1979

5) Davies KJ, Quiutanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 107 : 1198-1205, 1982

6) Freeman BA and Crapo, JD. Biology of disease, free radicals and tissue injury, *Lab Invest,* 47 : 412-426, 1982

7) Reid MB, Hack KE, Frankchk KM, Valberg PA, Kobzik L, West MS. Reactive oxygen in skeletal muscle. Intracellular oxidant kinetics and fatigue in vitro. *J App Physiol* 73 : 1797-1804, 1992

8) Sen CK. Oxidants and antioxidants in exercise. *J Appl Physiol,* 79 : 675-686, 1995

9) Kumar CT, Reddy VK, Prasad M, Thyagaraju K, Reddanna P. Dietary supplementation of vitamin E protects heart tissue exercise induced oxidant stress. *Mol Cell Biochem* 111 : 109-115, 1992

10) Powers SK, Criswell D, Lawler J, Ji LL, Martin D, Herb RA, Dudley G. Influcence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. *Am J Physiol* 266 : 375-380, 1994

11) Somani SM, Ravi R, Rybak LP. Effect of exercise training of antioxidant system in brain regions in rat. *Pharm Biochem Behavi* 50 : 635-639, 1995

12) Baek YH. Effect of garlic intake on the antifatigue and fatigue recovery during prolonged exercise. *J. Korean Soc Food Nutr* 24 : 970-977.1994

13) Soliman MA, El-Sawy AA, Fadel HM, Osman F. Effect of antioxidants on the volatiles of roasted sesame seeds. *J Agric Food Chem* 33 : 523-527, 1985
14) Kim MJ and Rhee SJ. Effects of green tea on hepatic antioxidative defense system and muscle fatigue recovery in rat after aerobic exercise. *J Korean Soc Food Sci Nutr* 31 : 1058-1064, 2002
15) Kim EH and Kim DH. Antioxidant activity of ethanol-extracts of defatted soybean, sesame and perilla flours in a soybean oil water emulsion system. *Korea J Food Sci Technol* 13 : 283-289, 1981
16) Choi HM, Lee SC, Ryu SP, Rhee IK, Joo GJ, Rhee SJ. Effects of glucuronic acid derivertives isolated from on antioxidative defense system and muscle fatigue recovery after aerobic exercise. *Korean J Nutrition* 34 : 872-880, 2001
17) Kim KR and Rhee SJ. Effect of glucuronic acid derivatives isolated from xylan on antioxidative defense system in rat white gastrocnemius after aerobic exercise. *Korean J Nutrition* 35 : 729-736, 2002
18) Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamate pyruvic transaminase. *Am J Clin Pathol* 28 : 56-63, 1957
19) Stripe F and Della Corte. The regulation of liver xanthine oxidase. *J Bio Chem* 244 : 3855-3863, 1969
20) Marklund S and Marklund G. Involvement of the superoxide anion radical in antioxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47 : 467-469, 1974
21) Lawrence RA and Burk RF. Glutathione peroxidase. Activity in selenium deficiency rat liver. *Biochem Biophys Res Commun* 71 : 952-958, 1976
22) Habig WH, Pabst MJ, Jakoby WB. Glutathione s-transferase the first enzymatic step in mercapturic acis formation. *J Biol Chem* 249 : 7130-7139, 1974
23) Satoh K. Selenium lipid peroxidase in cerebrovascular disorders determined by a new colormetric method. *Clinica Chemica Acta* 90 : 37-43, 1978
24) Lowry OH, Rosenbrough NJ, Farr AL and Randalll RJ. Protein measurement with the folin phenol reagent. *J Bio Chem* 193 : 265-275, 1951
25) Parikh DJ, Ramanathan NL. Exercise induced serum enzyme changes in untrained subjects. *Indian J Physiol Pharmacol* 21 : 175-180, 1997
26) Duke EJ, Joyce P, Ryan JP. Characterization of alternative molecular forms of xanthine oxidase in the mouse. *J Biochem* 131 : 187-190, 1973
27) Laughlin MH, T Simpson, WL Sexton, OR Brown, JK Smith and RJ Korthuis. Skeletal muscle oxidative capacoty, antioxidant enzymes and exercise training. *J Appl Physiol* 68 : 2337-2343, 1990
28) Bus JS, Aust SD, Gibson JE. Superoxide and singlet oxygen catalyzed lipid peroxidation as apossible mechanism for paraquat(methyl viologen) toxicity. *Biophys Res Commun* 58 : 749-753, 1974
29) Fridovich I. The biology of oxygen radicals, the superoxide radical is an agent of oxygen toxicity : superoxide dismutase provide an important defense. *Science* 201 : 875-880, 1978
30) Ji LL. Antioxidant enzyme response to exercise and aging. *Med Sci Sports Exerc* 25 : 225-231, 1993
31) Speisky H, Kera Y, Penttla KE, Israel Y, Lindors KO. Depletion of hepatic glutathione by ethanol occurs independently of ethanol metabolism. *Alcol Clin Exp Res* 12 : 220-232, 1988
32) Alessio HM. Exercise-induced oxidative stress. *Med Sci Sports Exerc* 25 : 218-224, 1993
33) Tamura S, Tsutsumi S, Ito H, Nakai K, Masuda M. Effects of glucuronolactone and the other carbohydrates on the biochemical changes produced in the living body of rats by hard exercise. *JPN J Pharmacol* 18 : 30-38, 1968