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Effects of Ginseng Saponin on Serum Alanine Aminotransferase Activity in Trained Rats

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The effects of ginseng saponin on the activity of serum alanine aminotransferase (ALT) in trained rats were examined. The trained group was given a chronic swimming bout (approx. 90 min/day) for 50 days, and ginseng group was given an oral administration of ginseng saponin (150mg/kg/day) for 2 weeks. Ginseng treated-trained group was given an oral administration of ginseng saponin for 2 weeks prior to the termination of a swimming bout. In this experiment, male rats of Sprague-Dawley strain (250 ± 20 g) were used. The activities of serum ALT in trained and in ginseng groups increased 72.89% ($P < 0.01$) and 57.14% ($P < 0.01$) than in control groups, respectively. Also, the activities of serum ALT increased 69.66% ($P < 0.01$) in saline treated-trained group, and 79.31% ($P < 0.01$) in ginseng treated-trained group than in control groups which were given saline solution and kept sedentary. The effect of ginseng saponin, as revealed by comparing the ginseng treated-trained group with the saline treated-trained group, was not significant. The present study suggests that training and ginseng saponin significantly increased the activity of serum ALT in rats, but that, in ginseng treated-trained group, ginseng saponin did not raise any further the increased activity of serum ALT by training.

KEY WORDS: Ginseng saponin, Alanine aminotransferase

It is now known that the degradation of muscle protein can contribute to an energy source during exercise (Felig and Wahren, 1971; Odessey *et al.*, 1974; Dohm *et al.*, 1977; Chang and Goldberg, 1978; Dohm *et al.*, 1980; Booth and Watson, 1985; Dohm *et al.*, 1985). Also, training was associated with an increased muscle glycolytic capacity and related to the ability of liver to increase gluconeogenic capacity (Huston *et al.*, 1975; Dohm *et al.*, 1981; Donovan and Brooks, 1983).

The activity of citric acid cycle enzyme in muscle was increased by training and decreased by exhaustion of trained animals (Holloszy and Booth, 1976; Dohm *et al.*, 1973; Dohm *et al.*, 1985). Also, the training or single bout of exhaustive exercise increased the total activity of lysosomal enzymes. It might represent an adaptation of the muscle to "clean up" damaged or partially degraded proteins as part of the recovery process (Dohm *et al.*, 1980).

The level of alanine aminotransferase activity (ALT; E. C. 2. 6. 1. 2.) that synthesizes alanine from pyruvate and branched-chain amino acids (BCAA) was found to increase in muscle and liver of the trained rats (Mole *et al.*, 1973; Holloszy and Booth, 1976; Ji *et al.*,1987). However, Ji *et al.*(1988) showed that maximal activity of rat skeletal muscle mitochondrial ALT, as well as several other mitochondrial enzymes involved in various metabolic function, was significantly suppressed after a single bout of acute or exhaustive treadmill running.

A large number of studies of ginseng extract have been undertaken to clarify its effects on enzymatic activities (Kim *et al.*, 1975; Joo and Han, 1976a; Kang and Joo, 1985; Kim and Joo, 1985). Joo and Kim (1977) demonstrated that the oxida-

tion of pyruvate was increased in the presence of ginseng saponin, one of the components of Korean ginseng root(*Panax ginseng* C. A. Meyer) and that it was probably accomplished through the activation of mitochondrial enzyme by the saponin. Ginseng saponin at optimal concentration was observed to increase the activity of human serum ALT *in vitro*(Kim *et al.*, 1977; Park *et al.*, 1978).

In this study, it was attempted to observe whether ginseng saponin orally administered for 2 weeks influences serum ALT activity in trained rats.

Materials and Methods

Preparation of Ginseng Saponin

Ginseng saponin was obtained through the modified method of Joo and Han (1976b). Thirty grams of powdered Korean white ginseng roots (*Panax ginseng* C. A. Meyer) were placed in 760 ml chloroform-methanol-water mixtures (1 : 2 : 0.8, v/v/v) and the mixture was continuously stirred for one and half days at room temperature. After filtration under the reduced pressure, the insoluble precipitates were placed in 100 ml of the above chloroform-methanol-water mixtures and stirring was continued for another two days and filtered. The combined filtrate was then diluted with 250 ml of chloroform and 250 ml of water to make the volume ratio of chloroform-methanol-water in the mixture being 1 : 1 : 0.9, so that a good separation into two phases (chloroform phase and methanol-water phase) might be obtained. The methanol-water phase, or higher phase, when concentrated to remove the methanol and then lyophilized, yielded about 4.2g of the crude saponin preparation. This crude saponin preparation was used for this experiment without further purification.

Experimental Animals and Training Program

Male rats of a Sprague-Dawley strain, weighting approximately 250 ± 20 g at the start of the study, were allowed with ordinary diet (Daejong saryo Co. Ltd., Seoul) and water *ad libitum*. The temperature of animal room was maintained at 24 ± 4°C.

First, animals were randomly grouped into the sedentary and the training groups. The sedentary group remained sedentary in their cages. The training group was given a chronic swimming bout and carried out in a normal plastic water tank for an exercise session. The exercise session was 10 minutes from the first to the third day, 15 minutes from the 4th to the 6th day, 30 minutes from the 7th to the 10th day, 60 minutes from the 11th to the 14th day, 90 minutes from the 15th to the 28th day, and 120 minutes from the 29th to the 50th day. The water temperature was maintained at 30 ± 2°C.

Half of the animals in each group were given an oral administration of ginseng saponin (150 mg /kg/day) for 2 weeks prior to the termination of an experimental swimming bout. The remaining half of the animals in each group served as the controls and were given an oral administration of saline solution.

Preparation of Enzyme

The trained rats were not exercised for 72 hours prior to sacrifice in order to avoid any chronic effects of exercise. After overnight starvation, the animals were anesthetized with ethyl ether. Blood sera were prepared by placing the freshly drawn blood into a centrifuge tube without anticoagulant and incubating it in a water-bath (37°C) for about 30 minutes. The clot was sedimented by centrifugation (CL International clinical centrifuge, Damon, U. S. A.) for 15 minutes with 3,000 rpm and serum was taken for analysis. Blood sera served as the enzyme source and were kept in the refrigerator until required.

Assay Method of Enzymatic Activity

The alanine aminotransferase (ALT) activity was determined according to Reitman-Frankel (1957) by assaying pyruvate formed during ALT catalyzed reaction using ALT Reagent Kit (Sigma Co.). One ml of substrate was pipetted into a test tube, and placed in a water-bath at constant temperature (37°C) for 10 minutes. Upon the addition of 0.2 ml of serum, the contents were mixed, and after an incubation period of exactly 30 minutes, the tube was removed from the water-bath. One ml of the 2.4-dinitrophenly hydrazine reagent was added immediately, thereby stopping the reaction.

After the tube was permitted to stand at room temperature (18-26°C) for a minimum of 20 minutes, 10 ml of 0.4 N sodium hydroxide solution was added, and the contents were mixed by inversion. After 5 minutes, the optical density of the solution at 546 nm was read using UV visible spectrophotometer (DMS 90, Varian, Australia) using water as the blank. The ALT activities were described as Sigma-Frankel (SF) Unit that forms 4.82×10^{-4} μmol glutamate/minute at pH 7.5 and 25°C.

Results

The effect of training on the activity of serum alanine aminotransferase (ALT) in rats is shown in Fig. 1. The activities of serum ALT were 9.96 ± 0.47 Unit/ml in control group, kept sedentary, and 17.22 ± 0.70 Unit/ml in trained group, given a chronic swimming bout (approx. 90 min./day) for 50 days. It was that the activity of serum ALT in trained rats increased 72.89% higher levels than in control group. This difference was statistically significant ($P < 0.01$). Therefore, training influenced on the activity of serum ALT in rats.

The effect of ginseng saponin orally administered on the activity of serum ALT in rats is shown in Fig. 2. The activities of serum ALT were 10.15 ± 0.51 Unit/ml in control group, fed a saline solution, and 15.95 ± 0.85 Unit/ml in ginseng group, given an oral administration of ginseng saponin (150 mg/kg/day) for 2 weeks. Therefore, the activity of serum ALT in ginseng group was 57.14% greater than in control group, and it was statistically significant ($P < 0.01$). From this results, ginseng saponin had an effect on the increase of serum ALT activity in rats.

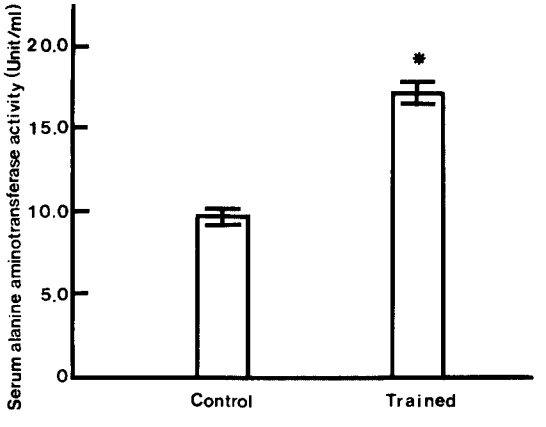

Fig. 1. Effect of training on the activity of serum alanine aminotransferase in rats. Control group was kept sedentary, and trained group was given a chronic swimming bout (approx. 90 min./day) for 50 days. *P < 0.01.

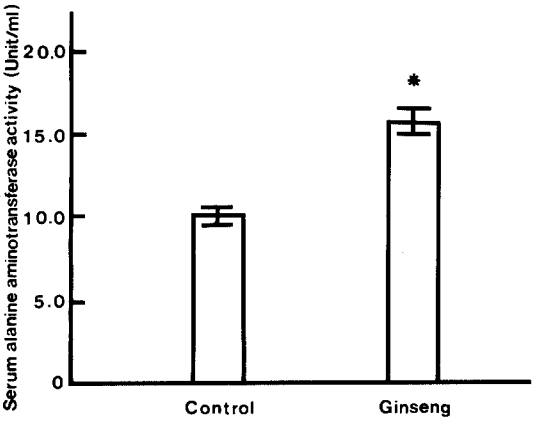

Fig. 2. Effect of ginseng saponin on the activity of serum alanine aminotransferase in rats. Control group was fed a saline solution, and ginseng group was given an oral administration of ginseng saponin (150 mg/kg/day) for 2 weeks. *P < 0.01.

The effect of ginseng saponin on the activity of serum ALT in trained rats is also shown in Table 1. Control group was given a saline solution and

Table 1. Activity of serum alanine aminotransferase in ginseng treated-trained rats. Control group was fed a saline solution and kept sedentary. Saline treated--trained group was fed a saline solution and given a chronic swimming bout (approx. 90 min./day) for 50 days. And ginseng treated-trained group was given an oral administration of ginseng saponin (150 mg/kg/day) for 2 weeks and given a chronic swimming bout for 50 days. Values are means ± SE.

Group	Numbers of rats	Alanine aminotransferase activity (Unit/ml)
Control	13	10.15 ± 0.51
Saline treated-trained	13	17.22 ± 0.70*
Ginseng treatedtrained	13	18.20 ± 0.61*

*P < 0.01.

kept sedentary. The activity of serum ALT increased from 10.15 ± 0.51 Unit/ml in control group to 17.22 ± 0.70 Unit/ml in saline treated-trained group. Saline treated-trained group was given a saline solution and a chronic swimming bout for 50 days. The activity of serum ALT in ginseng treated-trained group which was given an oral administration of ginseng saponin for 2 weeks and a chronic swimming bout for 50 days increased 18.20 ± 0.61 Unit/ml than in control group. Saline treated-trained group increased 69.66% higher levels of serum ALT activity than control group ($P < 0.01$). And ginseng treated-trained group was higher than control group by 79.31% ($P < 0.01$). Although the activity of serum ALT was increased 5.69% in ginseng treated-trained compared with saline treated-trained group, the significant difference in the activity of serum ALT was not observed between saline treated-trained group and ginseng treated-trained group.

From the above results, the activity of serum ALT in rats increased with training. Also, ginseng saponin stimulated the activity of serum ALT, but, in ginseng treated-trained group, ginseng saponin had no effect on the raise of the increased activity of serum ALT by training.

Discussion

Amino acid catabolism increased during exercise training and the muscle enzymes involved in leucine oxidation adapted to endurance training in a manner similar to the enzymes of carbohydrate and fat catabolism (Dohm *et al.*, 1977; Withe and Brooks, 1981; Dohm *et al.*, 1985).

Booth and Watson (1985) described that rates of muscle protein degradation decreased during exercise in rats and men if exercise duration is less than 12 hours, but increased when exercise is continued for a day.

Ji *et al.* (1987) observed that ratios of plasma branched-chain amino acids (BCAA) were dramatically lowered by exercise in the trained rats, but did not change in the resting state. It demonstrated that trained animals have a greater capacity to metabolize BCAA.

Amino acids released from this net loss of tissue proteins, mostly in the form of alanine, provided more than 50% for the precusors for gluconeogenesis of liver and kidney in rats and men (Felig and Wahren, 1971; Goldberg and Odessey, 1972; Felig *et al.*, 1973; Odessey *et al.*, 1974; Chang and Goldberg, 1978). The gluconeogenic process is a potentially important pathway for the utilization of amino acids, because it would contribute to the supply of glucose to prevent hypoglycemia during exercise in rats and men (Felig, 1973; Holloszy and Booth, 1976; Dohm *et al.*, 1985). Also, Huston *et al.*(1975) suggested that training was associated with an increased muscle glycolytic capacity and related to the ability of liver to increase gluconeogenic capacity in rats.

The activity of mitochondrial ALT for alanine synthesis from pyruvate and BCAA increased after training (Mole *et al., 1973). But Kindira and Rajendra (1987) and Ji et al.*(1988) showed that maximal activity of rat skeletal muscle mitochondrial ALT was significantly suppressed after a single bout or exhaustive treadmill running, and this enzymatic "down regulation" was maintained 24 and 48 hours post exhaustion.

A pathway for pyruvate removal in skeletal muscle was the conversion to alanine via the ALT reaction (Felig and Wahren, 1971; Ruderman and Berger, 1974). From this point of view, this adaptation could result in conversion of a greater proportion of the pyruvate formed in muscle to alanine and less to lactate, and thus help protect against the development of acidosis in muscle during strenuous exercise (Holloszy and Booth, 1976). Accordingly, Pösö *et al.* (1987) suggested that plasma alanine concentration serve as an indicator of the training and recovery status after training.

In this study, the activity of serum ALT in trained rats, given a chronic swimming bout (approx. 90 min./day) for 50 days, significantly increased 72.89% higher level than in control rats, kept sedentary. This result is in good agreement with that of Mole *et al.* (1973). Therefore, it appears that training had an effect on the activity of serum ALT in rats.

Nonspecific enzyme stimulating effects of ginseng saponin might be mainly due to their surface activity (Kim *et al.*, 1985). Also, these saponin might bring about a slight change of the enzyme

conformations (Kang and Joo, 1985; Kim and Joo, 1985). Park *et al.* (1978) found that the highest ALT activity was observed when the saponin concentration in the assay mixture was 1×10^{-7}%. Also, Kim *et al.*(1977) reported that the petroleum ether-alcohol extract of Korean ginseng roots (WG pet) stimulated the ALT activity. Joo *et al.* (1976) also showed that the moderate amounts of ginseng saponin ($10^{-6} - 10^{-4}$% in the assay mixture) stimulated purified pig cardiac ALT.

As shown in Fig. 2, the activity of serum ALT was significantly increased 15.95 ± 0.85 Unit/ml, 57.14% in ginseng group which was given an oral administration of ginseng saponin (150mg/kg/day) for 2 weeks. Therefore it can be suggested that ginseng saponin stimulate the activity of serum ALT in rats, and may bring about an increase of amino acid metabolism.

Avakian and Evonuk (1977) speculated that ginseng extract could affect the carbohydrate sparing actions during acute exercise in rats by stimulating increased synthesis of certain regulation enzymes involved in skeletal muscle and/or adipose tissue intermediary metabolism.

From this experimental results, it was concluded that the activity of serum ALT in saline treated-trained group, fed a saline solution and given a chronic swimming bout (approx. 90 min/day) for 50 days was significantly higher than in control group by 69.66% ($P < 0.01$). And in ginseng treated trained group which was given an oral administration of ginseng saponin (150 mg/kg/day) for 2 weeks and given a chronic swimming bout for 50 days, it was 79.31% higher than in control group. The activity of serum ALT was increased 5.69% in ginseng treated-trained group compared with saline treated-trained group. But this difference was not significant. From the above considerations, training increased the activity of serum ALT in rats. Also, it seems that ginseng saponin had an effect on the increased of serum ALT activity, but did not raise any further the increased activity of serum ALT by training.

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인삼 사포닌이 훈련된 흰 쥐의 혈청 Alanine Aminotransferase 활성에 미치는 영향

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본 연구는 인삼 사포닌(*Panax ginseng* C. A. Meyer)이 수영으로 훈련된 웅성 흰 쥐(Sprague-Dawley 계, 250 ± 20 g)의 혈청 alanine aminotransferase(ALT) 활성에 미치는 영향을 알아보았다. 훈련군은 50일간(약 90분/일) 수영훈련시켰으며, 인삼군은 체중 Kg 당 150 mg의 인삼 사포닌을 2주간 구강 투여하였다. 인삼 사포닌을 투여한 훈련군은 수영훈련 종료일 직전 2주간에 걸쳐 체중 Kg 당 150 mg의 인삼 사포닌을 구강 투여하였다. 훈련군의 혈청 ALT 활성과 인삼군의 혈청 ALT 활성은 대조군 보다 각각 약 72.89%와 57.14%로 유의성 있게 증가하였다. 실험군인 인삼 투여 훈련군과 인삼 비투여 훈련군의 혈청 ALT 활성은 대조군인 비투여 및 비훈련군 보다 각각 79.31% 및 69.66%로 유의성 있게 증가하였다. 인삼 투여훈련군을 인삼 비투여 훈련군과 비교하여 볼 때면 인삼 사포닌의 영향은 무의성으로 나타났다. 따라서 훈련 및 인삼 사포닌은 각각 흰 쥐의 혈청 ALT 활성을 뚜렷하게 증가시키나, 인삼 투여 훈련군의 경우 인삼 사포닌은 훈련으로 인해 증가된 흰 쥐의 혈청 ALT 활성을 더욱 상승시키지는 않는 것으로 생각된다.