J Food Sci Nutr
Vol 9, p 245～252(2004)

J Food Science and Nutrition

Hepatoprotective Effects of *Allium monanthum* MAX. Extract on Ethanol-Induced Liver Damage in Rat

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Abstract

This study investigated the effects of an ethanol extract of *Allium monanthum* MAX. (AME) on ethanol-induced hepatotoxicity in rat liver. Sprague-Dawley rats weighing 100～150 g, were divided into 5 groups; normal group (NOR), AME 200 mg/kg treated group (S1), ethanol (35%, 10 mL/kg) treated group (S2), AME 200 mg/kg and ethanol (35%, 10 mL/kg) treated group (S3) and AME 400 mg/kg and alcohol (35%, 10 mL/kg) treated group (S4). AME was fractionated by the following solvents: n-hexane, chloroform, EtOAC and n-BuOH. Antioxidant index of the n-BuOH fraction was 600 ppm, highest among fractions. The growth rate and feed efficiency ratio were decreased by ethanol, but gradually increased to the corresponding level of the normal group by administering AME. The serum ALT activities that were elevated by ethanol were significantly decreased by AME administration. It was also observed that the hepatic activities of SOD, catalase, xanthine oxidase and GSH-Px that were increased by ethanol were also markedly decreased in the AME treated group with compared to ETH. These results suggest that ethanol extracts of *Allium monanthum* MAX. may have a protective effect on ethanol-induced hepatotoxicity in rat liver.

Key words: *Allium monanthum* MAX., hepatoprotective effects, SOD, GSH, ethanol-induced liver damage, ALT, AST

INTRODUCTION

Numerous kinds of medicinal plants grow in the fields and mountains of Korea, and of these, many kinds have been used as foods or medicines (1). Recently, many studies have actively evaluated various bioactive phytochemicals with therapeutic potential as herbal medicines. Furthermore, some of these plants have been developed as high value-added products and are commercially used (2). In particular, with the reports on the harmful effects of synthetic antioxidants on the human body (3), many studies have focused on safe and effective natural antioxidants (4-8). Several studies have reported on the biological functions of natural products such as tocopherol (9), phenolic compounds (10), browning products formed by Maillard reactions (11), amino acids, and peptides (12), etc.

Allium monanthum MAX. is a perennial herbaceous plant belonging to genus *Allium* of the *Liliacae* family, and is also a wild plant broadly distributed in Northeast Asia regions such as Korea, Japan, China and Mongolia (13).

In oriental medicine, *Allium monanthum* MAX. is called as Ya-San (13) or Hae-Baek (14), and is known to be effective in stanching, nourishing the blood and tranquilization of nerves, etc. (15). Especially, the bulb of *Allium monanthum* MAX. is used for stomachache, normalizing intestinal function, and burn (16,17). Minerals (Ca, P, Fe, etc.) and amino acids have been identified in the bulb, roots and leaves of *Allium monanthum* MAX. (18).

It was reported that the leaves and bulbs of *Allium monanthum* MAX. contain vitamins and sugars such as fructose, glucose, sucrose and maltose (19). The unique substances isolated from *Allium monanthum* MAX. were alliin, methyl alliin and scorodose as secondary metabolites having antibacterial actions (20). Recently, Baek et al. (21) separated and isolated galactoglyceride compounds such as 1-O-linolenoyl-2-O-linolenoyl-3-β-D-galactopyranosyl-*sn*-glycerol. Ahn et al. (22) separated and isolated 2 kinds of compounds including: 3-O-[β-D-glucopyranosyl(1→4)-D-glucopyranosyl] kaempferol and 3-O-[β-D-glucopyranosyl(1→4)-d-glucopyranosyl] kaemp ferol, a flavonoid glycoside from the n-BuoH fraction of *Allium monanthum* MAX. Major studies of *Allium monanthum* MAX. have mostly focused on taxonomy

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(23,24), cultivation (25) and sterilization (26), etc. Choi et al. (27) reported that chronic administration of *Allium monanthum* MAX. affected the levels of serum lipids, sugar and protein in rats. Some studies have reported that several glycosylglyceride compounds are associated with anticancer effects (28) and hyperlipidemia improvements (29). Recently, the toxic effects of alcohol have been reported to be induced by very strong free radicals that are generated as by-products from its metabolism, because they produce oxidative damage in the cells, but not by the direct action of alcohol (30-32). Free radical oxygen produced as a by-product of alcohol metabolism is produced when oxygen is unstably reduced in the respiration of higher animals and plants or of aerobic microorganisms. If oxygen, essential for maintaining the life of an organic body, is activated *in vivo*, it is converted into very toxic superoxide anion ($O_2^{\cdot}$). This changes into H_2O_2, hydroxyl radical (-OH), and $^1O_2$ (singlet oxygen), and these chain reactions of active oxygen accelerate lipid peroxidation and degenerate the lipid in biological membranes, contributing to the etiology of geriatric diseases such as arteriosclerosis, diabetes, stroke, cancer, etc., and also promotes cellular aging when it is a continuous stimuli (33,34). Accordingly, inhibiting the production of free radicals *in vivo* is an important disease prevention strategy, and consequently, some anti-aging studies have been being performed using antioxidant substances for inhibiting biological free radical production (35).

This study measured the antioxidant activity of each fraction of the Allium monathum MAX. ethanol extract (AME) *in vitro*, and estimated the growth rate, feed efficiency ratio (FER), anlanine aminotranferase (ALT) and asparate aminotransferase (AST) activities to investigate whether *Allium monathum* MAX. can prevent the oxidative cellular injury induced by oxygen free radicals produced by alcohol in the liver. For the verification of the inhibitory effects against hepatotoxicity, this study compared the estimation of the activities of free radical generating enzymes: xanthine oxidase (XO), free radical scavenging enzyme, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), and the levels of thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) in liver tissue.

MATERIALS AND METHODS

Materials

Fresh *Allium monanthum* MAX. was purchased from a market in 2002 and dried in the shade before used. Alcohol and 100 g of sample were mixed at a 80:20 (v/w) ratio, ground in a blender (Braun, MR350 CA), extracted twice for 12 h using a reflux condenser at 65°C, and filtered through a millipore filter (type FH 0.5 μM).

The filtrate was concentrated and fractionated with n-hexane, chloroform, EtOAC and n-BuOH. After the solvent was removed by using a rotary vacuum evaporator in a water bath at 45°C, each fraction was vacuum-concentrated and dried.

Antioxidative activity was measured by using a Rancimat 676 (Metrohm, Swiss). Antioxidant index (AI) was calculated by dividing the induction period of an experimental group where each fraction was added by the induction period of a control group.

Animal experiments

Male Sprague-Dawley rats weighing 100~150 g were fed a commercial non-purified pellet diet (Samyang, Korea) for a 1-week adjustment period after arrival. They were assigned by randomized complete block design into groups of normal rats fed a control diet (NOR, n=10) and four experimental rats (n=10 per group): *Allium monathum* MAX. ethanol extract (200 mg/kg of body weight/day) group (S1), alcohol (10 mL of 35% ethanol/kg of body weight/day) group (S2), alcohol (10 mL of 35% ethanol/kg of body weight/day) and AME (200 mg/kg of body weight/day) group (S3), alcohol (10 mL of 35% ethanol/kg of body weight) and AME (400 mg/kg of body weight/day) group (S4), respectively. The rats were individually housed in stainless steel cages and fed for six weeks (Table 1). According to the method of Fujii et al. (36), 35% alcohol (10 mL/kg of body weight/day) was administered. AME was orally administered for 6 weeks by being dissolved in a saline solution to contain 200 mg or 400 mg per kg of body weight based on a preliminary experiment. During the experiment, body weight was measured at one week intervals and feed intake every two days, The feed efficiency ratio (FER) of each experimental group was calculated by dividing total body weight increase by the total feed intake for the period. Each rat was fasted for 16 h before sacrifice and blood

Table 1. Composition of experimental diet

Groups	Diet compositions
NOR	basal diet[1)]
S1	basal diet+AME200[2)]
S2	basal diet+EtOH[3)]
S3	basal diet+AME200+EtOH
S4	basal diet+AME400[4)]+EtOH

[1)]AIN-93 diet.
[2)]*Allium monanthum* MAX. ethanol extract (200 mg/kg of body weight/day).
[3)]35% ethanol (10 mL/kg of body weight/day).
[4)]*Allium monanthum* MAX. ethanol extract (400 mg/kg of body weight/day).

samples were collected from the carotid artery. The serum was separated from collected blood by centrifugation at 3000 rpm for 15 min at 4°C. Livers were surgically removed, cleaned, blood removed with 0.9% saline solution, weighed and then stored in a cryogenic freezer at -70°C until used for estimating the enzyme activity. All rats were allowed free access to water and pellet diet during the experiment period.

Preparation of enzyme source from liver

One gram of liver in 4 mL 0.25 M sucrose buffer (pH 7.5) was homogenized in ice using an ultra turax homogenizer. The homogenate was centrifuged at 600 g for 10 min at 4°C to remove the nucleus and non-homogenized parts, and the supernatant centrifuged again at 15,000×g for 20 min for assaying SOD, catalase, xanthine oxidase and GSH-Px. Serum was separated and used to measure the activities of ALT and AST.

Measurement of enzyme activities

Activities of xanthine oxidase, SOD, catalase, GSH-Px in liver tissue were assayed by the methods of Downey et al. (37), Crapo et al. (38), Aebi (39) and Flohe et al. (40), respectively. The level of peroxide was measured by the colorimetrical titration of malondialdehyde with thiobarbituric acid (41), and the level of glutathione was determined by the method of Tietze (42). The activities of serum ALT and AST were estimated using a kit (Asan Pharm, Korea) based on the method of Reitman-Frankel (43).

Protein contents and statistical analysis

Protein concentrations were assayed according to the method of Lowry et al. (44) using bovine serum albumin (Sigma) as the standard. The data were analyzed using SPSS Package and represented as mean±standard error (SE). Statistical significance among groups was evaluated by Tukey's test ($p<0.05$) after one-way analysis of variance.

RESULTS AND DISCUSSION

Estimation of antioxidative activities of the fractions

Table 2 shows the comparison of induction time of each fraction of *Allium monathum* MAX. ethanol extract (AME) by Rancimat's test method. The antioxidant index (AI) of each fraction was 1.62 for n-BuOH, 1.43 for EtOAC, 1.38 for n-hexane, 1.28 for chloroform fraction and 1.12 for water fraction in order, and all of these were much higher than those of the control group. Based on these results, the n-BuOH fraction showed the highest AI. These results correspond to the reports by Baek (21) and Ahn (22) that various flavonoids in the phenolic compounds in *Allium monathum* MAX. were transferred to the n-BuOH fraction.

Table 2. Antioxidative activities of each fraction of *Allium monanthum* MAX. ethanol extract on soybean oil

Fraction[1)]	IP[2)]	AI[3)]
Control	08.08 h	1.00
n-Hexane	11.17 h	1.38
Chloroform	10.32 h	1.28
Ethylacetate	11.63 h	1.43
n-Butanol	13.08 h	1.62
Water	09.07 h	1.12

[1)]Fractions were separated by separatory funnel.
[2)]Induction period (IP) of oil was determined by Rancimat's test at 110°C.
[3)]Antioxidant index (AI) was expressed as IP of oil containing various fraction/IP of soybean oil.

Growth rate and feed efficiency ratio (FER)

The growth rate and FER are shown in Fig. 1 and 2 for the six week experimental period. Growth rates were not significantly different among the groups from week 1 to 4. However, some differences were found from week 5. At week 6, the alcohol treated (S2) group (2.79±0.064) showed a significant slowdown of the growth rate than the normal control (NOR) group (3.12 ±0.087) ($p<0.05$), and the alcohol and AME (S3) group showed an elevated ratio close to that of the NOR group ($p<0.05$). These results were in concordance with the reports of Decarli (45) and Halsted (46) that the administration of alcohol reduced the body weight.

The growth rate was unchanged until week 4, but was slowed from week 5 onward, which suggests that alcohol was normally metabolized in the liver by ADH until week 4 to produce NADH and ATP and did not significantly affect the body weight loss, and thereafter, the alcohol metabolism was dependent on MEOS which caused alcohol levels to remain higher, which might

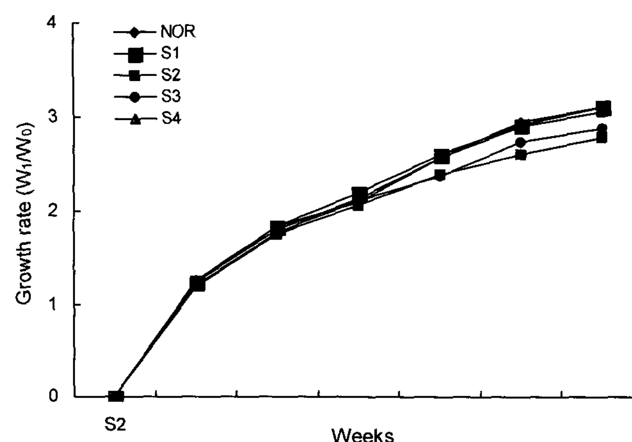

Fig. 1. The growth rate of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX. Abbreviations: See Table 1.

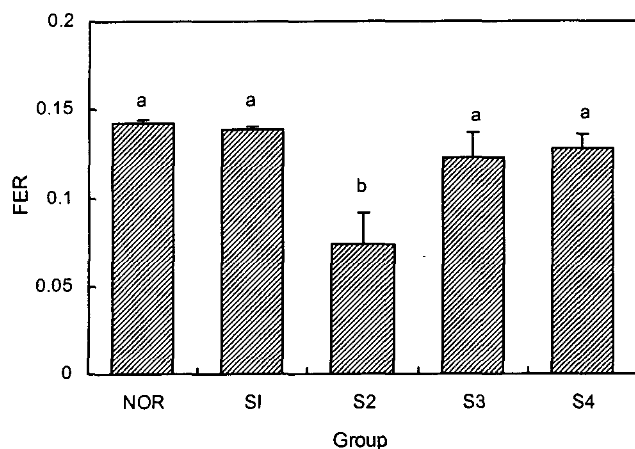

Fig. 2. The feed efficiency ratio (FER) of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX. Abbreviations: See Table 1. Values are mean±SE of 10 rats per each group and different superscript letters indicate significant differences at $p<0.05$.

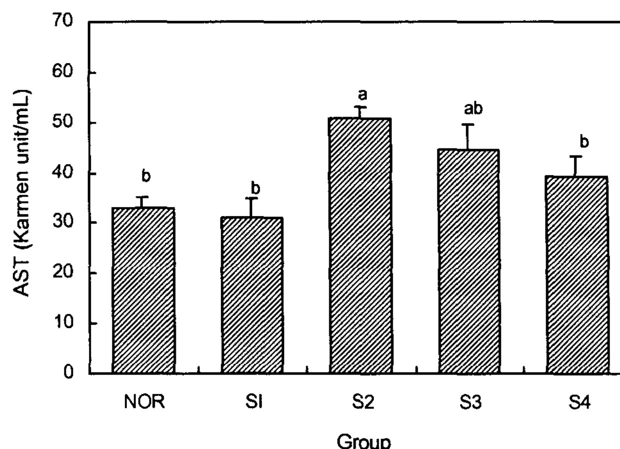

Fig. 3. The activities of AST in serum of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX. Abbreviations: See Table 1. Values are mean±SE of 10 rats per each group and different superscript letters indicate significant differences at $p<0.05$.

cause a significant slowdown body weight gain due to thermal generation without producing ATP (47). Also, it might be explained that the interaction of complex causes such as reduced nutrient absorption rate due to the alcohol-induced digestibility decline or small intestine mucosal damage, or a diminution of energy consumption efficiency due to the excessive intake of alcohol, etc.

The growth rate that was reduced by the administration of alcohol was restored to nearly the same as the NOR group by the combined administration of alcohol and AME, which suggests that AME relieved the toxic effects of alcohol and restored normal metabolism. FER was significantly reduced by the administration of alcohol in comparison with the NOR group, which can be caused by the reduction in feed consumption with the calories from the diet replaced by calories from alcohol. On the other hand, the combined administration of alcohol and AME greatly increased the FER but there were no significant difference between the two doses used.

Activities of ALT and AST in serum

Fig. 3 and 4 show the activities of serum ALT and AST used as indices of liver damage measured after the 6-week administration of alcohol and AME at 200 (S3) or 400 mg/kg of body weight/day (S4). The activities of serum ALT and AST in liver are indicative of the presence of damage-inducing substances produced during alcohol metabolism, and, moreover, aminotransferase present in the liver indicates progress of liver cell necrosis and liver tissue destruction resulting in the enzyme being released into the blood thereby increasing the serum activity (48,49). Serum ALT activity in the S2 group (50.86±2.11 Karmen unit/mL) showed a great increase owing to the alcohol administration as comparing with the NOR group (32.97±2.20 Karmen unit/mL) ($p<0.05$). However, the S3 and S4 groups exhibited the decreased ALT activities compared to the S2 group and the decrease was greatest in the S4 group, the higher dosage treated group ($p<0.05$). The serum AST activity was similar to the serum ALT activity. That is, the activity of S2 group (149.85±5.37 Karmen unit/mL) was significantly increased by alcohol administration compared to the NOR group (117.63±3.70 Karmen unit/mL) ($p<0.05$). However, the S3 and S4 groups had lower activities compared with the S2 group, and especially, with the greatest reduction in the S4 group ($p<0.05$). Based on the fact that the increased serum ALT and AST activities by alcohol were significantly reduced due to

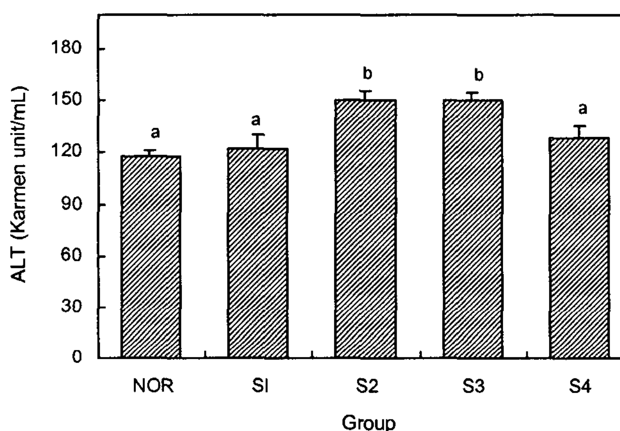

Fig. 4. The activities of ALT in serum of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX. Abbreviations: See Table 1. Values are mean±SE of 10 rats per each group and different superscript lettersindicate significant differences at $p<0.05$.

the administration of AME in this experiment in comparing with the NOR group, it can be concluded that AME can protect liver cells from alcohol-induced damage.

Activities of antioxidant enzymes in liver tissue

Free radicals produced during alcohol metabolism in the liver cause various cellular and molecular injuries which can cause liver damage and diseases, and the mechanisms of cell damage have been already verified in many studies (50,51). To examine whether AME can help damaged liver tissue recover from alcohol injury, alcohol with or without AME was administered for 6 weeks, and then we measured the activities of enzymes related to the free radical scavenging such as SOD, catalase and GSH-Px, and to monitor the activation of the free radical generation, xanthine oxidase (XO) in the liver tissue, as shown in Table 3. The free radical generating enzyme, XO, participates in purine metabolism and produces uric acid from xanthine or hypoxanthine. The massive accumulation of these substances might cause gout (51,52). The S2 group showed a significant increase of XO activity in the liver tissue with 116.39± 9.62 compared with the NOR group (37.33±4.45 NHDPH mol/min/mg protein) ($p<0.05$), which corresponds to the report of Oei et al. (53) that the XO activity was increased in rats where alcohol was chronically administered. The combined administration of AME and alcohol decreased the XO activity in the S3 group (90.09 ±3.28 NHDPH mol/min/mg protein) but not significantly, whereas the reduction was greater and statistically significant in the S4 group (76.55±7.27 NHDPH mol/min/mg protein). These results suggest that AME can inhibit the generation of oxygen free radicals that are increased due to alcohol.

Among free radical scavenging enzymes, catalase is widely distributed in mammalian tissues with especially with especially high concentrations in liver (54). Catalase together with GSH-Px decomposes H_2O_2 that is produced by autooxidation of fatty acids and oxidation of organic substances *in vivo* (55). In this 6-week experiment, the catalase activity of the S2 group (1550.00±19.87 H_2O_2 μmol/min/mg protein) increase greatly compared to the NOR group (1033.00±88.52 H_2O_2 μmol/min/mg protein) ($p<0.05$). However, its activities in the S3 and S4 groups were reduced to close to that of the NOR group ($p<0.05$). These results are consistent with the reports of Antonenkov and Panchenko (56) and Kino (57) that chronic administration of alcohol increases catalase activity. In the present experiment, the increased catalase activity due to alcohol was significantly reduced in the S3 and S4 groups, which suggests that the administration of the AME can inhibit active oxygen like H_2O_2 (58).

SOD is a scavenging enzyme that converts two molecules of oxygen free radicals into oxygen and peroxide (59), and is increased in the presence of free radicals like intracellular superoxide anion (O_2^-) (60). Keen et al. (61) reported that the chronic administration of alcohol increases O_2^- production which, in turn, increases SOD activity. Hilton et al. (62) explained that the increase of SOD activity is a physiological phenomenon to scavenge the alcohol-induced active oxygen. In the current experiment, the SOD activity in S2 group (95.37 ±12.66 mU/g protein) was increased above that of the NOR group (75.75±5.07 mU/g protein). However, its activities in the S3 and S4 groups were significantly reduced compared to the S2 group ($p<0.05$).

GSH-Px, which is typically distributed in the cytoplasm and mitochondria of cells, is an enzyme that detoxifies H_2O_2 by producing H_2O from H_2O_2, with a concomitant oxidation of reduced glutathione (GSH) to form oxidized glutathione (GSSG) with $NADP^+$ as an electron receptor during the reaction. Moreover, GSH-Px tends to be active at low concentration of H_2O_2 in comparison with catalase (63-65). The activity of GSH-Px was increased by alcohol administration to 269.6±34.08 H_2O_2 μmol/min/mg protein, which was significantly el-

Table 3. The activities of catalase, GSH-Px, SOD and XO in liver of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX.

Enzyme	6 weeks				
	NOR[1)]	S1	S2	S3	S4
Catalase[2)]	1033.00±8.85[a6)]	1033.00±6.85[a]	1550.00±19.87[bc]	1550.00±17.31[b]	1375.00±15.35[a]
GSH-Px[3)]	176.80±16.16[a]	172.20±50.30[a]	269.60±34.08[b]	226.00±22.29[b]	212.80±33.06[b]
SOD[4)]	75.75±5.07[a]	76.15±8.87[a]	95.37±12.66[b]	80.75±8.02[a]	77.78±3.50[a]
XO[5)]	37.33±4.45[a]	38.39±1.31[a]	116.39±9.62[c]	90.09±3.28[c]	76.55±7.27[b]

[1)]See the legend of Table 1.
[2)]μmol/min/mg protein.
[3)]Decreased H_2O_2 μmol/min/mg protein.
[4)]mU/g protein.
[5)]Decreased NADPH μmol/min/mg protein.
[6)]Values with different superscripts in the same row are significantly different ($p<0.05$) between groups by Tukey (T) test.

evated above that of the NOR group showing 176.8± 16.16 H_2O_2 μmol/min/mg protein ($p<0.05$). This results was accordant with the report of Speisky et al. (66) explaining that alcohol intake reduces the level of GSH in liver tissue and secondarily promotes lipid peroxidation through free radical production. However, the GSH-Px activity elevated by alcohol was decreased by the combined administration with the AME and alcohol during the experiment period, but not significantly.

Level of TBARS in liver tissue

Fig. 5 shows the changes liver TBARS levels. The level of TBARS was significantly increased in the S2 group (10.44±0.34 MDA nmole/g liver) compared to the NOR group (7.48±0.45 MDA nmole/g liver) ($p<0.05$). However, they were reduced to be close to the level in the NOR group because of the combined administration with the AME ($p<0.05$) and alcohol. In the present experiment, the result of the massive increase in the level of TBARS corresponded to the reports of Yunice et al. (67) and Harata et al. (68) that steady alcohol intake can raise the level of lipid peroxide. Moreover, it is well known that the increase of lipid peroxide affects the deterioration of the cell membrane stability and drug metabolizing enzyme system, and is known to induce hepatocyte damage (69).

Level of GSH in liver tissue

Fig. 6 shows the level of GSH in liver tissue, which was measured to verify the effects of non-enzymatic antioxidants. GSH generally exists in a reduced form *in vivo*, and in involved with the control of the enzyme activity and the prevention of the cell damage caused by active oxygen and free radicals directly or indirectly

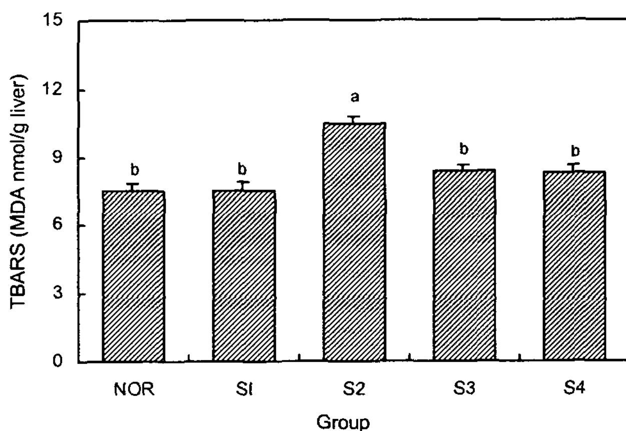

Fig. 5. The contents of TBARS in liver of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX. Abbreviations: See. Table 1. Values are mean±SE of 10 rats per each group and different superscript letters indicate significant differences at $p<0.05$.

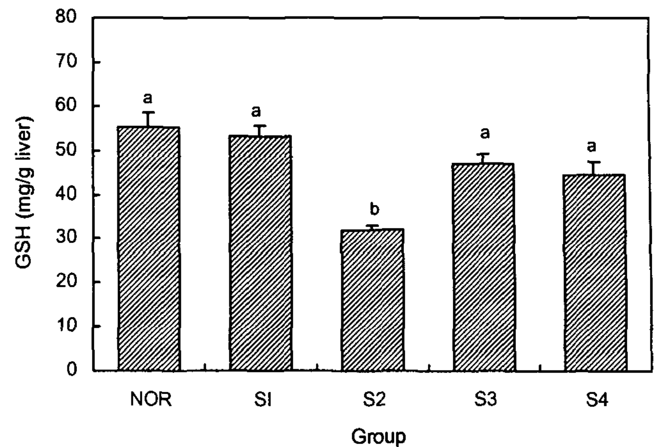

Fig. 6. The contents of glutathione (GSH) in liver of rats treated with alcohol and/or ethanol extract of *Allium monanthum* MAX. Abbreviations: See Table 1. Values are mean±SE of 10 rats per each group and different superscript letters indicate significant differences at $p<0.05$.

(70). In addition, GSH has detoxifying effects and plays important roles in DNA synthesis and other various *in vivo* reactions. Changes in liver GSH concentrations can be helpful in verifying not only the antioxidative status of the liver. But also the effects of oxidative stress (71). The level of GSH in the liver tissue was significantly reduced to 31.84±1.23 mg/g liver by the ethanol administration as compared to the NOR group (55.36± 3.02 mg/g liver), and was consistent with the reports by Ferandez-Richie et al. (72-74) that the chronic administration of ethanol significantly reduced the level of GSH in liver tissue. However, its level was significantly increased in the S3 and S4 groups to be 47.16±2.01 mg/g liver and 44.31±3.21 mg/g liver, respectively.

The mechanism by which GSH levels are decreased by ethanol has not yet been verified. However, there are some reports that alcohol promotes the secretion of hormones such as epinephrine, cortocosteroide and others that increase the release of GSH from the liver, and accordingly, the GSH level is reduced to induce lipid peroxidation by free radicals (75,76). Moreover, there is a study by Videla et al. (77) reporting that the lipid peroxide produced by ethanol is oxidized by reacting with GSH, which reduces the levels of GSH. Accordingly, the decrease in GSH level by the ethanol administration can be linked to the increase of the level of TBARS and lipid peroxide. Meanwhile, the GSH levels in the S2 and S3 groups were significantly increased as compared to the group receiving only ethanol. This result can be probably attributed to the reduction in the TBARS level due to the increased GSH level in the supplemented group.

These results provide compelling evidence that *Allium*

monanthum MAX. extract provides significant protection against liver damage in chronic alcoholic rats.

ACKNOWLEDGEMENT

This paper was supported by NON DIRECTED RESEARCH FUND (2002), Sunchon National University.

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(Received June 17, 2004; Accepted August 23, 2004)