GENETICS AND CELL BIOLOGY

PROTECTIVE EFFECTS OF SERICIN PROTEIN ON ALCOHOL-MEDIATED LIVER DAMAGE IN MICE

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Abstract — Aims: The purpose of this study was to investigate the protective effects of sericin protein (SP) on alcohol-induced hepatic injury in mice and the possible mechanisms. **Methods:** SP (0.375, 0.75 and 1.50 g/kg body weight) was dissolved in distilled water and given to mice by gavage 1 hour before the alcohol (56% wt/vol, 14.2 ml/kg b.w.) treatment for 30 days, then blood, urine and liver were collected, processed and used for alcohol concentration mensuration, various biochemical estimations and histopathological examination. **Results:** The concentration of alcohol evidently decreased in serum and increased in urine in SP treated mice as compared to alcohol-administered animals. Chronic alcohol administration resulted in significantly increase in the levels of transaminase (AST and ALT) and malondialdehyde (MDA) but decrease of glutathione (GSH), glutathione peroxidase (GSH-PX), catalase (CAT) and superoxide dismutase (SOD) in the serum and liver. Hepatic triglyceride (TG) also increased. When mice ingested high doses of SP (0.75 and 1.50 g/kg b.w.) the levels of antioxidant enzymes in the serum were restored to normal. However, hepatic CAT and GSH were still below normal, although a trend of significant increases was observed in comparison with alcohol treatment group. **Conclusions:** The results indicated that SP was able to hasten the alcohol elimination through urine directly and enhance the ethanol oxidation rate in liver. Simultaneously, SP may exert a protective effect against lipid peroxidation by scavenging reactive oxygen species and elevating the activity of antioxidant enzymes, in consequence prevented the peroxidative deterioration of structural lipids in membranous organelles, especially mitochondria and karyon.

INTRODUCTION

Alcoholicliver disease (ALD), the common consequence of prolong and heavy alcohol intake, is one of the most common causes of chronic liver disease in the world (Diehl, 2002). The increased production of reactive oxygen species (ROS) by the heavy intake of ethanol and consequently the enhanced per-oxidation of lipids, protein and DNA have been extensively studied using human and animal cells as well as animal models (Lindros, 1995; Zima *et al.*, 2001). Although the underlying mechanism of ALD still remains unclear, multiple mechanisms are likely to be involved in the pathogenic process including the generation of the toxic substances and the oxidative stress. It is believed that ROS and other free radicals generated from many sources such as the activation of Kupffer cells (Wheeler *et al.*, 2001) and ethanol oxidation by cytochrome P4502E1 (Albano, 2002) are the key factors causing ALD (Lindros, 1995). Since the oxidative stress plays a central role in liver pathologies and progression, antioxidants has been proposed as therapeutic agents and drug coadjuvants to prevent liver damage (Zima *et al.*, 2001).

Sericin is a natural macromolecular protein derived from silkworm *Bombyx mori*. As a water-soluble protein consisting of 18 amino acids, sericin is constructed by strong polar side groups such as hydroxyl, carboxyl and amino groups (Zhang, 2002). When sericin is involved in physical, chemical or enzymological process, it is degraded into a peptide or hydrolyzate with lower molecular weight, <20 kDa, which was designated as sericin protein (SP) (Zhang, 2002). Due to the unique biological functions including antioxidation (Kato *et al.*, 1998), tyrosinase inhibition (Kato *et al.*, 1998), anticoagulation (Tamada, 1997), anticancer activities (Zhaorigetu *et al.*, 2001)

as well as digest promotion (Sasaki *et al.*, 2000), sericin peptide and its hydrolyzate have been used extensively in many commercial products such as food, pharmacological and cosmetic goods. Up to now, many studies have focussed on SP because of its powerful antioxidant potential. It has been reported recently that SP has protective effect against 1,2-dimethylhydrazine and UVB-induced acute damage and tumour promotion in mice by reducing oxidative stress (Zhaorigetu *et al.*, 2001, 2003). It would be very interesting to see whether SP has similar antioxidant and hepatoprotective effects on ethanol-mediated toxicity in ALD. Thus, the purpose of the present study was to investigate the possible protective effect of SP on the ethanol-induced hepatic injury and the potential mechanisms of hepatoprotection involved.

MATERIALS AND METHODS

Materials

Alcohol (56%, wt./vol) was purchased from a commercial brewery in China (Hongxing). All other chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation and analysis of sericin protein powder

The cocoons of silkworm *Bombyx mori* were provided by Sericultural Research Institute, Zhejiang Academy of Agricultural Science. The sericin protein used in the present experiment was prepared by the procedure reported previously (Zhang, 2002). After crude sericin powder was obtained by using a spray dryer, the spray-dried powder was redissolved into distilled water and further purified using HPLC (Waters, USA). Purified solution was concentrated and lyophilized to dryness at low pressure at −50°C. Then a given amount of sericin powder was hydrolyzed in 6.0 mol/l HCl at 110°C for 24 hours. After removing HCl from the solution, the hydrolyzed sericin was used for

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determination of amino acids composition with Sykam433 model autoanalyzer (Sykam, Germany).

Animals and experimental design

Male ICR mice (25 ± 2 g) obtained from Zhejiang Academy of Medical Sciences were housed in plastic cages under controlled conditions of a 12 h light/12 h dark cycle, 60% humidity and 25 ± 1°C. After 1 week of acclimatization, the mice were randomized into five groups. Each group contained ten animals and five animals were kept in one cage. The standard pellet diet and water were provided *ad libitum*. Group 1 was the control, receiving distilled water. ALD was induced in rest of groups (group 2–5) by daily gastric intubations of 14.2 ml/kg body weight of 56% alcohol for 30 days. SP (0.375, 0.75, 1.50 g/kg b.w.) were dissolved in distilled water and given to group 3–5 respectively by gavage 1 hour before the alcohol treatment. Duration of the experiment was 30 days. At the end of the experiment, the mice were fasted overnight, anaesthetized with pentobarbital sodium (40 mg/kg b.w.) and sacrificed by decapitation. Blood and tissue were immediately collected, processed and used for various biochemical estimations. All animals used in the current study were handled and treated in accordance with guidelines of National Institutes of Health for experimental care and use of animals.

Sample preparation

Blood samples were collected in tubes and allowed to clot. Meanwhile, the bladders were rapidly removed, put into tubes and preserved order to collect urine. The serum and urine samples were collected by centrifugation at $3000\times$ g for 10 min at 4°C and were stored at −80°C until further analysis. The livers were also rapidly removed and rinsed in cold 0.9% saline. A portion of the liver was fixed for histopathology, while another portion was homogenized in cold 0.9% saline. Homogenization procedure was performed as quickly as possible under completely standardized conditions. The homogenates were centrifuged at $12,000\times$ g for 5 min at 4°C and kept on ice until assayed.

Determination of the alcohol concentration by gas chromatography (GC)

The alcohol concentration was determined by GC according to the method of Ding *et al.* (2002) with some modifications. A total of 100 μl of concentrated perchloric acid was added to a 0.2 ml serum (or urine) in a 1.5 ml tube, and isopropanol was adjusted to a final concentration of 100 ppm as an internal standard. The mixture was briefly vortexed and then centrifuged at $3000\times$ g for 10 min; the clear supernatant was transferred to a clean tube for GC analyses.

GC analyses were performed on a Model 6890N gas chromatograph coupled to a 5972 series mass-selective detector (Agilent, USA). The capillary column (db-624; 30 m × 0.32 mm) was coated with cross-linked methyl silicone (1.80 μm film thickness). We used an automatic injection system with an injector port temperature of 250°C. The initial oven temperature of the gas chromatograph was 120°C. After maintaining it at that temperature for 8 min, the oven temperature was increased at a rate of 25°C/min to reach a final temperature of 300°C. This temperature was maintained constant for another 30 min in order to clean the column. The carrier gas was helium, constant flow was 2.0 ml/min, and the ionization energy was 70 eV.

Biochemical estimations

Total protein concentration in all samples was determined by the method of Lowry *et al.* (Lowry *et al.*, 1951). Malondialdehyde (MDA) (Ohkawa *et al.*, 1979); aspartate transaminase (AST) and alanine transaminase (ALT) (Reitman and Frankel, 1957); triglyceride (TG) (Butler *et al.*, 1961); reduced glutathione (GSH) (Jollow *et al.*, 1974); superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971); glutathione peroxidase (GSH-PX) (Paglia and Valentine, 1967); and catalase (CAT) (Claiborne, 1985) were estimated in the serum and liver according to the procedures described in the respective references cited.

Histopathological examination

For histopathological study, the tissues of three animals from each group were separated and one part of the tissues was separated and stored in 10% formalin. They were later sectioned using a microtome, dehydrated in graded alcohol, embedded in paraffin section and stained with haematoxylin and eosin, then they were viewed using a Lecia-DM2500 (Germany) microscope. The other parts were fixed in 2.5% glutaraldehyde. They were dehydrated in graded alcohol, embedded in Sturr, coloured with uranyl acetate and reynolds, sectioned using a microtome, and observed using JEOL-1230 (Japan) transmission electron microscopy.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially available statistics software package (SPSS for Windows, V. 12.0, Chicago, USA). Results were presented as mean ± SD P-values <0.05 were regarded as statistically significant.

RESULTS

Amino acid composition

To understand the properties of SP, the amino acid compositions were measured using a Sykam433 model autoanalyzer (Sykam, Germany). Table 1 shows the amino acid composition of the SP – it is constituted by 14 kinds of amino acids (date not shown), the main bulk of which is made of serine and asparagine (about 48%), and the amino acids with antioxidant properties (Ser, Gly, Ala, etc.) occupy approximately 56% of the total amount. These results were in agreement with Kato *et al.* (1998).

Effect on the alcohol relative concentration in serum and urine

The concentration of alcohol in serum decreased evidently in mice with administration of SP at the doses of 0.75 and 1.50 g/kg b.w., while increased in urine in all SP + alcohol administered mice as compared to only alcohol administered animals (Table 2).

Table 1. Amino acid composition of the sericin protein

Amino acid	Mol (%)
Asp	19.18
Thr	7.81
Ser	28.89
Glu	6.98
Gly	10.75
Ala	4.65
Cys	<0.05
Val	3.12
Met	<0.05
Iso	0.83
Leu	1.12
Tyr	4.24
Phe	0.58
Lys	3.51
His	3.95
Arg	4.39
Pro	<0.05

Table 2. Effect on the ethanol relative concentration in serum and urine among different group mice 1h after administering alcohol (56%)

Treatment ($n = 10$)	Ethanol relative concentration	
	Serum	Emiction
Ethanol (E, 14.2 ml/kg)	3.8 ± 0.5	3.7 ± 0.6
SP (0.375 g/kg) + E	3.5 ± 0.7	4.1 ± 0.4
SP (0.75 g/kg) + E	2.4 ± 0.5**	5.5 ± 0.6**
SP (1.5 g/kg) + E	2.1 ± 0.3**	5.4 ± 0.5**

Ethanol relative concentration means the contents of ethanol divided by isopropanol. Values are mean ± SD; $n = 10$.
**Significant difference from ethanol ($P < 0.05$).

Table 3. Effect of SP on the content of MDA and activities of AST, ALT in the serum of control and ethanol-administered mice

Treatment ($n = 10$)	MDA (nmol/ml)	AST (U/l)	ALT (U/l)
Control	12.3 ± 1.5	43.9 ± 5.7	29.7 ± 4.2
Ethanol (E, 14.2 ml/kg b.w.)	35.8 ± 5.6*	70.6 ± 7.0*	62.1 ± 6.1*
SP (0.375 g/kg b.w.) + E	12.9 ± 4.9**	24.1 ± 4.2*,**	29.4 ± 6.5**
SP (0.75 g/kg b.w.) + E	11.3 ± 2.3**	27.3 ± 4.9*,**	21.9 ± 5.5**
SP (1.50 g/kg b.w.) + E	13.7 ± 2.1**	36.2 ± 2.9**	17.7 ± 3.7*,**

Values are mean ± SD; $n = 10$; SP: sericin protein.
*Significant difference from control ($P < 0.05$), **Significant difference from ethanol ($P < 0.05$).

Effect of SP on biochemical parameters

Table 3 showed administration of alcohol produced severe liver damage as indicated by markedly increased AST, ALT activities and MDA in serum, and results shown in Table 4 also indicate that hepatic MDA and TG contents increased significantly by ethanol exposure alone. However, on SP administration, the content of MDA was restored to normal and the AST, ALT activities decreased distinctly in serum. In liver, the levels of MDA decreased evidently and TG restored to normal at the doses of 0.75 and 1.50 g/kg b.w.

In serum determination (Table 5), the activities of antioxidant-related enzymes were significantly deteriorated by ethanol administration. Pretreated with SP, the amount of antioxidants such as GSH, GSH-PX, CAT and SOD increased, in which GSH-PX and SOD restored to normal at the dose of 1.50 g/kg b.w., GSH restored to normal at all three doses, and

Table 4. Effect of SP on the contents of MDA and TG in the liver of control and ethanol-administered mice

Treatment ($n = 10$)	MDA (nmol/mg protein)	TG (mmol/g protein)
Control	2.0 ± 0.2	0.5 ± 0.1
Ethanol (E, 14.2 ml/kg b.w.)	2.5 ± 0.3*	1.2 ± 0.2*
SP (0.375 g/kg b.w.)+E	2.0 ± 0.2**	1.0 ± 0.2*
SP (0.75 g/kg b.w.)+E	1.5 ± 0.3*,**	0.6 ± 0.1**
SP (1.50 g/kg b.w.)+E	1.6 ± 0.1*,**	0.5 ± 0.2**

Values are mean ± SD; $n = 10$; SP, sericin protein.
*Significant difference from control ($P < 0.05$), **Significant difference from ethanol ($P < 0.05$).

the CAT was significantly increased as compared to control at the doses of 0.75 and 1.50 g/kg b.w.

The antioxidant enzymes' activities in the liver of control and experimental animals are given in Table 6. Administration of SP increased the activity of GSH-PX and SOD towards normal after 30 days. The levels of CAT and GSH increased significantly compared to alcohol treatment mice, but still lower evidently than control.

Histopathological studies

Histopathological changes in liver are shown in (Figure 1) (H&E, ×200). The liver samples of alcohol-administered mice showed feathery degeneration, micro- and macro-cellular fatty changes, periportal fibrosis and vascular congestion (Figure 1B). On treatment with SP, the abnormalities were gradually remedied and showed normal histology with mild congestion of central vein at the dose of 1.50 g/kg b.w. Under low magnification through TEM (×4K), histopathological examination of the liver cells of alcohol-treated mice revealed evidently degenerative changes such as mitochondria tumefaction and vacuolization, rarefaction of protoplasm as well as karyon fragment in comparison to control (Figure 2A and B). The abnormalities were also gradually remedied and the cell structure was restored nearly normal when highest dose (1.50 g/kg) was administrated (Figure 2C–E). Since the structural integrity of hepatic mitochondria directly reflects the degree of the damage, hepatic mitochondria were particularly observed through higher magnifying electron micrograph (Figure 3). The structure of hepatic mitochondria from ethanol-administered mouse was deteriorated, in which the mitochondria were tumid, christae mitochondriales disrupted and even disappeared, matrix granules were rarefactive and appeared to be vacuoles (Figures 2, 3B). In SP (1.50 g/kg b.w.) treated mice, the structure of hepatic mitochondria restored to normal (Figure 3E). The results from histopathological studies (Figures 1–3) also provided supportive evidence for these biochemical analyses (Tables 2–6).

DISCUSSION

Ethanol is a fat-soluble non-electrolyte, which is readily absorbed from the gastrointestinal tract, diffuses rapidly into circulation and is distributed uniformly throughout the body (Godde *et al*., 1989). So the absorption rate of alcohol could be reflected by the ethanol concentration in serum, and the ethanol concentration in urine means the quantity of one part of elimination. Our results (Table 2) show that, in the animals pre-treated with SP, the concentration of ethanol in serum

Table 5. Effect of SP on the content of GSH and activities of GSH-PX, CAT and SOD in the serum of control and ethanol-administered mice

Treatment ($n = 10$)	GSH (mmol/l)	GSH-PX (A)	CAT (U/ml)	SOD (U/ml)
Control	0.69 ± 0.08	97.3 ± 14.1	18.3 ± 1.4	146.6 ± 17.7
Ethanol (E, 14.2 ml/kg b.w.)	0.35 ± 0.08*	71.2 ± 6.2*	6.6 ± 2.1*	69.6 ± 18.3*
SP (0.375 g/kg b.w.)+E	0.62 ± 0.06**	68.4 ± 9.0*	18.3 ± 7.3**	91.1 ± 10.2*
SP (0.75 g/kg b.w.)+E	0.84 ± 0.09**	89.2 ± 6.2**	37.9 ± 9.1*,**	78.6 ± 25.2*
SP (1.50 g/kg b.w.)+E	0.82 ± 0.07**	102.8 ± 14.5**	33.6 ± 4.0*,**	131.6 ± 16.6**

Values are mean ± SD; $n = 10$; SP, sericin protein; A, μmol GSH reduced/min/ml.
*Significant difference from control ($P < 0.05$), **Significant difference from ethanol ($P < 0.05$).

Table 6. Effect of SP on the content of GSH and activities of GSH-PX, CAT and SOD in the liver of control and ethanol-administered mice

Treatment ($n = 10$)	GSH (mmol/mg protein)	GSH-PX (a)	CAT (U/mg protein)	SOD (U/mg protein)
Control	98.4 ± 14.1	336.2 ± 42.9	72.6 ± 11.7	100.4 ± 15.3
Ethanol (E, 14.2 ml/kg b.w.)	45.9 ± 7.8*	155.3 ± 29.3*	8.6 ± 5.8*	61.2 ± 8.2*
SP (0.375 g/kg b.w.) + E	79.8 ± 10.1*,**	230.0 ± 14.3*,**	31.1 ± 2.8*,**	82.5 ± 9.3
SP (0.75 g/kg b.w.) + E	78.2 ± 11.4*,**	222.8 ± 11.0*,**	34.9 ± 9.2*,**	76.6 ± 4.9*
SP (1.50 g/kg b.w.) + E	70.4 ± 6.2*,**	307.8 ± 33.0**	28.7 ± 4.7*,**	100.2 ± 16.7**

Values are mean ± SD; $n = 10$; SP, sericin protein; A, μmol GSH reduced/min/mg protein.
*Significant difference from control ($P < 0.05$), **Significant difference from ethanol ($P < 0.05$).
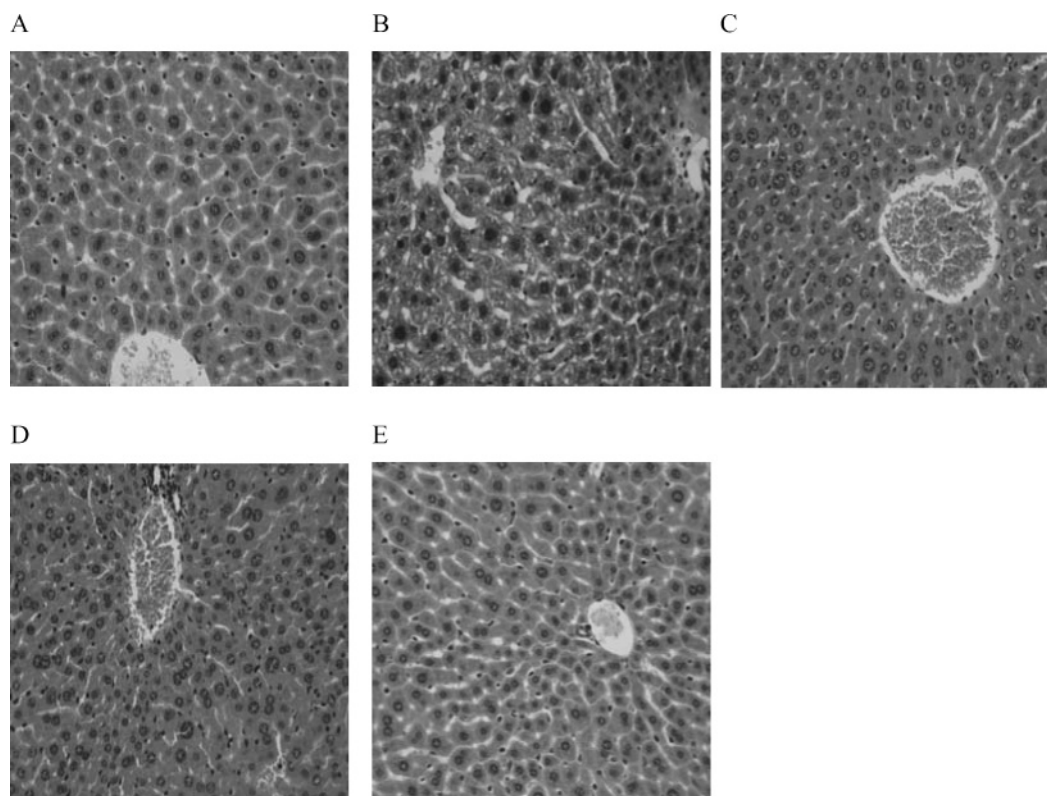

Fig. 1. Effect of SP on alcohol-mediated experimental liver damage, H&E staining, magnification ×200. A, Control; B, Ethanol (56% wt/vol 14.2 ml/ kg b.w.); C, SP (0.375 g/kg b.w.) + Ethanol; D, SP (0.75 g/kg b.w.)+ Ethanol; (E): SP (1.5g/kg b.w.) + Ethanol.

decreased and concomitantly enhanced in urine, which implied that the SP could interfere in alcohol absorption at gastrointestinal tract and decrease the concentration of ethanol in blood by hastening the elimination of ethanol through urine directly, so as to alleviate the ethanol-induced damage to gastrointestinal mucosal.

But the ethanol absorbed by gastrointestinal mucosal has been nearly exclusively metabolized in the liver. Ethanol is cleared from the body primarily by hepatic oxidation catalyzed initially by alcohol dehydrogenase (ADH), the NAD$^+$-dependent cytoplasmic enzyme, to form acetaldehyde (Theorell and Bonnichsen., 1951; Theorell and Chance., 1951). Subsequently, the acetaldehyde is oxidized to acetate by mitochondrial aldehyde dehydrogenase. The resulting acetate is utilized in the mitochondrial Krebs cycle to provide energy. Continued oxidation of ethanol following alcohol intoxication
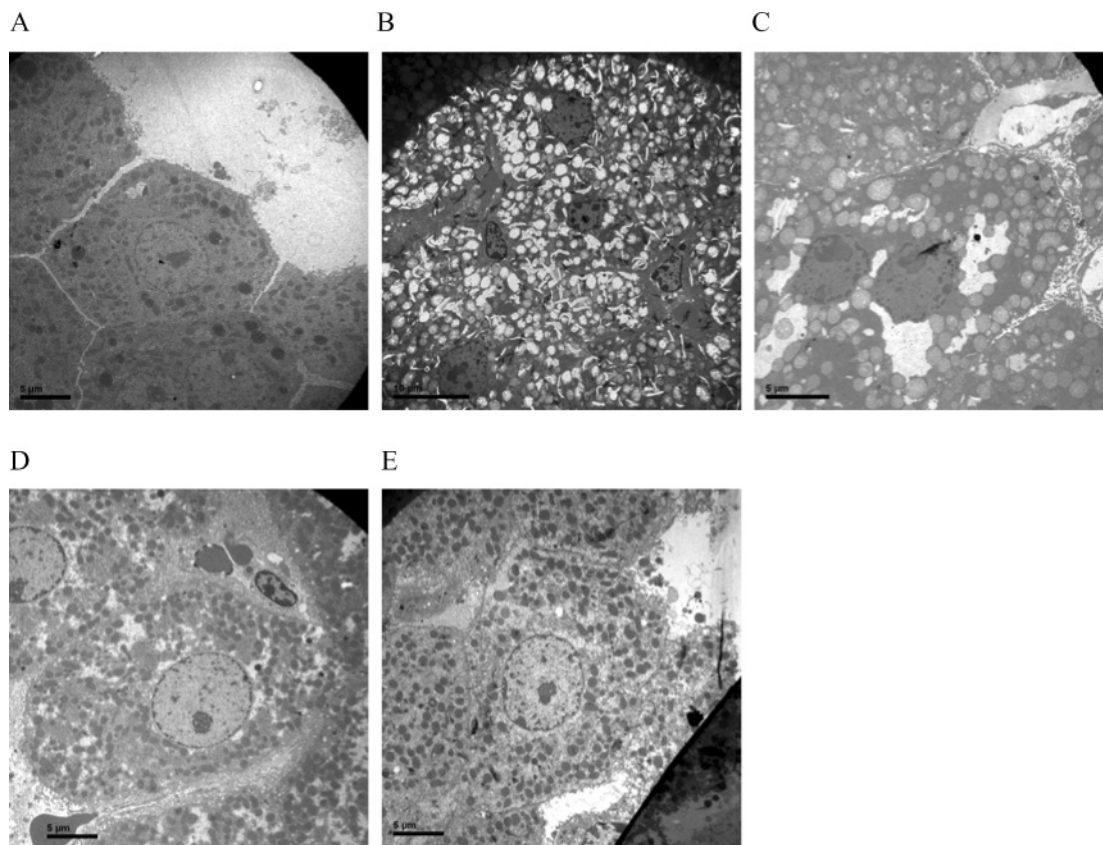
Fig. 2. Micrographs of mice hepatic cell;Magnification, TEM ×4K. A, Control; B, Ethanol (56% wt/vol, 14.2 ml/kg b.w.); C, SP (0.375 g/kg b.w.) + Ethanol; D, SP (0.75 g/kg b.w.) + Ethanol; E, SP (1.50 g/kg b.w.) + Ethanol.

results in shifting of the intracellular NAD^+ to NADH. Under such a condition, due to low concentration of NAD^+ and the high concentration of NADH, the ethanol oxidation rate would be slowed and, also, as the consequence of such redox state changes, fatty acid oxidation would be hindered, thus causing a fatty liver due to excess accumulation of TGs in liver cells (Lieber and Savolainen., 1984). Because the inner membrane of hepatic mitochondria is impermeable to NADH, thus, several hydrogen shuttles, including the malate–aspartate shuttle, have been proposed to be responsible for the transfer of these ethanol-derived reducing equivalents into the mitochondrion (Williamson *et al*., 1971; Cederbaum *et al*., 1977; Sugano *et al*., 1990). Subsequently, Sugano *et al*. (1990) found that both acute and chronic ethanol treatment could increase the activity of the hepatic malate–aspartate shuttle. As the main amino acids of SP were serine and asparagine (Table 1), so upon ingestion of SP, the cytoplasmic asparagine level could be enhanced. Increased asparagine will be catalyzed to oxalacetic acid by glutamic–oxalacetic transaminase (GOT). As oxalacetic acid is an NADH carrier, an increased oxalacetic acid level may accelerate the NADH transfer from cytoplasm into mitochondria. Additionally, a key change in hepatic mitochondria after chronic ethanol exposure is a decrease in the rate of ATP synthesis. So the higher ADP/ATP and NADH/NAD ratios in hepatic mitochondria will increase the oxidation of NADH to NAD^+ via the oxidative phosphorylation system. Thus, it appears that ready availability of NAD^+ increases the turnover rate of ADH and enhances the ethanol oxidation rate. Results (Tables 2 and 4) indicate that not only the serum alcohol concentrations but also the hepatic TG contents increased significantly by ethanol exposure alone but decreased after SP administration for 30 days. The results also agree with the histopathological changes that took place in liver (Figure 1).

Ethanol administration can disturb the delicate balance between the pro- and antioxidant systems of the organism, leading to oxidative stress (Lindros, 1995; Zima *et al*., 2001). Increased generation of ROS/free radicals is able to cause auto-oxidation of the hepatic cells, resulting in marked hepatic lesions (Suzuki *et al*., 1998). The obvious sign of hepatic injury is the leakage of cellular enzymes into plasma (Baldi *et al.,* 1993). In the present study, the increased activities of serum enzymes (AST and ALT) have been detected in alcohol-administered mice (Table 3), implying the increased permeability, damage and necrosis of hepatocytes. These results agree with the results of Goldberg and Watts (1965). Because the enzyme ALT is located in the cytoplasm and the soluble enzyme AST is located mainly in organelles such as mitochondria (Senthil *et al*., 2003), increased levels of AST and ALT suggested damage of both hepatic cellular and mitochondrial membranes in alcohol-administered mice. When SP was administered into the mice, levels of ALT and AST in serum were reverted to the levels similar as the control; these results verified the hypothesis that SP may possess the capability confined to inducing some mechanism which in turn preserves the structural integrity
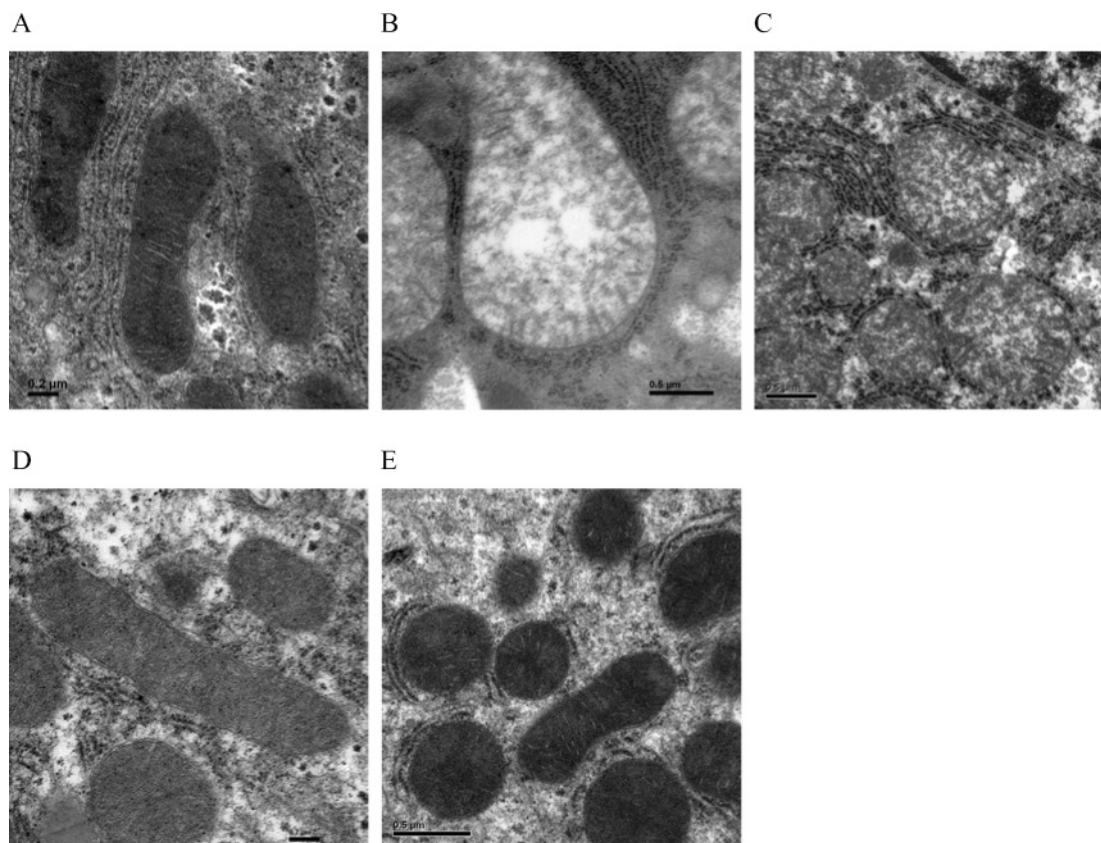
Fig. 3. Micrographs of mouse hepatic mitochondria Magnification, TEM ×60K. A, Control; B, Ethanol (56% wt/vol, 14.2 ml/kg b.w.); C, SP (0.375 g/kg b.w.) + Ethanol; D, SP (0.75 g/kg b.w.)+ Ethanol; (E): SP (1.50 g/kg b.w.) + Ethanol.

of liver cells and hepatic mitochondria from the adverse effects of ethanol.

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity (Thampi, *et al*., 1991). Hepatic lipid peroxidation associated with chronic ethanol administration, as an indicator of oxidative stress, has been often determined in both animal models and human clinical trials. Excess lipid peroxidation as measured by formation of MDA has been found in most studies (Venditti and Di, 1997). In agreement with these findings, ethanol-administered mice showed increased levels of MDA as compared to control. SP intake at the three different doses for 30 days significantly reduced the levels of MDA in comparison to the untreated animals (Tables 3 and 4). This observation demonstrates the antiperoxidative and antioxidant effects of SP.

The liver is the primary organ for the metabolism of ethanol where ROS/free radicals are generated. The potential harmful effects of these species are controlled by the cellular antioxidant defence system (Thampi *et al*., 1991). Glutathione (GSH) is a major non-protein thiol in living organisms, which plays a central role in co-ordinating the antioxidant defence processes in our body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reactions (Gueeri, 1995). Mice on alcohol treatment had reduced levels of GSH in serum and liver as compared to the control. These lowered levels may be due to the increased utilization of GSH by antioxidant enzymes such as GSH-PX, which scavenge H_2O_2 (Anand *et al*., 1996). Activities of SOD and CAT – the two enzymes that help to scavenge superoxide ions and hydroxyl ions, respectively – were also significantly lower in alcohol-administered mice as compared to control. Lowered activities of SOD and CAT result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function (Senthil *et al*., 2003). Because SP is made of 14 kinds of amino acids, among which the main amino acids L-serine, L-glycine and L-alanine have been shown to exert cytoprotection against free radical–induced injury in hepatocytes (Ishizaki-Koizumi *et al*., 2002). L-histidine has long been known as a fairly efficient scavenger of both the hydroxy radical and singlet oxygen ($^1O_2$), the interactions may involve free histidine, small histidine-containing peptides such as carnosine, and histidine residues in proteins (Wade and Tucker, 1998). Moreover, the combination arginine, histidine, taurine, lysine together with ascorbic acid possesses the greatest positive effect on the antioxidant system in rat liver, the activities of the SOD and glutathione system (glutathione reductase/peroxidase, GR/GPO) activities are increased nearly twofold and the GSH level elevated by 70% in the liver homogenates (Petushok and Miskevich, 2003). Current results indicated that SP exerted a protective effect against lipid peroxidation by scavenging reactive oxygen species and elevating

the activity of antioxidant enzymes, the increased trend of the hepatic SOD, CAT and GSH-PX activities on treatment with SP may be due to the direct stimulatory effect of SP on serum SOD, CAT and GSH-PX. Although the SP demonstrated the positive effects, the concentration of ROS in the hepatic cells may exceed the capacity of the antioxidant defence system to eliminate them, which could be one of the explanations that why the content of GSH and the activity of hepatic CAT enzyme were not restored to normal.

Under normal physiological conditions the mitochondrion is the major source of ROS production in the hepatocyte. However, recent findings suggest that chronic ethanol-related disturbances in mitochondrial structure and function might be responsible for an increase in the production of ROS by mitochondria (Bailey *et al.*, 1999). As shown in Figures 1–3, we found that ethanol-administered mice hepatic mitochondria were severely destroyed. When the mice were fed SP at 1.50 g/kg b.w., the persistent damage disappeared and a part of mitochondria restored to normal, while another portion of mitochondria were still little tumid as shown by distorted christae (Figure 2E). Those results indicated the experimental dose of SP needed for further investigation. It has been reported that the key metabolic change in hepatic mitochondria after chronic ethanol exposure was manifested by the decline in the rate of ATP synthesis via the oxidative phosphorylation system. Evidence from previous experiments suggested that chronic ethanol consumption depresses the activities of all the mitochondrial respiratory chain segments, except complex II (Hoek, 1994) by means of lifting the transformation of electrons to molecular oxygen, consequently increased ROS production (Bailey *et al.*, 1999), destroyed the mitochondria structure. Live cell studies revealed that the consequent decrease in NADH oxidizing capacity was associated with altered mitochondrial morphology (Koopman *et al.*, 2005), ATP handling (Visch *et al.*, 2006) and increased rates of ROS production (Iuso *et al.*, 2006). Our present study showed that SP administration demonstrated a clear hepatic mitochondria protective effect by elevating antioxidant potential, as a potential result of increase in the oxidation of NADH to NAD$^+$, thus enhancing the overall rate of alcohol oxidation. Meanwhile, in histopathological examination (Figure 2), we found that the hepatic karyon were also significantly destroyed, the karyotheca were draped and misshapen, and nucleoli were fragmented and even disappeared. However, in SP (0.75 and 1.50 g/kg b.w.) supplemented groups, the karyotheca and nucleoli were normal and the mechanism involving the potential protective effects on the karyotheca and nucleoli is currently being investigated in our lab.

Results from the current experiments demonstrated that SP has positive effects in treatment of alcohol-induced hepatic lesions in mice. One protective effect of SP might be due to interference in alcohol absorption, hastening the ethanol elimination through urine directly and enhancing the ethanol oxidation rate in liver. The other protective mode of SP may be the inhibition of free radical-induced chain reaction by its antioxidant activity, resulted in the prevention of peroxidative deterioration of structural lipids in membranous organelles. Current results will lead to the future utilization of SP as an effective medicine resource for the prevention and treatment of alcohol-induced hepatic lesions.

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