Effect of the Saponin Fraction of Korean Ginseng on the Ethanol Metabolism in the Animal Body

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Abstract : Ethanol exerts different effects on hepatic cellular metabolism, depending mainly on the duration of its intake. In the presence of ethanol following an acute load, a number of hepatic functions are inhibited, including lipid oxidation and microsomal drug metabolism.

In its early stages, chronic ethanol consumption produces adaptive metabolic changes in the endoplasmic reticulum which result in incresed metabolism of ethanol and drugs and accelerated lipoprotein production. Prolongation of ethanol intake may result in injurious hepatic lesions such as alcoholic hepatitis and cirrhosis.

A number of such metabolic effects of ethanol are directly linked to the two major products of its oxidation; hydrogen and acetaldehyde. The excess hydrogen from ethanol unbalances the liver cell's chemistry. In the presence of excess hydrogen ions the process is turned in a different direction

In this study, it was attempted to observe the effect of ginseng saponins on alcohol dehydrogenase(ADH), aldehyde dehydrogenase(ALDH) and microsomal ethanol oxidizing system(MEOS) *in vivo* as well as *in vitro*. Furthermore, the effect of ginseng saponin on the hydrogen balance in the liver and the hepatic cellular distribution of [1-^{14}C] ethanol, its incorporation into acetaldehyde and lipids was also investigated.

It seemed that ginseng saponin stimulated the above enzymes and other related enzymes in ethanol metabolism, resulting in a rapid removal of acetaldehyde and excess hydrogen from the animal body.

Introduction

Ethanol is one of the favorite mood-altering drug and its psychic effects, both pleasant and unpleasant, are well known enough but what is less known is that alcohol is a toxic drug; its overconsumption taxes the body's economy, produced a number of pathological changes particularly in the liver and impairs biological functions.

Unlike carbohydrate and fats, alcohol is essentially foreign to the body and it is known that the body get rid of it by oxidizing alcohol mainly in the liver. Present knowledge on alcohol metabolism showed that over-consumption of alcohol cause cirrhosis and death not only because alcoholism promotes malnutrition but also because alcohol and its metabolic effects are reported directly linked to the first two products of its oxidation, hydrogen and acetaldehyde.

In the factual scene, when ginseng extract was administered to an animal following the medication of ethanol, there had been reports that the consumption rate of ethanol speeds up.

This paper described the effect of saponin fraction of *Panax ginseng* C.A. Meyer on rat hepatic alcohol dehydrogenase, aldehyde dehydrogenase, aldehyde oxidase, xanthine oxidase and microsomal ethanol oxidizing system(MEOS) *in vitro* as well as *in vivo*. Furthermore, the preventive effect of the saponin fraction against ethanol intoxication on rat liver was demonstrated.

Materials and Methods

Ginseng saponin fraction(1.6g) was obtained

fraction(Table 1). It is not clear, however, whether a little activity of the ALO in mitochondrial and microsomal fraction was present *in situ* or contaminated during cell fractionation procedure.

2. Distribution of alcohol and aldehyde in rat hepatic cell organelles following ethanol injection

1.0 m*l* of 10% ethanol containing[1-^{14}C] ethanol(2.5μCi) were injected intraperitoneally to Wistar rats(150-200g, ♂)., 30 min later, the rats were killed and livers were taken. The liver was then homogenized and fractionated as described in the experimental part.

Radioactivity distribution in rat liver showed that about 50% of the recovered radioactivity was found in cytoplasmic fraction and 21% was in mitochondrial fraction and 14% in microsomal fraction as shown in Table 2.

It is not clear from the radioactivity distribution alone whether the radioactivity comes from ethanol and/or aldehyde. Analysis of ethanol and aldehyde in cell organelles showed that(73%) of non-oxidized ethanol remained in cytoplasmic fraction and 16% was found in microsomal fraction but little ethanol was found in mitochondrial fraction. On the other hand, aldehydes were clouded in both mitochondrial and cytoplasmic fraction, but a little in microsomal fraction as shown in Table 3.

This result suggest that ethanol might be oxidized mainly by cytoplasmic ADH and partly by microsomal ethanol oxidizing system(MEOS) and resulted aldehyde may be transported to mitochondria where most of aldehyde is to be oxidized. However, it is not clear whether aldehyde formed by MEOS is oxidized by local microsomal ALDH or by mitochondrial ALDH.

Table 2. *Distribution of radioactivity in subcellular fractions of the liver of rat fed with ethanol. The rats were killed at 30 min. after intraperitoneal injection of 10% ethanol containing 2.5 μCi[1-^{14}C]-ethanol(1 ml).*

Subcellular fraction	Radioactivity (DPM)	Relative Ratio (%)
Crude homogenate	245, 350	100
Mitochondria	52, 175	21
Cytoplasm	119, 460	49
Microsome	34, 022	14

Table 3. *Distribution of ethanol and acetaldehyde in subcellular fractions of the liver of rat fed with ethanol. The rats were killed at 30 min. after intraperitoneal injection of 10% ethanol(1 ml).*

Subcellular fraction	Ethanol (μmole/ whole liver)	Acetaldehyde (μmole/ whole liver)
Crude homogenate	174	5. 22
Mitochondria	2 (1)	2. 70 (51. 7)
Cytoplasm	127 (73)	1. 95 (37. 4)
Microsome	29 (16)	0. 52 (10. 0)

* Figures in brackets are relative percentage distribution.

3. Variation of alcohol dehydrogenase(ADH), aldehyde dehydrogenase(ALDH), aldehyde oxidase(ALO), microsomal ethanol oxidizing system(MEOS) activities and [NAD(P)$^+$]/[NAD(P)H] ratio of prolonged ethanol fed rat liver.

Wister rats(150-200g, ♂), were fed freely with 10% ethanol instead of water for 6 days and the liver was analysed. Liver homogenate was used for ADH, ALDH, ALO and MEOS activities as shown in Table 4.

For the determination of [NAD^+]/[NADH] ratio, the rats were killed by dislocation of the neck. The liver was rapidly removed and pressed between metal clamps previously cooled in liquid N_2. The average time between dislocation of the neck and deep-freezing the tissue was 10 seconds. The frozen liver was pulverized in a mortar to a fine powder, with frequent addition of liquid N_2. The powder was transferred to a weighed plastic centrifuge tube containing 6 m*l* of ice-cold 0.6 N(w/v) $HClO_4$. After a rapid reweighing, the tissue(2g) was mixed with the $HClO_4$ and immediately homogenized in the centrifuge tube. This was continued for about 2 min. Protein was removed by centrifugation in the cold at 30,000×g for min. The supernatant fluid was adjusted to pH 5-6 with 2 N(w/v) KOH and, after standing for

from 100g of Korean white ginseng(Keumsan, 4 years, 50 pieces/300g) according to Kim *et al.* (1983). The chromatogram(solvent; chlorform: methanol: water, 65:40:9, v/v/v) of the above saponin preparation showed that the saponins with Rf values of 0.22 and 0.27 were most abundant, the saponins with Rf values of 1.47, 0.43, 0.41, 0.36, 0.34, 0.20, 0.17 were less abundant and those of Rfs of 0.71, 0.65, 0.59, 0.52 were the least. Purified ginsenoside Rb_1, Rb_2, Rc, Re, Rg_1 were obtained from Korea Ginseng and Tobacco Research Institute.

The liver slices of Wistar rats(200-250g, ♂), were homogenized in 0.25 M sucrose containing 10 mM Tris-HCl buffer(pH 7.4) and made up to 10%(w/v) homogenate(total fraction) and centrifuged at 600×g for 10 min, to remove the nuclei and cell debris. The supernatant was then centrifuged at 9,500×g for 15 min. The precipitates were suspended in a known volume of 0.25 M sucrose containing the 10 mM Tris-HCl buffer(mitochondrial fraction). The supernatant was further centrifuged at 12,000×g for 15 min to remove the precipitates(lysosome fraction). The supernatant was removed(cytoplasmic fraction) and the precipitate was suspended in 0.15 M KCl containing to 10 mM Tris-HCl buffer and again ultracentrifuged at 105,000×g for 30 min. The precipitates were then resuspended in a known volume of 0.15 M KCl containing the 10 mM Tris-HCl buffer(microsomal fraction). The combined supernatant is cytoplasmic fraction. These all procedures were carried out at 4℃, other experimental methods were given in corresponding sections, tables and figures respectively.

Results and Discussion

I. Distribution of aldehyde dehydrogenase, aldehyde oxidase and xanthine oxidase in rat liver cell organelles.

Acetaldehyde is produced during ethanol metabolism and is known to be oxidized mainly by aldehyde dehydrogenase(ALDH) but is also oxidized by aldehyde oxidase(ALO) and xanthine oxidase(XO). There is, however much controversy on the distribution and consequently about the oxidation site of acetaldehyde. Walkenstein and Weinhouse(1953) and Glenn and Venko(1959) reported a mitochondrial ALDH with broad substrate specificity in rat liver. Hedlund and Kiessling(1969) and Hassinen *et al*(1970) found two locations for aldehyde oxidation in rat liver mitochondria, one associated with respiratory chain and one probably associated with the outer membrane. On the other hand, Marjanen(1972) found 80% in the mitochondrial fraction and 20% in the cytosol of the total aldehyde dehydrogenase activity of rat liver homogenate but no activity was found in the microsomal fraction. However Tottmar *et al.*(1973) found that on ALDH, designated ALDH-I, was exclusively in the mitochondria and another ALDH, designated ALDH-II was localized in both mitochondrial and microsomal fraction. Tietz *et al.*(1964) reported an enzyme in the soluble and microsomal fractions of rat liver which could oxidize long-chain aldehyde with either NAD^+ or $NADP^+$ as cofactor.

Present study showed that the ALDH activity was found mainly in the mitochondrial fraction but a significant ALDH activity was also present in microsomal fraction. A small ALDH activity was in the cytosol fraction as shown in Table 1. On the other hand, both ALO and XO were present exclusively in cytosol fraction. A little activity of aldehyde oxidase was detected in mitochondrial and microsomal fraction, but no xanthine oxidase activity was detected either in mitochondrial or in microsomal

Table 1. *Subcelluar distribution(%) of aldehyde dehydrogenase(ALDH), aldehyde oxidase(ALO) and xanthine oxidase(XO) activity in rat liver*

Subcellular fraction	ALDH	ALO	XO
Mitochondria	35	3	ND*
Microsome	37	4	ND*
Cytoplasm	17	93	92

* N.D: not detected

Table 4. *Variation of alcohol dehydrogenase(ADH), aldehyde dehydrogenase(ALDH), microsomal ethanol oxidizing system(MEOS) and aldehyde oxidase(ALO) in prolonged ethanol fed rats in vivo. The rats were fed with 10% ethanol(test) instead of water(control) for 6 days. The values are mean±S.D. of three determinations.*

Group	ADH (units[a]/mg protein)	ALDH (units[a]/mg protein)	MEOS (units[b]/mg protein)	ALO (units[c]/mg protein)
Control	8.743±0.159	3.076±0.600	3.165±0.472	2.765±0.059
	(100)	(100)	(100)	(100)
Test	10.136±0.221	2.303±0.661	4.443±0.681	0.174±0.087
	(116)	(75)	(140)	(6)

a) One unit of enzyme was defined as one n mole of NADH formed per min, b) One unit of enzyme was defined as μ mole of acetaldehyde formed per min, c) One unit of enzyme was defined as 0.01 increment of optical density per min. at 420 nm. Figures in brackets are relative activities assuming that of control being 100.

30 min, in the cold, the precipitate of $KClO_4$ was centrifuged off. The yellow supernatant fluid was used for the analysis, and lactate, pyruvate, α-ketoglutarate, isocitrate, ammonia and glutamate were determined and the [NAD^+]/[NADH] ratio in cytoplasm was calculated using Keq(1.11×10^{-4}) for lactate dehydrogenase reaction(lactate+$NAD^+\rightarrow$ pyruvate+NADH+H^+) and that in mitochondria was calculated using Keq(3.87×10^{-6} M) for glutamate dehydrogenase reaction(glutamate+$NAD^+\rightleftharpoons$ α-ketoglutarate+NH_4^++NADH+H^+) at pH 7.0, 38°C and I 0.25. [$NADP^+$]/[NADPH] ratio in cytoplasm was calculated using Keq(1.17 M) for isocitrate dehydrogenase reaction(isocitrate+$NADP^+\rightleftharpoons$ α-ketoglutarate+CO_2+NADPH+H^+) at pH 7.0, 38℃ and I 0.25. The concentration of CO_2 was taken to be 1.16 mM. As shown in Table 5 [NAD^+]/[NADH] ratio of the ethanol administered rat liver was lowered significantly but [$NADP^+$]/[NADPH] ratio of the test group was not much differ from that of control group.

Table 5. *[NAD^+]/[NADH] ratio and [$NADP^+$]/[NADPH] ratio in the liver of ethanol administered rat. Wistar rats were fed freely with 10% ethanol instead of water for 6 days and the liver was analyzed for lactate, pyruvate, α-ketoglutarate, isocitrate, ammonia and glutamate, and the [NAD^+]/[NADH] ratio and [$NADP^+$]/[NADPH] ratio were celculated as descrided in the text.*

		Control	Test
[NAD^+]/[NADH]	Cytoplasm	866	507
	Mitochondria	7.58	4.68
[$NADP^+$]/[NADPH]	Cytoplasm	2.9×10^{-3}	3.2×10^{-3}

Table 6. *The effect of ginseng saponin on partially purified rat hepatic alcohol dehydrogenase(E.C.1.1.1.1)in vitro. Assay mixture(1 ml) contained(final concentration) 48 mM glycine-NaOH buffer(pH 9.6), 0.8 mM NAD^+, 3 mM ethanol and various concentration of saponin. The values are mean±S.D. of 3 determinations.*

Added saponin concenatration(%)	Enzyme activity*	Relative activity**
0	6.58±0.79	100
10^{-9}	7.05±0.05	107
10^{-8}	6.65±0.05	101
10^{-7}	7.38±0.22	113
10^{-6}	6.80±0.22	103
10^{-5}	6.10±0.37	93
10^{-4}	5.83±0.39	89

* Unit of enzyme activity was defined as 0.01 increment of optical density per min. at 340 nm.
** Relative activity was defined assuming that of control being 100.

Table 7. *The effect of ginseng saponin on rat hepatic mitochondrial and cytoplasmic aldehyde dehydrogenase. Reaction mixture(3 ml) contained(final concentration) 16.7 mM pyrophosphate buffer(pH 9.2), 5 mM acetaldehyde, 0.3 mM NAD+, 20 mM nicotinamide and various concentration of saponin. The values are mean of 3 determinations.*

saponin concentration	total homogenate	mitochondrial ALDH	cytoplasmic ALDH
0	0.051 (100)	0.061 (100)	0.012 (100)
10^{-8}	0.058 (114)	0.066 (108)	0.017 (142)
10^{-7}	0.062 (122)	0.075 (123)	0.020 (166)
10^{-6}	0.058 (114)	0.069 (113)	0.020 (166)

* The enzyme activities were expressed by the increase of optical density at 340 mm during 9 min. incubation at room temperature.
** Figures in brackets were relative activities assiming those of corresponding control being 100.

Table 8. *The effect of ginseng saponin on the partially purified rat hepatic microsomal aldehyde dehydrogenase in vitro. Assay mixture(1 ml) contained(final concentration) 70 mM sodium pyrophosphate buffer(pH 8.0), 1 mM NAD+, 2 mM pyrazole, 1 mM acetaldehyde and various concentrations of saponin.*

Added saponin concentration (%)	Enzyme activity (units*/mg protein)	Relative activity**
0	2.028	100
10^{-11}	2.072	102.2
10^{-10}	2.206	108.8
10^{-9}	2.478	122.2
10^{-8}	2.222	109.6
10^{-7}	2.117	104.4
10^{-6}	2.133	105.2
10^{-5}	2.156	106.3
10^{-4}	1.911	94.2
10^{-3}	1.983	97.8

* One unit was defined as an optical density increment of 1 per min.
** Relative activity was defined assuming that of control being 100.

4. The effect of ginseng saponin in alcohol dehydrogenase(ADH), aldehyde dehydrogenase (ALDH), xanthine oxidase(XO), aldehyde oxidase(ALO) and microsomal ethanol oxidizing system(MEOS) of rat liver *in vitro*.

Enzymes such as ADH, ALDH, XO, ALO which are related to ethanol metabolism were partially purified as described elsewhere [Hyun and Joo (1986) for ALO and XO, Joo *et al.*(1987) for ALDH and Tae and Joo(1984) for MEOS]. As shown in Table 6,7,8,9,10,11, there were optimum concentration of ginseng saponin for the maximum activity of the above enzymes respectively. Maximum activity of rat hepatic ADH was observed at 10^{-7}% of the saponin concentration in the reaction mixture and those of mitochondrial and cytoplasmic ALDH were at 10^{-7}% and that of microsomal ALDH was at

Table 9. *The effect of ginseng saponin on partially purified rat hepatic MEOS in vitro.*

Concentration of ginseng saponin (%)	Acetaldehyde formed (μmole/15min./mg)	Relative activity
0	42.54	100
6.67×10^{-10}	44.40	104
6.67×10^{-9}	48.52	114
6.67×10^{-8}	47.93	113
6.67×10^{-7}	49.28	116
6.67×10^{-6}	59.67	140
6.67×10^{-5}	58.86	138
6.67×10^{-4}	43.28	102
6.67×10^{-3}	47.73	112

Activity of MEOS was determined as follows(Lieber *et al.*, 1978) 0.16 M phosphate buffer(pH 7.4) containing 15 mM semicarbazide-HCl was placed in the center well of warburg's manometric flask and 3 ml of reaction mixture containing 1.0 mM sodium EDTA, 1.0 mM sodium azide, 1.14 M ethanol, ginseng saponin fraction(varius concentration) and MEOS preparation was placed in the main vessel. In the side arm, 70% TCA(0.6 ml) was placed. After 10 mins' preincubation at 37℃, NADPH was added to be 0.4 mM in the reaction mixture and the flask was stoppered. After 15 mins' incubation at 37℃, the reaction was terminated by adding TCA in the side arm and then the mixture was stood for 12 hours. The adsorbed acetaldehyde in semicarbazide in the center well was assayed.
* The relative activities were expressed assuming the activity of control being 100.

Table 10. *The effect of ginseng saponin fraction on aldehyde oxidase.*

Conc. of ginseng saponin (w/v %)	Activity (unit)*	Relative activity**
Control	3.184	100
10^{-8}	3.200	101
10^{-7}	3.604	113
10^{-6}	3.496	107
10^{-5}	3.568	112
10^{-4}	3.420	107
10^{-3}	3.376	106
10^{-2}	2.440	77

The activites of aldehyde oxidase were measured in the reaction mixture which contained 50 mM phosphate buffer(pH 7.8) containing 5×10^{-3}% EDTA, 0.04 mM 2,6-dichlorophenol-indophenol, catalase(200 unit), 0.3 M ethanol, 10μM allopurinol, 20 mM acetaldehyde and enzyme preparation. The values are mean value of three determinations.
* One enzyme unit was difined as an optical density decrement of 0.1 per min. ** The relative activities were expressed assuming the activity of control being 100.

10^{-9}% respectively. The maximum activities of both XO and ALO were seen at 10^{-5}%. MEOS was most active when the concentration of ginseng saponin was about 10^{-5}%.

During the past decade the effect of saponin fraction of *Panax ginseng* C.A. Meyer on various enzymes such as alcohol dehydrogenase, mitochondrial dehydrogenases, lipases and transaminases has been investigated and found that moderate amounts of the saponin fraction stimulated the enzyme catalyzed reactions unexceptionally so far tested in this laboratory as shown in Fig.1 and present data were also the case.

This nonspecific enzyme stimulation effect of the saponin fraction led us to suggest that the surface activity of the saponin might play a significant role in enzyme catalyzed reactions [Joo(1984)].

The Michaelis constants(Km) of various enzymes for their substrates were lowered in the presence of moderate amounts of the saponin fraction [Joo(1984)]. UV difference spectra, electrophoretic mobility, DTNB titration and substrate-enzyme binding data demonstrated that moderate amounts of the saponin might bring about a slight change of the enzyme conformations, which would be in favour of the reactions being accelerated [Joo and Kim(1984), Kang and Joo(1985)].

5. Prevent effect of ginseng saponin against ethanol intoxication of the liver of prolonged ethanol fed rat.

The livers of three groups of Wister rats(150-200g, ♂) were examined biochemically and morphologically. Normal group(6 rats) was fed with ordinary diet. Control group(6 rats) was fed with ordinary diet and 12% ethanol(free access) instead of water for 6 days and test group(6 rats) was fed with ordinary diet and 12% ethanol containing 0.1% ginseng saponin instead of water for same period.

The rats were anethesized using ethyl ether and blood was taken by heart puncture and livers were removed.

For the determination of $[NAD^+]/[NADH]$

Table 11. *The effect of ginseng saponin fraction on xanthine oxidase.*

Conc. of ginseng saponin (w/v %)	Activity (unit)*	Relative activity**
Control	1.520	100
10^{-8}	1.680	111
10^{-7}	1.828	120
10^{-6}	1.600	105
10^{-5}	1.868	123
10^{-4}	1.800	118
10^{-3}	1.800	118
10^{-2}	1.320	87

The activies of aldehyde oxidase were measured in the reaction mixture which contained 50 mM phosphate buffer(pH 7.8) containing 5×10^{-3}% EDTA. 0.04 mM 2, 6-dichlorophenol-indophenol, 200 unit of catalase, 0.3 M ethanol, 6μM menadione, 20 mM acetaldehyde and enzyme preparation. The values are mean value of three determinations.
* One enzyme unit was difined as an optical density decrement of 0.1 per min. ** The relative activities were expressed assuming the activity of control being 100.

	conc. of ginseng saponi:. in assay mixture							
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1(%)
SDH(chicken liver)			↑		↓			
MDH(chicken liver)		↑			↓			
α-KGDH(chicken liver)					↑			
ICDH(NAD^+)(pig heart)						↑	↓	
ICDH($NADP^+$)(pig heart)				↑				
L-GlDH(rat liver)			↑			↓		
TP(rat liver)					↑			
AP(rat liver)				↑				
GOT(human serum)			↑	↑				
GPT(human serum)				↑				
AlDH(rat liver)	↑			↓				
ADH(horse liver)				↑			↓	
ADH(horse liver)					↑	↓		
LPL(rat pancrease)				↑		↓		
G6PDH(human blood cell)			↑	↓				
MOase(rat liver)		↑						
MEOS(rat liver)			↑					
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1(%)

Figure *The effect of ginseng saponin on enzyme catalyzed reactions. The corresponding enzyme reaction rate reached maximum at the concentrations shown by mark (↑) and inhibited when the saponin concentration was over those shown by mark (↓).*

Abbreviation: Succinate dehydrogenase(SDH), malate dehydrogenase(MDH), α-Ketoglutarate dehydrogenase(α-KGDH), Isocitrate dehydrogenase(ICDH), L-Glutamate dehydrogenase(GlDH), Glutamate- oxaloacetate transaminase(GOT), Glutamate-pyruvate dehydrogenase(GPT), Aldehyde dehydrogenase(AlDH), Alcohol dehydrogenase(ADH), Lipoprotein lipase(LPL), Alkaline phosphatase(AP), Tryptophan pyrolase (TP), Glucose 6-phosphate dehydrogenase(G6PDH), Monoamine oxidase(MOase), Microsomal ethanol oxidizing system(MEOS).

Table 12. *[NAD+]/[NADH] ratio and [NADP+]/[NADPH] ratio of the liver of rat fed with ordinary diet and 12% ethanol along with(test) and/or without(control) 0.1% ginseng saponin instead of water(free access) Normal group was fed only ordinary diet and water Calculation of [NAD+]/[NADH] ratio and [NADP+]/[NADPH] ratio was described in the text.*

		Normal	Control	Test
[NAD+]/[NADH]	cytoplasm	866	507	676
	mitochondria	7.58	4.68	6.12
[NADP+]/[NADPH]	cytoplasm	2.9×10^{-3}	3.2×10^{-3}	2.5×10^{-3}

ratio, the liver was rapidly removed and pressed between metal blocks previously cooled in liquid N_2, and analyzed as described in section 3. As shown in Table 12, [NAD+]/[NADH] value of test group was recovered close to normal level.

ADH activity level of both control and test group was higher than that of normal group but that of test group was lower than of control, suggesting that the ginseng saponin may depress the ADH activity increase by ethanol administration.

It is known that ADH activity is usually stimulated by ethanol feeding at initial stage but it became gradually steady after prolonged ethanol feeding. Present experimental results showed that slightly raised ADH activity might come from the increased concentration of cytoplasmic ethanol but the ADH activity migth be inhibited by the saponin absorbed. ADH is slightly inhibited at cytoplasmic saponin concentration which is expected to be 10^{-5}-10^{-4}% after one week feeding of the saponin from our previous data.

On the other hand, ALDH activity of both control and test group was lowered than that of

Table 14. *Acetaldehyde concentration in the liver and serum of the rat fed with 12% ethanol and 0.1% ginseng saponin for 6 days prior to the intraperitoneal injection of 20% ethanol(1 ml). The rats were killed at 30 min. after the ethanol injection.*

Group	Liver (nmole/g liver)	Serum (nmole/ml serum)
Normal	210.669± 98.611 (100)	12.139±3.540 (100)
Ethanol fed	304.703±119.506 (145)	17.594±3.521 (145)
Ethanol and saponin fed)	238.343± 24.540 (113)	13.297±2.512 (110)

* Numbers in bracket are the relative ratios that were expressed assuming that of control being 100.

Table 13. *The effect of ginseng saponin on alcohol dehydrogenase(ADH), aldehyde dehydrogenase(ALDH), microsomal ethanol oxidizing system(MEOS) and aldehyde oxidase(ALO) in prolonged ethanol fed rats in vivo. The rats were fed with 10% ethanol(control) and/or 10% ethanol containing 0.1% ginseng saponin(test) instead of water for 6 days.*

Group	ADH (unit[a]/mg protein)	ALDH (unit[a]/mg protein)	MEOS (unit[b]/mg protein)	ALO (unit[c]/mg protein)
Normal	8.743±0.159 (100)	3.076±0.600 (100)	3.165±0.472 (100)	2.765±0.059 (100)
Control	10.136±0.221 (116)	2.303±0.661 (75)	4.443±0.681 (140)	0.174±0.087 (6)
Test	9.242±0.123 (106)	2.678±0.015 (87)	7.028±0.775 (222)	0.390±0.057 (14)

a) One unit of enzyme was defined as one n mole of NADH formed per min, b) One unit of enzyme was defined as one μ mole of acetaldehyde formed per min, c) One unit of enzyme was defiend as 0.01 increment of optical density per min. at 420 nm, d) Figures in brackets are relative activities assuming that of normal being 100.

Table 15. *Ethanol level in the liver and serum of rat fed with 12% ethanol and 0.1% ginseng saponin for 6 days.*

Group	Liver (μmole/g liver)	Serum (nmole/ml serum)
Control (Ethanol fed)	2.442±0.017 (100)	59.405±12.470 (100)
Test (Ethanol and saponin fed)	2.648±0.032 (108)	256.925±22.448 (432)

* Numbers in brackets are the relative ratios assuming those of corresponding control being 100.

normal group. It is well known that aldehyde inhibites ALDH activity. However, the ALDH activity of test group was less inhibited than that of control group. Aldehyde analysis showed that the levels of both liver and serum of test group were much lower than those of control group as shown in Table 14.

Analysis of alcohol and aldehyde levels of liver and blood serum(Table 15) showed that alcohol level of test group was considerably higher than control. This again suggests that ethanol is less oxidized to acetaldehyde in the

Table 16. *Distribution of radioactivity(DPM) of hepatic lipids of rat which received intraperitoneal injection of 1 ml of 10% ethanol(containing [1-^{14}C] ethanol, 5 μCi). The rats were killed 30 min. later. Rats were fed with 12% ethanol(control) or 12% ethanol containing 0.1% saponin(test) instead of water for 6 days prior to [1-^{14}C] ethanol injection.*

Lipid fraction	Radioactivity (DPM)			relative ratio	
	Normal	Control	Test	C/N	T/N
total lipid	114,089 (100)	176,867 (100)	142,637 (100)	155.0	125.0
phospholipid fraction	49,045 (43.0)	12,407 (7.0)	34,322 (24.1)	25.3	70.0
cholesterol 〃	10,528 (9.2)	10,248 (5.8)	10,267 (7.2)	97 .3	97.5
fatty acid 〃	22,895 (20.1)	49,820 (28.2)	47,119 (33.0)	217.6	205.8
triglyceride 〃	35,817 (31.4)	73,141 (41.4)	59,326 (41.6)	204.2	165.6

Table 17. *Light microscopic observation of the effect of ginseng saponin on hepatocytes of rats dosed with 12% ethanol instead of water for 6 days.*

Group		dilatation of the sinusoids	dilatation of the veins	Fat deposition	vacuolic degeneration	pyknosis	occurence of binuclear cells	irregularity of the nuclear size	appearance of large cells	disappearence of nucleus
Control	1	+	+	++	++	+	++	++	−	++
	2	+	+	+++	++	++	+	++	+	++
	3	−	−	++	+	+	+	++	+	+
	4	+	+	++	+	++	+	+	−	++
	5	+	+	++	+	+	+	++	−	++
Test I	1	−	−	+	−	−	−	−	−	+
	2	+	+	+	−	−	+	−	−	−
	3	−	−	−	−	−	+	−	−	−
	4	−	−	−	−	+	+	+	−	−
	5	−	−	−	−	−	+	−	−	−

Control group was fed with 12% ethanol only instead of water for 6 days. Test group was fed with 0.1% ginseng saponin in 12% ethanol instead of water 6 days.
− : normal + : mild ++ : moderate +++ : severe

livers of ginseng administered rat resulting in less amount of aldehyde fomation, probably because ADH will be less active in ginseng fed group than control. But ALDH activity will be higher in saponin fed group control.

Ethanol containing [1-^{14}C] ethanol (5 μCi)

Table 18. *Electron microscopic observation of the effect of ginseng saponin on hepatocytes of rats dosed with 12% ethanol instead of water for 6 days.*

Group		swelling & disruption of mitochondria	dilatation & vesiculation of RER	proliferation of SER	pyknosis	fat deposition
Control	1	+++	+++	+++	+	++
	2	+++	+++	+++	+	+++
	3	+++	++	++	+	++
	4	++	++	++	+	++
	5	++	+++	++	+	++
Test I	1	−	+	+	−	−
	2	−	−	−	−	+
	3	−	−	−	+	−
	4	+	+	−	−	+
	5	+	−	−	−	−

Control group was fed with 12% ethanol only instead of water for 6 days. Test 1 group was fed with 0.1% ginseng saponin in 12% ethanol instead of water.
− : normal + : mild ++ : moderate +++ : severe

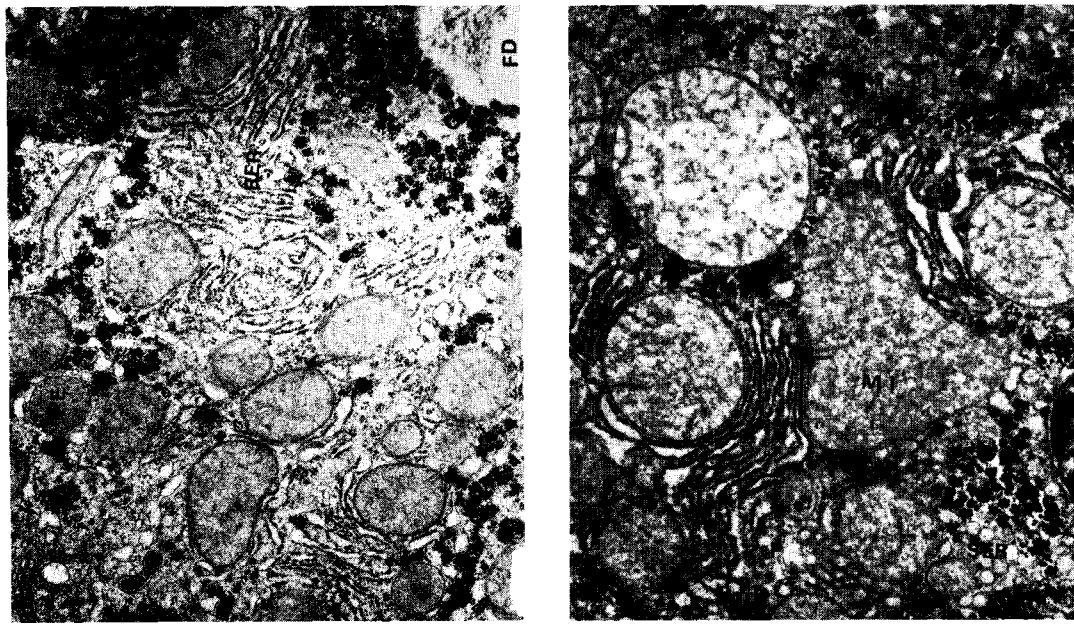

Fig.2. *Electron micrograph of hepatocytes of rats dosed with 12% ethanol instead of water for 6 days(x20,000). Mitochondria are swollen and disrupted severely. The RER are dilated and vesiculated and the SER are proliferated. Peroxisomes are increased in number and prominant golgi apparatus are seen. Pyknosis occur and large fat droplets are seen.*

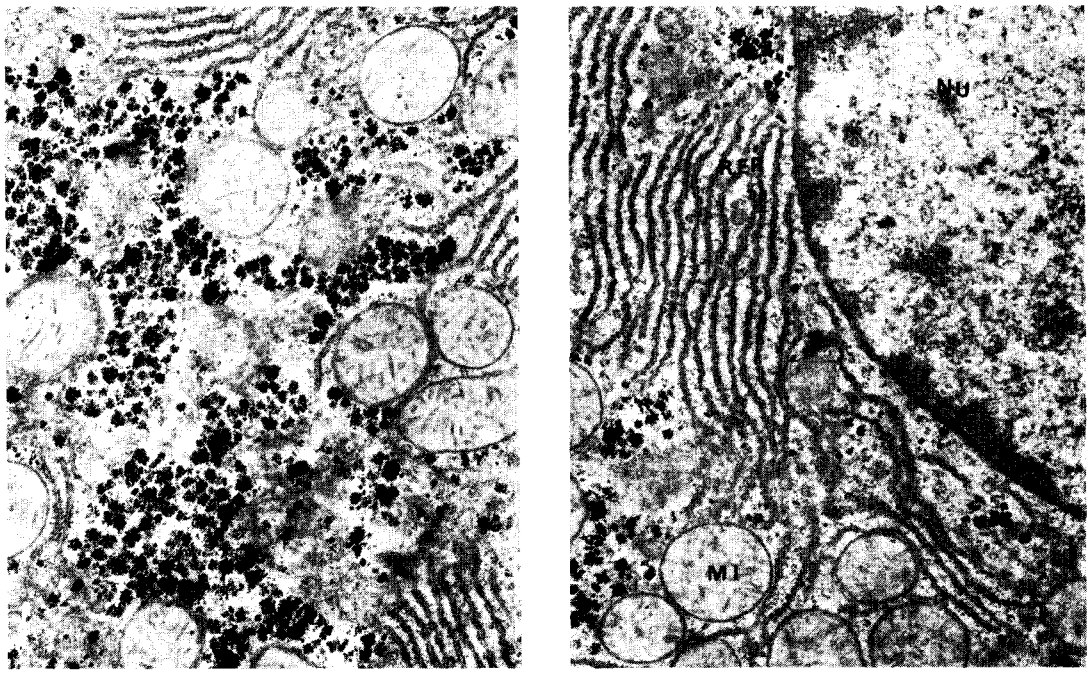

Fig.3. *Electron micrograph of hepatocytes of rats dosed with 0.1% saponin in 12% ethanol instead of water for 6 days. Swollen or disrupted mitochondria can not be seen. Dilated or vesiculated RER are very few(x20,000)*

was injected to the above three groups. 30 min later, the distribution of radioactivity of hepatic lipids was investigated. As shown in Table 17, radioactivity of hepatic lipids of both control and test group was higher than that of normal group, but that of test group was much lower than that of control.

By the analysis of individual lipids such as phospholipids, cholesterol, fatty acid and triglycerides, it was realized that phospholipid biosynthesis is significantly impaired and fatty acid and triglyceride biosynthesis was greatly stimulated. However, the saponin prevented the phospholipid biosynthesis depression and triglyceride biosynthesis stimulation considerably as shown in Table 16.

Electron microscopic observation showed that the hepatic cell of control group was significantly damaged. Mitochondria were swollen and disruptly severely. The rough endoplasmic reticulum(RER) were dilated and vesiculated and smooth endoplasmic(SER) were proliferated. Peroxisomes were increased in number and prominant golgi apparatus were seen, and pyknosis occurred and large fat droplets were seen. However, hepatocytes of test group showed that swollen or disrupted mitochondria were not seen, and dilated or vesiculated RER were very few(Table 17, 18 and Fig.2, 3).

From the above results, it is likely that under the present feeding condition, less amount of acetaldehyde was formed, resulted from the saponin, which inhibits ADH but activates ALDH at their expected concentration in cytoplasm(10^{-5}%) and in mitochondria(10^{-8}%) respectively. Moreover the saponin was shown to raise the lowered the $[NAD^+]/[NADH]$ ratio resulted from ethanol administration.

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