

Effects of Plant Vinegar Extract on the Reduction of Blood Concentration of Alcohol and Acetaldehyde in Alcohol Administrated Rats

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ABSTRACT – Excessive drinking causes ‘alcohol hangover’ within 8-16 hours. The cause of ‘hangover’ has not been elucidated exactly until now, but it is reported that it is caused by the creation of blood ethanol and acetaldehyde as ethanol metabolites. In this study vinegar extract of wood (VE) or OC-1, to which the powder extract of green tea leaves extract is added, was administered to the rats 30 minutes before the oral administration of ethanol (3 g/kg) and the blood ethanol and acetaldehyde concentration was measured in order to evaluate the efficacy of the beverage material for detoxification. As a result, the blood ethanol concentration in the group of the VE-1 (vinegar crude extract) and VE-2 (double diluted solution) is statistically lower ($p < 0.05$) than the exclusive alcohol administered control group. The blood acetaldehyde concentration of all groups of VE and OC-2, which is the double dilution of OC-1, is statistically low after 7 hours following ethanol administration. Especially, the AUC value of OC-2 group is statistically low compared to the control group. Accordingly, it indicates the conclusion that VE and OC-1, reducing the blood ethanol and acetaldehyde concentration which are two leading factors of ‘hangover’ after drinking, are worthwhile to be developed as beverage materials to eliminate ‘hangover’.

Keywords □ Vinegar extract of wood, OC-1, blood ethanol concentration, blood acetaldehyde concentration, hangover

INTRODUCTION

In Korea, the drinking population is comparatively larger than that of developed nations, and thanks to the drinking culture and encouragement of repetitive intake of excessive ethanol (alcohol), many people are exposed to alcohol chronically. In event of chronic intake of alcohol, disorders such as pancreatitis, myocardial infarction, nerve disorders, tuberculosis, and so on appear, and fatal damage to the structure and function of liver (An *et al.*, 1999; Lieber *et al.*, 1985) can be brought about. In addition, the symptoms such as nausea, vomiting, dizziness, thirst, lethargy, headache, and muscular pain can cause social and economic damage due to the deterioration of duty efficiency (Kerb, 1948).

Alcohol exists in the interior tissue of the body, which includes a lot of water with a strong hydrophilic property. Absorbed alcohol is delivered to each body through the blood and the alcohol concentration in the body tissue and the blood

is balanced by being indiscriminately widespread. The central nerve systems is under the severest influence of alcohol. Accordingly, the restraint degree of the central nerve system is directly related to the blood alcohol concentration (Kim, 1999).

Alcohol is absorbed by the simple diffusion method from the gastrointestinal tract to the blood (Kolb *et al.*, 1982). In the case of drinking after fasting, 20-25% is absorbed in the stomach and 70-75% is absorbed in the small intestines. After fasting, the maximized range of alcohol concentration is from 1 hour to 6 hours, according the person (Lieber *et al.*, 1986).

As the blood alcohol concentration increases, the reaction of a person decreases. An alcohol-addicted person is numbed and is not stable either physically and mentally. The American Medical Association defined in 1987 that the blood alcohol concentration of 0.04 g/100 ml or 0.04 g/210 L (respiration rate) induces instability in all kinds of people. According to the classification of the stages of alcohol addiction, 0.01-0.05 g/100 ml blood alcohol concentration is ahead of the clinical stage, 0.03-0.12 g/100 ml is the stage of talking too much and being distracted with an enhanced mental state, 0.09-0.25 g/100 ml is the stage of emotional instability, the degradation of sensual function and eyesight and the imbalance of nerves and

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movement, 0.18-0.30 g/100 ml is the emotional disturbance stage, 0.25-0.30 g/100 ml is the stage of numbness and coma, 0.35-0.50 g/100 ml induces trance, and 0.45 g/100 ml is the stage of death with the apnea (Lieber *et al.*, 1992).

Ethanol, absorbed into the body, is chiefly metabolized in the liver and is oxidized into acetate through acetaldehyde (Wiese *et al.*, 2000; Kai *et al.*, 1977; Winkler *et al.*, 1969; Chance *et al.*, 1974). In the oxidization process, from ethanol to acetaldehyde, alcohol dehydrogenase (ADH) functions, and it is known that acetaldehyde dehydrogenase (ALDH) is working in the process of oxidization from acetaldehyde to acetate (Svanas *et al.*, 1985). In addition, when ethanol is absorbed acutely or in small amount, it is metabolized by ADH or cytochrome P-450 and changed into acetaldehyde. However, when ethanol is absorbed chemically or in large amount, the generation amount of acetaldehyde, due to the microsomal ethanol oxidizing system (MEOS), increases. Acetaldehyde, as a strong poisonous substance, is changed into tetrahydroisoquinone through a condensation reaction with biological amines, which causes chronic addiction of ethanol (Deitrich *et al.*, 1972).

'Hangover' means physical and mental discomfort after drinking, and the degradation of recognition, mobility, and the blood and hormone changes are objective symptoms of it (Wiese *et al.*, 2000).

Even though the cause of 'hangover' has not been clearly established, there are many hypotheses such as dehydration, toxicity of alcohols (ethanol and methanol), alcohol metabolites (acetaldehyde, formaldehyde, acetone, etc), and the deficiency of nutrients (the deficiency of blood sugar, vitamin, and mineral) due to the disorder of absorption. Recently, methanol within alcohol beverage and formaldehyde were pointed out as causes of 'hangover' (Jones *et al.*, 2003., Sainio *et al.*, 1976; Bagchi *et al.*, 1993), and various findings that 'alcohol hangover' is due to acetaldehyde and acetone, have been published (Tomita *et al.*, 1990; Tsukamoto *et al.*, 1998; Tsukamoto *et al.*, 1989; Wall *et al.*, 2000).

With the emergence of 'hangover' causes, various studies on materials for ameliorating 'hangover' have been actively carried out. In Japan, there was a patent, containing the content that fermented products of rice bran greatly contribute to the degradation of acetaldehyde concentration than the blood alcohol concentration and there was a report (Paek, 1990) that the concentration of acetaldehyde is higher for the people who have serious 'hangover' symptoms than the people who have low 'hangover' symptoms, while there is no difference in the blood ethanol concentration. Therefore, the focus is on the the-

ory that the cause of 'hangover' is acetaldehyde. Accordingly, numerous researches have been carried out in order to reduce the concentration of acetaldehyde as well as alcohol.

When a kind of oak is heated in the less airy places like the charcoal pit, carbonization occurs and wood becomes charcoal. In this process, the occurring smoke is naturally cooled and this aqueous solution is called 'vinegar liquid', extracted from charcoal. After passing through a purification process of several stages, safe and edible vinegar extract is obtained. Acetic acid, propionic acid, organic acid, phenolics, and minerals such as calcium, magnesium, and zinc are included in the vinegar extracts (Swift *et al.*, 1998). It is especially known that in case of acetic acid, one of the major ingredients, it makes metabolism in the Krebs cycle, and plays an important role in the disintegration and elimination of body waste materials (Wang, 1988).

In terms of the efficacy of vinegar extract, there are many reports (Tsukamoto *et al.*, 1998) such as the detoxification effect of the oral administration of carbon tetrachloride (CTC) to the rat, the detoxification of alcohol towards the healthy male, and the insulin secretion, the study on immune modulating activity improvement of the oak wood vinegar extract and the preventive effect on cancer, and the blood circulation through the inhibition on platelet aggregation. In addition, it is known that vinegar rapidly eliminates waste materials such as acidic and poisonous substances, generated by alcohol intake, from the body system and thereby, 'hangover' symptoms such as headache, whole body fatigue, tiredness, phsyconia, and vomiting due to drinking are removed (Lee *et al.*, 2000). However, it is difficult to find research on whether the extract of wood vinegar liquid has the substantial alcohol disintegration effect or not, in the case of alcohol drinking.

Accordingly, through investigating the vinegar extract on the alcohol, the active use of vinegar extract for detoxification should be studied. From this point, this study investigates the blood alcohol concentration, and the effect on the change of acetaldehyde concentration, and compares vinegar extract (VE) and OC-1, to which extract powder of green tea leaves (the content over poly-phenol 40%) is added, in order to analyze detoxification efficacies to reduce the blood alcohol and acetaldehyde concentration.

MATERIALS AND METHODS

Preparing of samples

Oak tree extract (*Quercus serrata T*) in this experiment was

supplied by Bio Oaky Co., Ltd and extract powder of green tea leaves and vitamin C were supplied by Samjo Cell Tech Co., Ltd.

Extraction method of VE was as follows : If heated in the air-thin place as in a charcoal kiln, trees like conifer, broad-leaved tree, bamboo cause carbonization, thus becoming charcoal. Crude plant vinegar is the solution obtained by naturally cooling the fume produced in the process. The plant vinegar is produced through the refinement process of the crude plant vinegar.

OC-1 was manufactured by adding 0.05 g vitamin C (0.05%) that was expected to have synergic effect on antioxidant activity with vinegar extract after fully mixing 100 ml oak tree vinegar extract and 0.5 g (0.5%) green tea leaves extract powder (the content 40% poly-phenol) in the temperature of 40.

Experimental animals

As Sprague Dawley Rats, pre-pubertal male rats were purchased from Samtako BIO KOREA Co. Ltd and the rat was used when its weight was 200-250 g after preliminary rearing for two weeks, supplied with free solid feed and water. The breeding temperature was maintained to be 25°C, relative humidity was 50~60% and illumination was regulated every 12 hours by taking turns day and night.

Measurement of blood ethanol concentration and acetaldehyde concentration

Before the test, rats were fasted from food for 18 hours and among VE administration groups, VE, VE-1 (crude extract), VE-2 (double diluted extract), VE-3 (4 times extract) were orally administered, and OC-1 administration groups were orally administered by being divided into OC-1 (crude liquid), and OC-2 (4 times diluted liquid). After 30 minutes of administration, alcohol was orally administered by using an oral feeding tube with 99% alcohol as 3 g/kg. 1 hour later, 3 hours later, and 5 hours later, blood was collected from the orbit respectively and 7 hours later, blood was collected from the heart. Collected blood was centrifuged at 3,000 rpm for 15 minutes and serum was separated. The ethanol measurement kit (Roche, Cat. No. 10176290035) was used for the measurement of blood ethanol concentration, and the acetaldehyde measurement kit (Roche, Cat. No. 066861) was used for the measurement of blood acetaldehyde concentration in the same way.

Analytical method

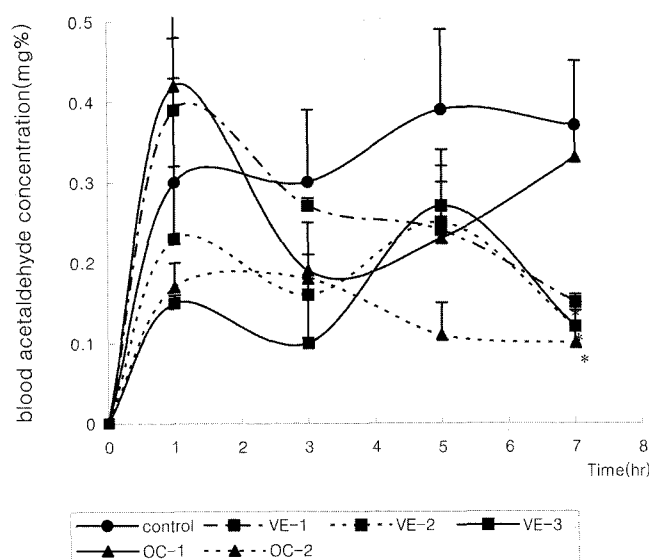


Fig. 1. Effect of plant vinegar extract (VE or OC-1) on blood ethanol concentration after oral administration of VE or OC prior to ethanol (3 g/kg) treatment *in vivo*. VE-1: an undiluted solution of plant vinegar extract (n=5), VE-2: two fold dilution of plant vinegar extract (n=5), VE-3: four fold dilution of plant vinegar extract (n=5), OC-2: a diluted solution of OC-1 (n=5). * $p < 0.05$, ** $p < 0.001$ significantly different from the control by t-test.

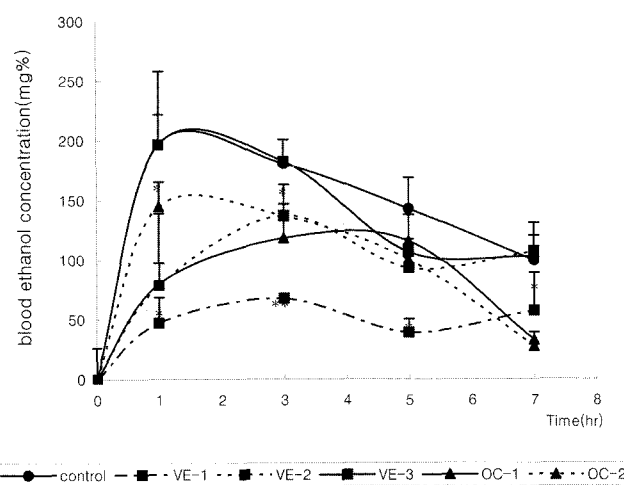


Fig. 2. Effect of plant vinegar extract (VE or OC) on blood acetaldehyde concentration after oral administration of VE or OC prior to ethanol (3 g/kg) treatment *in vivo*. VE-1: an undiluted solution of plant vinegar extract (n=5), VE-2: two fold dilution of plant vinegar extract (n=5), VE-3: four fold dilution of plant vinegar extract (n=5), OC-2: a diluted solution of OC-1 (n=5). * $p < 0.05$ significantly different from the control by t-test.

AUC, T_{max} and C_{max}, which are the pharmacokinetics parameters, are obtained from the concentration-time curve of the blood plasma of each substance. AUC (Shumate *et al.*,

Table I. Cmax, Tmax and AUC of blood ethanol and acetaldehyde concentration after oral administration of VE or OC prior to ethanol (3 g/kg) treatment *in vivo*

	Ethanol			Acetaldehyde		
	Cmax ²⁾ (mg%)	Tmax ³⁾ (hr)	AUC ⁴⁾	Cmax (mg%)	Tmax (hr)	AUC
Control	196.7±25.21	1	949.3±108.7	0.39±0.10	3	2.05±0.58
VE-1	67.4±4.2**	3	398.7±5.0*	0.39±0.09	1	1.57±0.24
VE-2	137.1±9.79*	3	672.8±270.5	0.25±0.05	5	1.11±0.28
VE-3	196.5±61.6	1	759.9±205.9	0.27±0.05	5	1.12±0.34
OC-1	118.3±28.5*	3	584.3±111.4*	0.42±0.29	1	1.60±0.58
OC-2	144.7±26.3*	1	655.0±126.2*	0.18±0.07	3	0.86±0.29*

¹⁾Mean ± SD

²⁾peak concentration

³⁾time of peak concentration

⁴⁾Area under the curve

VE-1: an undiluted solution of plant vinegar extract (n=5), VE-2: two fold dilution of plant vinegar extract (n=5), VE-3: four fold dilution of plant vinegar extract (n=5), OC-2: a diluted solution of OC-1 (n=5)

*p < 0.05, **p < 0.001 significantly different from the control by t-test.

1967) sought for the value until the final blood gathering point by using the calculation formula of the trapezoid area.

Statistical analysis

Every experimental result was marked with mean ± standard deviation and in the statistical significance, by applying the Student's t test, it was judged that statistical significance was found in the case of p* < 0.05, p** < 0.01. When the p value was under 0.05, it was judged to be statistically significant by applying ANOVA'S Duncan.

RESULTS AND DISCUSSION

Change of blood ethanol concentration

Shumate and others reported²⁶⁾ that 60~90% of the alcohol was absorbed within 30 minutes after being orally administered and 100% was absorbed within 90 minutes, so the blood alcohol concentration was the highest within 90 minutes after drinking.

In the control group of this experiment, blood alcohol concentration was recorded to be highest 60 minutes after alcohol administration. In control, in the group of VE-1 (wood vinegar extract), blood alcohol concentration was the highest after 3 hours of alcohol administration and the blood concentration appeared to be low in every measurement compared to the control group.

When comparing the VE-1 group with the control group, the blood alcohol concentration was statistically low at every time after administration of alcohol. It was reduced by about 76% after 1 hour following administration, and 3 hours later, 5 hours

later, and 7 hours later, it was reduced by 66%, 73%, and 43% respectively. Cmax was reduced by about 66% so it showed statistical difference (p < 0.001) and the value of AUC was reduced by about 58%, so it showed statistical difference (p < 0.05). Vinegar extract represented the effect, reducing the increased blood alcohol concentration after the administration of alcohol

After administration of alcohol, the blood alcohol concentration of the VE-2 group was reduced to 60% 1 hour later, and 24% 3 hours later in comparison with the control group, so that it was statistically low (p < 0.05). After 5 hours and 7 hours following administration, it tended to be on the decline but there was no statistical difference. The VE-3 group showed the decline tendency at every time but there was no statistical difference.

The AUC value of the VE-2 group and the VE-3 group appeared to be low, compared to the control group, but there was no statistical difference. Therefore, it is seen that as the concentration of vinegar extract is low, the reduction effect on blood alcohol concentration is low.

Tmax of the OC-1 group is 3 hours after the administration of alcohol, and the blood alcohol concentration after passing 1 hour, 3 hours, 5 hours, and 7 hours, is reduced by about 59%, 35%, 18%, and 66% respectively and it was statistically (p < 0.05) low when compared to control groups, and AUC was also statistically low.

In the case of the OC-2 group, the blood alcohol concentration of each time was reduced by 26%, 24%, 30%, and 73% and it showed the same tendency as OC-1.

Change of blood acetaldehyde concentration

In all the groups of VE-1, VE-2, and VE-3, blood acetaldehyde concentration appeared to be statistically ($p < 0.05$) low 7 hours after alcohol administration. There was no statistical difference of C_{max} between the control group and the vinegar extract administration group, but in case of T_{max} , the control group was 3 hours and in VE-1 it was 1 hour.

The blood acetaldehyde concentration of the OC-1 group was low, compared to the control group, but the C_{max} between the control group and OC-1 group didn't show a statistical difference. In the case of T_{max} , the control group was 3 hours but the OC-1 was 1 hour.

In the OC-2 group, blood acetaldehyde concentration after 7 hours appeared to be statistically low ($p < 0.05$) but AUC also appeared to be low.

Above results mean that, VE-1, VE-2, OC-1 and OC-2 are effective in reducing the increased blood ethanol concentration after the administration of alcohol and in the comparison with the AUC value, the blood ethanol concentration appeared to be statistically ($p < 0.05$) low in VE-1, OC-1 and OC-2.

VE-1, VE-2 and OC-2 are effective in reducing the blood acetaldehyde concentration, and in case of AUC of acetaldehyde, only OC-2 showed a statistical ($p < 0.05$) difference.

Therefore, it is concluded that the addition of green tea extract to the vinegar extract, which holds the efficacy to reduce the blood ethanol concentration, can reduce the blood acetaldehyde concentration more effectively.

Accordingly, VE and OC-1, which can reduce the concentration of ethanol and acetaldehyde, two factors causing 'hang-over', are worthwhile to be developed as materials for detoxification.

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