

# Effects of *Cudrania Tricuspidata* Root Extract (CTE) on Ethanol-Induced Hangover via Modulating Alcohol Metabolizing Enzyme Activities and Blood Gas Levels in Rats

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## 꾸지뽕나무 뿌리 추출물의 알코올 대사 효소 활성 및 혈액 산성화 기전 조절을 통한 숙취해소 효과

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**Abstract** To investigate the anti-hangover effects of *Cudrania tricuspidata* root extract (CTE), the blood alcohol metabolism and blood gas imbalance of CTE in rats treated with 10 ml/kg alcohol were examined. CTE (500 mg/kg and 750 mg/kg) was administrated after 30 minutes of alcohol consumption (10 ml/kg). Blood collection was implemented from the tails of the animals after 1, 3, and 5 hours post alcohol consumption. The Condition drink (a commercial anti-hangover beverage) was used as a positive control. Single administration by the oral route was performed. The consumption of CTE (500 mg/kg and 750 mg/kg) decreased the serum alcohol concentration by increasing and maintaining both the alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) enzyme activity levels in the blood and liver. In addition, CTE led to recovery from the imbalances in the blood gas levels, including carbon dioxide (CO<sub>2</sub>) and changes in pH, bicarbonate (HCO<sub>3</sub><sup>-</sup>) and lactic acid levels due to alcohol ingestion. In conclusion, CTE exerted a more pronounced anti-hangover effect than a commercial anti-hangover drink. Therefore, CTE can be a novel and safe anti-hangover natural product agent for the prevention or treatment of symptoms caused by excessive alcohol consumption.

**요약** 본 연구에서는 알코올을 섭취한 흰쥐에서 알코올 대사 효소 활성과 혈액가스 불균형에 대한 꾸지뽕 나무 뿌리 추출물 (이하 CTE)의 효과를 확인하고자 하였다. 알코올을 10 ml/kg의 농도로 흰쥐에게 경구 투여하였고, 섭취 30분 후 CTE를 500 mg/kg 및 750 mg/kg의 농도로 투여하였다. 혈액은 알코올 섭취 1시간, 3시간, 5시간 후 꼬리 정맥을 통하여 채취하였다. 양성 대조군은 시판되고 있는 숙취해소 음료 (컨디션)를 사용하였다. 약물 처리는 모두 단회 경구 투여로 진행되었다. CTE의 섭취는 흰쥐의 혈액과 간 조직 내 알코올 대사 효소인 알코올 분해효소 (alcohol dehydrogenase)와 아세트알데히드 분해효소 (acetaldehyde dehydrogenase)를 증가 또는 유지시킴으로써 혈중 알코올 농도를 감소시켰다. 또한, CTE는 알코올섭취에 의한 혈액가스 (pH, CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, lactic acid)의 불균형을 회복시켰다. 결론적으로, CTE는 현재 시판되고 있는 숙취해소음료 보다 우수한 숙취 해소 효과를 나타내었다. 따라서 본 연구자는 CTE를 숙취 해소 및 예방을 위한 천연물 유래의 안전하고 새로운 물질로써 제시하는 바이다.

**Keywords** : Acetaldehyde dehydrogenase, Alcohol dehydrogenase, Blood gas, *Cudrania tricuspidata*, Anti-hangover

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## 1. Introduction

*Cudrania tricuspidata* (*C. tricuspidata*), belonging to the Moraceae family, grows in East Asian countries, Korea, China and Japan [1]. Its root and cortex have been used as a medical herb for treatment of cancer in Korea and China [2,3]. Previous studies have shown that *C. tricuspidata* root has an anti-oxidant [4], hepatoprotective [5], blood pressure lowering [6], anti-atherosclerotic, and anti-inflammatory properties [7]. However, there have been no studies for demonstrating anti-hangover effects of *C. tricuspidata* root extract (CTE). Therefore, we investigated the effect of CTE on alcohol metabolism and blood gases that affects hangover symptoms.

The positive functions of proper alcohol consumption are release of stress and relaxation; however, alcohol abuse and chronic consumption can cause adverse health effects such as having poor nutrition, hangover, and alcoholic hepatitis [8]. For hangover, the typical symptoms may include headache, dry mouth, dizziness, and fatigue [9]. The major causes of a hangover are known to acetaldehyde accumulation, changes in the immune system, glucose metabolism, dehydration and metabolic acidosis [8]. Most of the absorbed ethanol is oxidized by alcohol dehydrogenase (ADH) to acetaldehyde, which is oxidized to acetate by acetaldehyde dehydrogenase (ALDH) in the liver [10]. In previous study, Taking alcohol can also lead to blood acidosis, resulting in generation of  $H^+$ . The blood's buffering capacity is dependent on the bicarbonate ( $HCO_3^-$ ) system with  $HCO_3^-$  being naturally produced by the reaction of  $CO_2$  with water ( $H_2O$ ) to produce carbonic acid ( $H_2CO_3$ ), which dissociates to  $HCO_3^-$  and  $H^+$  ( $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ ) [11]. Therefore, taking alcohol can lead to dehydration and cause thirst. Several alterations in the metabolic pathways due to intake of alcohol can result in low blood sugar levels and hypoglycemia. As alcohol metabolism leads to a buildup of an intermediate metabolic product, lactic acid [8,12], regulation of

blood gas including pH and lactic acid metabolism can help prevent hangover symptoms. In the present study, we investigated the effects of CTE on alcohol metabolism and blood gas imbalance after alcohol intake.

## 2. Materials and Methods

### 2.1 Materials

*C. tricuspidata* roots were collected from Yeosu, which is located in Jeollanamdo, South Korea. The Condition beverage was purchased from CJ (CJ CheilJedang Co., Korea). Ethanol assay kit was from Megazyme (Megazyme, Wicklow, Ireland). ADH and ALDH activity assay kits were purchased from Biovision (Biovision, Milpitas, CA, USA).

### 2.2 Methods

#### 2.2.1 Preparations of CTE

The roots were washed in tap water twice and dried at room temperature. They were then heated in boiling water with 70% ethanol for 1 hour and distilled water for 4 hours. The mixture was centrifuged at  $2,000 \times g$  for 10 min and the clear supernatant was collected. Collected supernatant was freeze-dried with a freeze dryer (IS Bio Co., Ltd., South Korea) and re-suspended in mineral water.

#### 2.2.2 Animal treatment and study design

Experiments were carried out on adult Sprague Dawley (SD) male rats weighing (200-250 g). The animals were kept for 1 week to allow acclimatization to the animal facility before starting the experiments. After 1 week, they were then randomly divided into 5 experimental groups ( $n = 6$  each) based on the following dietary regimens: a normal group provided with water (10 ml/kg, NC), an alcohol-fed control group provided with 40% alcohol (10 ml/kg, AC), alcohol + CTE 500 mg/kg group, given 40% alcohol and CTE 500 mg/kg after 30 min of alcohol

administration, alcohol + CTE 750 mg/kg group given 40% alcohol and CTE 750 mg/kg after 30 min of alcohol administration, and alcohol + Condition drink (1.7 ml/kg, CO) group given 40% alcohol and the Condition drink after 30 min of alcohol administration. The blood levels of alcohol, ADH, and ALDH were measured after 1, 3 and 5 h of alcohol administration. Blood gases (CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, pH and lactic acid), liver ADH and ALDH activities were measured after 5 h of alcohol administration (Fig. 1). The animal study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Konyang University.

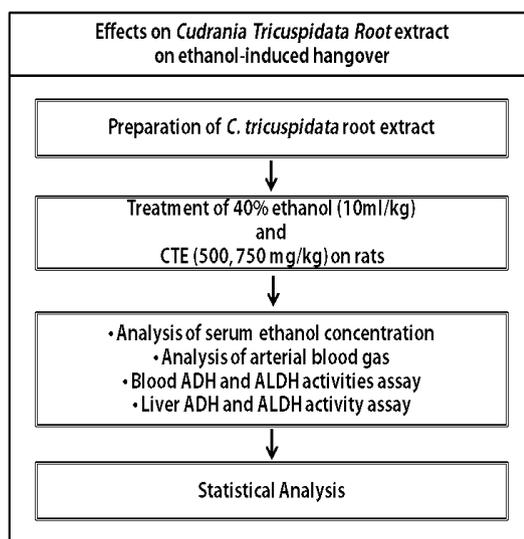


Fig. 1. The strategy of this study

### 2.2.3 Determination of serum ethanol concentration

Blood was collected from tail vein after a period following the alcohol administration and treatments. Serum was obtained by centrifugation at 3,500 rpm for 10 min. The serum ethanol concentration was determined enzymatically along with levels of ADH and ALDH using the respective kits.

### 2.2.4 Analysis of arterial blood gas

After alcohol administration, arterial blood was

drawn anaerobically from the abdominal artery with heparinized syringe under anesthesia. Blood gases were analyzed by pHox Ultra Blood Gas Analyzer (Nova Biomedical, Waltham, MA, USA).

### 2.2.5 Blood ADH and ALDH activities assay

The ADH enzymatic activity was measured with the ADH assay kit using a spectrophotometer set at 450 nm to measure the absorption of isopropanol with the substrate undergoing proportional color change and according to the manufacturer's protocol. For the ALDH enzymatic activity, it was measured with the ALDH assay kit using the 450 nm absorbance measure with the spectrophotometer for the colored product when acetaldehyde was oxidized by ALDH and following the manufacturer's instructions.

### 2.2.6 Liver ADH and ALDH activity assays

The liver tissues were removed as rapidly as possible from the euthanized rats, and homogenized in 3 volumes of iced cold ADH (or ALDH) assay buffer (Biovision). The homogenates were centrifuged at 3,500 rpm for 10 minutes to remove the insoluble material. Supernatant was then centrifuged 15 min at 14,000 rpm, and ADH and ALDH activities of the final supernatant (1 mg of protein) were determined by the methods described above.

### 2.2.7 Statistical analysis

The results were expressed as mean ± S. E. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Duncan's method for post hoc tests. Statistical significance was considered at  $p < 0.05$ .

## 3. Results

### 3.1 Effect of CTE on serum ethanol concentrations

In order to determine the effects of CTE on

relieving hangovers, blood alcohol concentration was investigated. In Table 1, blood drawn at 1 h had the highest alcohol concentration. However, blood alcohol concentration was significantly decreased by CTE (500 and 750 mg/kg) and more effectively than the Condition drink that served as a positive control. Especially for 750 mg/kg of CTE at 1 h after alcohol consumption, there was approximately 19.6% greater alcohol degradation over the CO group. These results indicate that CTE is more rapidly active in reducing blood alcohol levels than the Condition drink.

**Table 1.** Effect of CTE on serum ethanol concentrations (Unit: g/l).

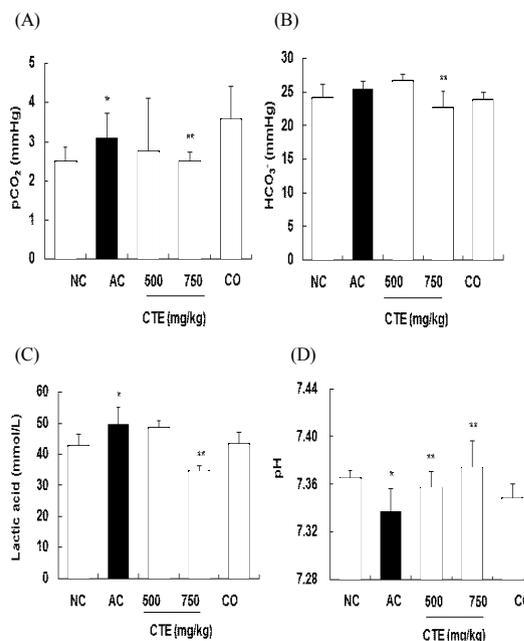
Group	1 hr	3 hr	5 hr
NC	0.000 ± 0.000	0.000 ± 0.001	0.000 ± 0.001
AC	0.223 ± 0.006	0.197 ± 0.006	0.188 ± 0.008
CTE 500 mg/kg	0.193 ± 0.001*	0.187 ± 0.004*	0.183 ± 0.009*
CTE 750 mg/kg	0.154 ± 0.018*	0.165 ± 0.024*	0.158 ± 0.033*
CO	0.198 ± 0.006*	0.175 ± 0.028*	0.188 ± 0.019*

NC: normal control, AC: alcohol control, CO: the Condition drink. Data are expressed as mean ± SD (n = 6). \* $p < 0.05$  was for comparison to AC.

### 3.2 Effect of CTE on arterial blood gas levels

We next investigated the effects of CTE on blood acid/base metabolism after alcohol consumption. In Fig. 2A, the concentration of  $p\text{CO}_2$  increased from  $42.7 \pm 3.8$  mmHg (NC) to  $49.7 \pm 5.3$  mmHg (AC). However, CTE recovered  $p\text{CO}_2$  concentration was increased by alcohol consumption (500 mg/kg of CTE:  $48.6 \pm 2.1$  mmHg, 750 mg/kg of CTE:  $34.5 \pm 1.7$  mmHg). The inhibitory effect of CTE on  $p\text{CO}_2$  was due to lowering of the  $\text{HCO}_3^-$  concentration (Fig. 2B). In addition, CTE recovered the pH levels that were increased by alcohol consumption to normal range (500 mg/kg of CTE:  $7.357 \pm 0.013$  and 750 mg/kg of CTE:  $7.386 \pm 0.022$ , Fig. 2C). Alcohol consumption can lead to hypoglycemia and a buildup of lactic acid. Therefore, we next investigated the effect of CTE on alcohol induced-alterations in blood glucose levels. In Fig. 2D, alcohol consumption increased blood lactic

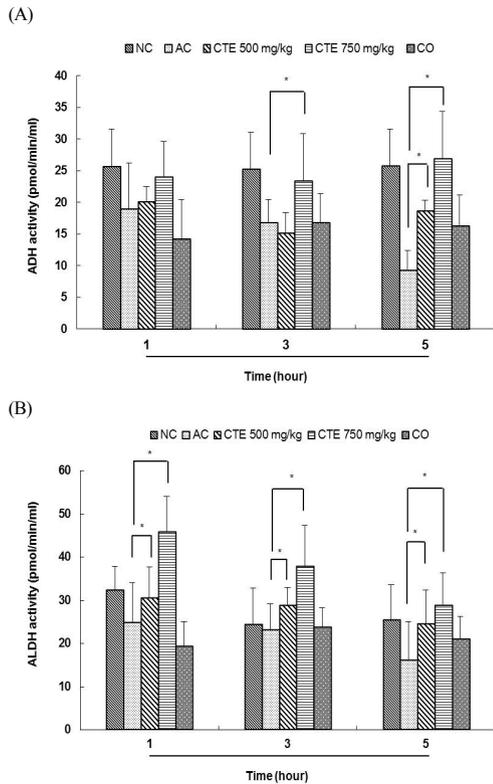
acid levels from  $2.5 \pm 0.4$  to  $3.1 \pm 0.6$  mmol/L. The levels of lactic acid after taking CTE were restored to normal levels (CTE 500 mg/kg:  $2.8 \pm 1.3$  mmol/L, CTE 750 mg/kg:  $2.5 \pm 0.2$  mmol/L). These results show that CTE may prevent hangover symptoms through regulation of blood acid metabolism and hypoglycemia.



**Fig. 2.** Effect of CTE on arterial blood  $p\text{CO}_2$  (A),  $\text{HCO}_3^-$  (B), lactic acid (C), pH (D). NC: normal control, AC: alcohol control, CO: the Condition drink. Data are expressed by mean ± SD (n = 6). \* $p < 0.05$  was compared to NC. \*\* $p < 0.05$  was compared to AC

### 3.3 Effect of the CTE on blood ADH and ALDH enzyme activity

In Table 1, CTE decreased blood alcohol concentrations in a dose dependent manner. This lowering of blood alcohol levels might be due to modulation of alcohol-metabolizing enzymes. Thus, we next investigated the effects of CTE on blood ADH and ALDH activity. In Fig. 3, the lowering of blood ADH and ALDH activities was detected in AC compared with NC after 1, 3, 5 h alcohol administration

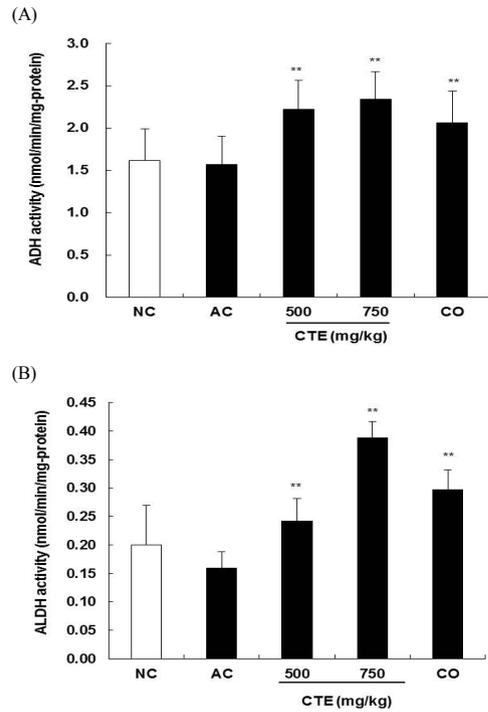


**Fig. 3.** Effect of CTE on blood ADH (A) and ALDH (B) activities. NC: normal control, AC: alcohol control, CO: the Condition drink. Data are expressed as mean  $\pm$  SD (n = 6). \* $p$ <0.05 was compared to AC.

(ADH:  $19.0 \pm 7.2$ ,  $16.8 \pm 3.6$ ,  $9.3 \pm 3.1$  pmol/min/ml, respectively, ALDH:  $24.9 \pm 9.2$ ,  $23.3 \pm 6.0$ ,  $16.2 \pm 8.8$  pmol/min/ml). However, CTE-500 mg/kg and 750 mg/kg markedly increased the blood ADH and ALDH activities compared with AC in a dose-dependent manner. In addition, at the 5 h point, the 750 mg/kg-CTE group had approximately 22.5% and 21.1% greater ADH and ALDH activities compared to the CO group. These results demonstrate that CTE decreased blood alcohol by modulating the activity of alcohol metabolizing enzymes.

### 3.4 Effects of the CTE on hepatic ADH and ALDH enzyme activity

In Fig. 3, CTE recovered the activity of ADH and



**Fig. 4.** Effects of CTE on liver ADH (a) and ALDH (b) activities. NC: normal control, AC: alcohol control, CO: the Condition drink. Data are expressed as mean  $\pm$  SD (n = 6). \*\* $p$ <0.05 indicated comparison to AC.

ALDH enzymes reduced by alcohol consumption. Since ADH and ALDH are most abundant in the liver, we next investigated the effects of CTE on hepatic alcohol metabolizing enzyme activity. In Fig. 4A, NC and AC showed similar ADH enzyme activity after the 5 h alcohol treatment ( $1.62 \pm 0.37$ ,  $1.57 \pm 0.33$  nmol/min/mg-protein, respectively). However, CTE increased liver ADH enzyme activity with  $2.23 \pm 0.34$  and  $2.34 \pm 0.33$  nmol/min/mg-protein at 500 and 750 mg/kg, respectively. This result means that CTE decreased blood alcohol concentration by enhancing hepatic ADH enzyme activity. In Fig. 4B, ALDH enzyme activity was  $0.24 \pm 0.04$ ,  $0.39 \pm 0.03$  and  $0.30 \pm 0.03$  nmol/min/mg-protein for 500, 750 mg/kg-CTE and CO, respectively. Especially for the 750 mg/kg-CTE group, there was a 12.5% greater enzyme activity compared to the CO group. Therefore, these

results indicate that CTE increased hepatic ALDH enzyme activity, reducing the systemic acetaldehyde levels and showing a greater potential as an anti-hangover beverage in reducing the metabolic effects of alcohol intake.

#### 4. Discussion

Metabolism of ethanol with ADH produces acetaldehyde, and at higher concentrations causes toxic effects, such as tissue damage, sweating, skin flushing, nausea, and vomiting [13], and then ALDH rapidly metabolizes acetaldehyde to acetate [14] to minimize the damaging effects of acetaldehyde. To prevent hangover, treatments that shorten the duration of acetaldehyde in the blood stream or combat the severity of its symptoms are required [15]. Candidates for an anti-hangover agent from natural plant products require either positive effects on alcohol metabolism, an increase in levels of hepatic ADH and ALDH or an overall decrease in the concentration of consumed alcohol absorbed [16]. In Fig. 2 and 3, CTE increased and maintained the alcohol metabolism enzymes in high concentration and blood ethanol levels, showing potency as an active agent in the properties outlined above.

*C. tricuspidata* is a traditional Chinese folk medicine, and is a rich source of bioactive flavonoids [17]. Cudraticusxanthone A from its roots has anti-inflammatory effects by regulating expression of cyclooxygenase-2 and inducible nitric oxide (NO) synthase (iNOS) in lipopolysaccharide (LPS)-stimulated macrophages [18]. In some cases, certain medications such as aspirin for reducing headache and muscle aches associated with a hangover should be used cautiously since alcohol metabolism enhances the medicine's toxicity to the liver [8]. A previous study suggested that methanol extract fractions such as cudraflavone B, gericudranin E from *C. tricuspidata* root barks have a hepatoprotective effect on human liver cells challenged

with tacrine-induced cytotoxicity [19]. Therefore, *C. tricuspidata* derived-natural agent may work via multiple mechanisms for improving symptoms, with efficacy not just in remediating hangovers, but also in other areas including reducing inflammation. Chronic ethanol consumption may influence metabolic disorders, such as ketoacidosis and lactic acidosis [20]. It may lead blood acidosis causes headache and nausea by increasing blood CO<sub>2</sub>. Most studies for relieving hangovers are related to regulate high concentration of acetaldehyde. However, toxic effects induced by blood gas imbalance have not been clarified until now. Therefore, regulation of blood gas metabolism could be a new strategy in reducing hangover symptoms.

#### 5. Conclusion

In conclusion, CTE was shown to reduce blood alcohol levels by increasing the enzymatic activity of ADH and ALDH in a rat model, and also allowing recovery from a blood acid/base imbalance by regulating pH, CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and lactic acid levels after alcohol consumption. Therefore, CTE may have a potential anti-hangover effect for prevention or treatment of symptoms caused by excessive alcohol consumption.

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<Research Interests>

Hematology, Neuroscience