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Hypoglycemic Effect and Hepatic Detoxification Activity of Extracts from *Crataegus fructus* and *Morus alba L.* in Alcohol-treated Rats

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To consider potentially new sources which have hypoglycemic effect and accelerating alcohol detoxification, this study was designed to investigate the effect of *Crataegus fructus* and *Morus alba L.* in alcohol-treated rats. I compared the body weight, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH) of rats administered both alcohol and extract of experimental plants to rats treated with alcohol alone. Administration of extracts of *C. fructus* and *M. alba*, respectively, resulted in a significant reduction in the blood glucose level and the activities of ADH of liver compared to the control rats, and administration of extract of *M. alba* showed significantly lower on bodyweight gain in the rats than in other treated rats. In contrast, the activities of ALDH of liver were increased. The activities of AST and ALT between the only alcohol-treated rats and the alcohol and experimental plants-treated rats were no significant difference. The results suggest that *C. fructus* and *M. alba* have a hypoglycemic effect, and reduce liver damage by accelerating acetaldehyde metabolism in alcoholic rats, so the combined effect of *C. fructus* and *M. alba* may be considered as an alternative remedy for hangovers, alcohol-induced overweight and alcohol-induced diabetes.

Key Words: *Crataegus fructus*, *Morus alba*, Alcohol metabolism, Hyperglycemia

INTRODUCTION

Alcohol ingestion is known to produce a variety of metabolic and pathological alterations in the liver (Lieber, 2003). Some 80% to 90% of ingested alcohol is oxidized in the liver, where alcohol is oxidized to acetaldehyde (Vidal et al., 1990; Lieber, 2004). Acetaldehyde is more toxic than ethanol and is responsible for many hangover symptoms. Some of these alterations indeed depend on changes in the redox state due to NADH generated via the liver's alcohol dehydrogenase (ADH) pathway, which are especially due to cell metabolic disturbances associated with ethanol oxidation and oxidative stress, which in turn affects the metabolism of lipids, carbohydrates, proteins and purines (Lieber, 2005). Furthermore, induction of the microsomal pathway contributes to increasing acetaldehyde generation; with protein adduct formation, enzyme inactivation, decreased DNA repair, reduced liver glutathione (GSH) depletion, free radical mediated toxicity and lipid peroxidation.

The liver is busy processing alcohol, it stops releasing glucose. This glucose-lowering effect can last for as long as 8 to 12 hours after drinking says the American Diabetes Association. Sometimes alcohol can cause blood sugar levels to go up instead of down, and this is a particular concern for people with diabetes. Alcohol can alter blood sugar levels and exacerbate or cause diabetes (Crane and Sereny, 1988; Sneyd, 1989; Gordon and Lieber, 1992; Avogaro et al., 1993). Many of alcohol's toxic effects in the liver have been ascribed to oxidative stress caused by ethanol metabolism, which induces marked hepatotoxicity by acting as a pro-oxidative agent or by reducing antioxidant levels, which also associated with hyperglycemia.

Recently, some medicinal plants have been reported to be useful in diabetes, or elimination of alcohol-induced hangover, or alcohol-induced liver disease. There have been numerous attempts to develop clinically useful plants to ameliorate or cure alcohol-related disorders (Overstreet et al., 1996a; Overstreet et al., 2003b; Park et al., 2002; Han

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et al., 2003; Choi et al., 2006). *Crataegus fructus*, the fruit of the species *Crataegus pinnatifida*, which are used to make many kinds of Chinese snacks. A number of studies have shown that extracts of the leaves, berries and flowers of *C. pinnatifida* can be anticholesterolemic (Chen et al., 1995), cardiotonic (Di et al., 1969; Holubarsch et al., 2000; Degenring et al., 2003; Pittler et al., 2003), hypotensive (Belz et al., 2002; Walker et al., 2002), stomachic (Min et al., 1999). *C. fructus* is helpful in treating mild-to-moderate congestive heart failure, improving symptoms, decrease blood pressure, help to reduce anxiety and anxious mood. *Morus alba* has been applied in the clinical treatment of various diseases in Oriental medicine. Recent evidence shows that the leaves and shoots from the mulberry tree possess several medicinal properties, including hypoglycemic, hypotensive, and diuretic effects. In our previous study, we have demonstrated the antidiabetic effect of *M. alba* in alloxan diabetic rats (Kim et al., 2006). The present investigation was undertaken to study the effect of *C. fructus* and *M. alba* on accelerating alcohol detoxification and preventing hyperglycemia in alcohol-treated rats. To avoid the risk of serious complications from alcohol's toxic effect, controlling weight and organ weights are necessary. I therefore determined the effects of the extracts of *C. fructus* and *M.alba* on body weight, organ weights, glucose, AST, ALT and alcohol metabolizing enzyme activities in this study.

MATERIALS AND METHODS

1. Experimental plants and chemicals

The *C. fructus* and *M. alba* were purchased from Kyungdong herbmarket, Korea. Two hundred grams of each plant was extracted individually, and soaked overnight in 200 ml of 95% ethanol. The ethanolic extracts were filtered using Whatman filter paper No. 40 to remove particulate matter, evaporated in a rotary evaporator at 40~50℃ under reduced pressure and freeze-dried as a powder. NAD, semicarbazide, propionaldehyde, pyrazole, 2-mer-captoethanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Unless specified otherwise, additional reagents used were of analytical grade.

2. Experimental animals

Male SD rats of body weight 280~320 g were obtained from the Korea Research Institute of Chemical Technology. The animals were housed in polycarbonated cages at a temperature regulated (22℃) and humidity (55%) controlled room with a 12-h light/12-h dark cycle.

3. Experimental design

In the experiment, a total of 32 rats were used. The rats were divided into 4 groups of 8 rats each. Group 1: normal rats. Group 2: alcohol-treated rats. Group 3: alcohol-treated rats given extract of *C. fructus*. Group 4: alcohol-treated rats given extract of *M. alba*. All rats received a normal diet of standard pellets and water throughout the experimental period. We checked body weight, food intake and water intake every other day. Alcohol-treated rats received an alcohol dose of 4 g/kg body weight diluted in water (50%, v/v). Alcohol was administered once a day orally for 6 weeks, and administered each extract in 1 h (100 mg/kg). Each extract was suspended in distilled water and administered orally through an intragastric tube at the dose of 100 mg/kg body weight for 6 weeks. At the end of the experimental period, after overnight fasting, all the rats were anaesthetized by pentobarbitone sodium (60 mg/kg), and opened at the abdomen. Blood was withdrawn from the abdominal aorta and centrifuged at 3,000 rpm for 10 min to obtain the serum and stored at -20℃. After the rats were euthanized, livers, brains, kidneys, spleens and hearts were weighed and frozen in liquid nitrogen and stored at -70℃.

4. Preparation of subcellular fractions

Fresh livers were washed with 0.9% NaCl and homogenized with 9 volumes of 25 mM Tris-HCl (pH 7.5) containing 0.25 M sucrose and 1 mM EDTA at 4℃. After the crude homogenate was centrifuged at 4℃ for 10 min at 600 x g to remove the nuclear fraction and cell debris, the supernatant was transferred to another tube, and centrifuged at 4℃ for 20 min at 10,000 x g. The mitochondrial fraction was contained in the precipitate. To isolate the cytosolic and microsomal fractions, the supernatant was further ultracentrifuged at 4℃ for 60 min at 105,000 x g. The precipitate (microsomal fraction) and supernatant (cytosolic fraction) were separated and stored at -70℃ until the enzyme assay was conducted. These fractions were used assays for alcohol-metabolizing enzymatic activities.

5. Biochemical analysis

Blood glucose was determined by the O-toluidine method.

Table 1. Effect of extracts from *C. fructus* and *M. alba* on body weight in alcohol-treated rats (g)

Experimental group	0 w	1 w	2 w	3 w	4 w	5 w	6 w
Normal	332±19.0	331±18.7	341±21.6	349±19.4	345±19.3	355±19.6	358±18.8
Alcohol	303±10.3	318±11.0	334±25.3	348±25.0	348±25.0	346±24.9	351±24.3
Alcohol + *C. fructus*	287±19.4	263±17.2	281±17.6	290±17.5	304±18.1	302±17.2	316±19.7
Alcohol + *M. alba*	313±22.1	315±20.5	319±21.8	325±23.5	323±22.0	328±22.8	334±22.0

Values are mean concentration ± S.E.M. (n=8)

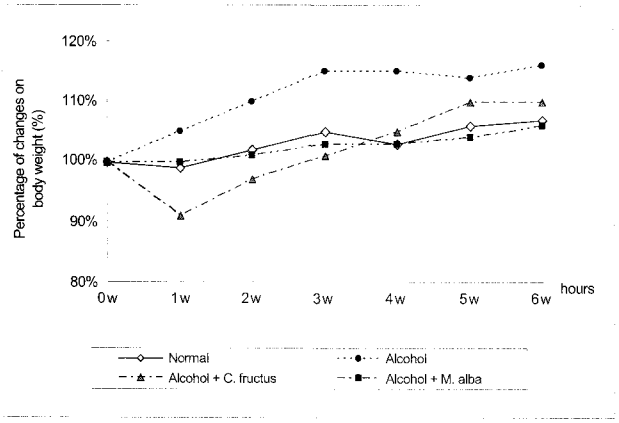

Fig. 1. Percentage of changes on body weights compared with 0 wk data in alcohol-treated rats (%)

AST (aspartate aminotransferase), ALT (alanine aminotransferase) were measured using Roche Diagnostic reagents on a Cobas Mira analyzer (Roche Diagnostic Pty Ltd.).

6. Enzyme assays

1) Alcohol dehydrogenase

Cytosolic ADH activities were determined with the hepatic cytosolic preparation according to the method of Lebsack and Anderson (1979). The assay mixture contained 0.2 M etanol 0.2 ml, 0.5 M semicarbazide 0.02 ml, 0.1 M NAD^+ 0.02 ml, 0.1 M Tris-HCl buffer (pH 8.5) 1.6 ml and 0.1 ml of sample, in a final volume of 2.0 ml. The mixture was incubated for 3 min at 37℃. ADH oxidizes ethanol to acetaldehyde and NADH at the expense of NAD^+. The absorbance of NADH was measured at 340 nm using a spectrometer. The enzyme activities were expressed as nM of NADH formed per min per mg protein of hepatic cytosolic preparations.

2) Aldehyde dehydrogenase

Aldehyde dehydrogenase activity was measured spectrophotometrically using propionaldehyde and NAD^+ as the substrates by following the NADH production as 340 nm according to the method of Lebsack and Anderson (1979). The reaction mixture contained 0.1 M propionaldehyde 0.06 ml, 1 M KCl 0.1 ml, 0.1 M pyrazole 0.02 ml, 1 M 2-mercaptoethanol 0.02 ml, 0.1 M NAD^+ 0.1 ml, 0.2 M tris-HCl buffer (pH 8.3) 1.25 ml with 0.1 ml mitochondrial preparations in final volume of 2.5 ml was incubated for 3 min at 25℃. The enzyme activities were expressed as in the ADH assay.

7. Statistical analysis

All data were expressed as means ± S.E. Significant differences among the groups were determined by one-way analysis of variance using the SPSS statistical analysis program. Statistical significance was considered at $P<0.05$.

RESULTS

1. Effect of experimental plants on body weight

Table 1 and Fig. 1 show the body weight (g) in all rats during the 6w. At the 6^{th} week, the body weight gain was significantly higher in the alcohol-treated rats than in the normal rats. The administration of extract of *C. fructus* with alcohol produced the reduction in body weight level of 91% at first week, however, a trend toward increased weight become evident. The administration of extract of *M. alba* with alcohol showed low weight gain rate, was same trend increasing weights was seen in normal rats.

2. Effect of experimental plants on brain weight, liver weight, kidney weight, spleen weight and heart weight

Table 2 shows the organ weights (g/100 g body weight). Compared with normal rats, the alcohol-treated rats showed a significantly decreased the brain weights, spleen weights and heart weights ($P<0.05$). The administration of extracts of *C. fructus* and *M. alba* significantly increased the brain weights, spleen weights and heart weights ($P<0.05$, $P<0.01$). The liver weights, as well as the kidney weights, showed no differences.

Table 2. Effect of extracts from *C. fructus* and *M. alba* on organ weights/body weight in alcohol-treated rats (g/100 g BW)

Experimental group	Brain	Liver	Kidney	Spleen	Heart
Normal	0.39±0.014	2.64±0.20	0.31±0.013	0.17±0.018	0.29±0.013
Alcohol	0.36±0.011▲	2.50±0.44	0.27±0.027	0.14±0.021▲	0.27±0.036▲
Alcohol + *C. fructus*	0.42±0.011*	2.60±0.35	0.30±0.052	0.18±0.025*	0.33±0.023**
Alcohol + *M. alba*	0.41±0.062*	2.70±0.17	0.30±0.026	0.18±0.03*	0.31±0.053*

Values are mean concentration ± S.E.M. (n=8). ▲; $P<0.05$ vs. Normal group, *; $P<0.05$, **; $P<0.01$ vs. Alcohol group

Table 3. Effect of extracts from *C. fructus* and *M. alba* on glucose, AST and ALT in alcohol-treated rats

Experimental group	Glucose (mg/dl)	AST (unit/l)	ALT (unit/l)
Normal	155±19.0	90.5±24.1	58.5±8.1
Alcohol	278±26.6▲▲	104±56.4	96.6±14.6
Alcohol + *C. fructus*	149±24.8**	101±22.2	74.0±31.8
Alcohol + *M. alba*	148±15.7**	98±34.8	72.4±36.8

Values are mean concentration ± S.E.M. (n=8).
▲▲; $P<0.01$, *vs.* Normal group, **; $P<0.01$ *vs.* Alcohol group

Table 4. Effect of extracts from *C. fructus* and *M. alba* on ADH and ALDH in alcohol-treated rats (nM NADH/min/mg protein)

Experimental group	ADH activities	ALDH activities
Normal	55.4±10.6	22.0±5.9
Alcohol	68.62±11.9▲	11.9±3.2▲
Alcohol + *C. fructus*	42.02±6.3*	26.6±1.3*
Alcohol + *M. alba*	45.1±7.2*	27.1±3.3*

Values are mean concentration ± S.E.M. (n=8).
▲; $P<0.05$, *vs.* Normal group, *; $P<0.05$ *vs.* Alcohol group

3. Effect of experimental plants on glucose levels, AST and ALT

Table 3 shows the level of blood glucose and the activities of AST, ALT of experimental rats. Compared with normal rats, alcohol-treated rats showed a significantly increased blood glucose level ($P<0.01$). Administration of extracts of *C. fructus* and *M. alba* tends to bring down the blood glucose level of alcohol-treated rats to near normal ($P<0.01$). The activities of AST and ALT was higher in the alcohol-treated group than in the normal rats, while there was no the statistical difference.

4. Effect of experimental plants on alcohol metabolizing enzyme activities

Table 4 shows the activities of ADH and ALDH of the liver in alcohol-treated rats. The activities of ADH in the alcohol-treated rats were significantly increased compared to the normal rats ($P<0.05$). The administration of extracts of *C. fructus* and *M. alba* tends to bring down the activities of ADH of alcohol-treated rats to near normal ($P<0.05$). In contrast, the activities of ALDH in the alcohol-treated rats were significantly decreased ($P<0.05$). The administration of extracts of *C. fructus* and *M. alba* increased the activities of ALDH compared to alcohol-treated rats ($P<0.05$).

DISCUSSION

Chronic heavy drinking has been associated with excessive blood glucose levels (hyperglycemia). It can reduce the body's responsiveness to insulin and cause glucose intolerance in both healthy individuals (Shah, 1988) and alcoholics with liver cirrhosis (Letiexhe et al., 1993). In fact, 45 to 70 percent of patients with alcoholic liver disease are glucose intolerant or are frankly diabetic (Gordon and Lieber, 1992). In the present study, alcohol-treated rats showed a significantly increased blood glucose level, but the administration of extracts of *C. fructus* and *M. alba* decreased the blood glucose levels in alcohol-treated rats (Table 3). The ethanol oxidation and oxidative stress could be occurred the increase in blood glucose levels, and glucose auto-oxidation secondarily increases the oxidative stress. *C. fructus* is mainly composed of flavonoids (Zhang et al., 2001a; Zhang et al., 2001b; Zhang et al., 2003), procyanidins, catechin, epicatechin (Chu et al., 2003), and ursolic acid (Jeong, 1999), known as its antioxidants. Previous studies have demonstrated that *M. alba* extracts showed strong antioxidant activities and also showed a protective effect on DNA damage caused by hydroxyl radicals (Choi et al., 2002). Based on above-mentioned reports, we suggest that the possible mechanism of action of extracts from *C. fructus* and *M. alba* could be related to antioxidants that aid to recover from impaired metabolism of glucose, which damaged by ethanol oxidation.

Many studies have reported that the supplement of alcohol to the rats caused significant increase in bodyweight and liver weight (Kwon et al., 2005). But, in our study, despite body weights were increased; the liver weights did not

show a significant change in alcohol-treated rats. In contrast, the brain weights, spleen weights and heart weights in the alcohol-treated rats were significantly decreased. The administration of extracts of *C. fructus* and *M. alba* significantly increased the brain weights, spleen weights and heart weights. It may be due to fill up the reduction of intake and absorption of nutrients by extracts of *C. fructus* and *M. alba*. Especially, the administration of extract of *M. alba* with alcohol showed the slower weight gain than in extract of *C. fructus*, was same trend increasing weights was seen in normal rats. It may be useful in preventing overweight induced by alcohol.

The activities of AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, we used the activities of AST and ALT in the circulation as indicators of hepatic damage. In the present study, the activities of AST and ALT were higher in the alcohol-treated rats than in the normal rats, while there was no significant difference (Table 3). Although the treated with experimental plant extracts reduced plasma AST and ALT activities in the alcohol-treated rats, there was no the statistical difference.

In the liver, the enzyme alcohol dehydrogenase (ADH) converts ethanol into acetaldehyde. It is then further converted into harmless acetic acid by acetaldehyde dehydrogenase (ALDH). ADH is located in the cytoplasm, catalase in the peroxisomes, the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum (Lieber, 2005). ALDH is located in the soluble and insoluble fractions of hepatocytes (Crow et al., 1974). Although ADH catalyzes the rate-limiting step in the ethanol metabolism, its physiological role is uncertain. And it is also unclear that how liver ADH activity changes with dose and time during acute alcohol intoxication (Haseba et al., 2003). As shown in Table 4, the alcohol-treated rats significantly increased the activities of ADH of the liver, but treatment with the extracts of *C. fructus* and *M. alba* decreased in the ADH activities of alcohol-treated rats. In contrast, the activities of ALDH of the liver in the alcohol-treated rats were significantly decreased, whereas treatment with extracts of *C. fructus* and *M. alba* increased in the ALDH activities of alcohol-treated rats. Therefore, the extracts of *C. fructus* and *M. alba* may improve the detoxification of alcohol and acetaldehyde by regulating the activities of alcohol-metabolizing enzymes, thereby preventing hepatic damage.

In conclusion, administration of extracts of *C. fructus* and *M. alba* not only reduced liver damage by accelerating the alcohol and acetaldehyde metabolism, but also corrected associated glucose levels caused by alcohol. Especially, *M. alba* produced beneficial effects on glucose homeostasis and body weight in chronic alcohol consumption. Taken together, the combined effect of *C. fructus* and *M. alba* may be considered as an alternative remedy for hangovers, alcohol-induced overweight and alcohol-induced diabetes.

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