

Effects of Galgunhaejutang on Alcohol Consumption in C57BL/6 Mice

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Abstract

Objectives :

Galgunhaejutang extract (GHT) is a Traditional Korean medical herbal mixture that has been traditionally used to treat alcohol consumption. This study was performed to evaluate the effects of GHT on alcohol consumption in C57BL/6 mice.

Methods :

Sixty three C57BL/6 mice were alcohol dependence-induced by limited access paradigm. Water, GHT 0.688 g/kg (GHT-L), or GHT 3.45 g/kg (GHT-H) were administrated for 10 days. The amounts of alcohol consumption for 2 hours, water consumption for 22 hours, food intake for 24 hours and body weight were measured.

Results :

There weren't significant differences in 2 hours of alcohol consumption, 22 hours of water consumption, 24 hours of food intake and body weight for ten days between vehicle group and GHT-L or GHT-H group.

Conclusions :

Further studies employing multi-dose and long term administration of GHT (more than 10 days) might be of benefit.

Key Words:

Korean traditional medicine, Herbal mixture, Alcohol consumption, C57BL/6.

Received : August 9, 2013; Revised : August 27, 2013; Accepted : August 27, 2013

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This work was supported by a grant from the Kyung Hee University in 2012 (KHU-20121742).

I. Introduction

Alcohol dependence is a chronic disease characterized by physical withdrawal symptoms in the absence of alcohol consumption or the compulsive need to drink substantially large amounts despite continued alcohol-related problems and cognitive, behavioral, and physiologic symptoms.

Behavioral, physical, or psychological symptoms of alcoholism are also described in Oriental medicine and studies of treatment modalities described in Oriental medicine have been increasingly reported.

Galgunhaejutang extract (GHT) is a Traditional Korean medical herbal mixture that has been traditionally used to treat alcohol dependence. Bang et al. observed significant changes in food-step number, rearing number, motor incoordination changes by Rota-Rod, and T-maze crossing time by GHT in alcoholism-induced rats¹⁾.

The effects of *Puerariae Radix* (a main herb of GHT) on alcoholism are observed by many studies. Keung et al. also observed daidzein and daizin, major components of *Puerariae Radix* extract, attenuated ethanol consumption in Syrian golden hamsters²⁾. Overstreet et al. reported that extracts of both *Puerariae Radix* plus *Citrus Reticulata* and *Puerariae Radix* suppressed alcohol intake in P and Fawn-Hooded rats (alcohol-preferring rats)³⁾.

Yoon and his colleague demonstrated that *Puerariae Radix* inhibited the desire for alcohol drinking in adult men who had recovered from acute alcohol intoxication or withdrawal symptoms⁴⁾. Kim et al. proved that *Puerariae Radix* did not elevate blood alcohol levels in both alcoholics and non-alcoholics^{5,6)}. Kang et al. *Puerariae Radix* suppressed desire for alcohol drinking and inhibited alcohol-induced increased alcohol craving⁷⁾.

Although many treatment modalities for alcoholics exist and are commonly used in Oriental medicine, studies as to determine the underlying mechanism and effect in controlled experimental settings are scarce. Thus, to elucidate the anti-alcohol drinking desire, herein the effect of GHT on alcohol consumption, preference, was determined.

II. Materials and Methods

1. Reagents

The contents of the prescription Galgunhaejutang used in this study are shown in Table 1. Total of 850 g of Galgunhaejutang was first distilled and 103.8 g of powder was obtained after lyophilization. Low dose (688 mg/kg/day, GHT-L) and high dose (3,450 mg/kg, GHT-H) of galgunhaejutang were administered. These doses of GHT were chosen on the 2 times and 10 times of the clinical dose.

Table 1. Contents of Galgunhaejutang

Botanical name	Dose
<i>Puerariae Radix</i>	12.0 g
<i>Puerariae Flos</i>	12.0 g
<i>Alpiniae officinarum Rhizoma</i>	8.0 g
<i>Crataegii Fructus</i>	8.0 g
<i>Cyperi Rhizoma</i>	8.0 g
<i>Aurantii immaturus Fructus</i>	8.0 g
<i>Atractylodis macrocephalae Rhizoma</i>	4.0 g
<i>Chaenomelis Fructus</i>	4.0 g
<i>Sophorae Radix</i>	4.0 g
<i>Citri Pericarpium</i>	3.0 g
<i>Zedoariae Rhizoma</i>	2.0 g
<i>Hovenia Semen</i>	12.0 g
Total amount	85.0 g

Table 2. Summary of Experimental Phase from Day 1 to Day 47

Experimental Phases										
Access to water	Free access to water, no alcohol	Limited access procedure (2 hrs alcohol, 22 hrs water)			Limited access procedure (2 hrs alcohol, 22 hrs water)					
Access to alcohol		Free access to alcohol no water								
Oral administration				Oral intake education of water		Oral feeding of water or GHT-L or GHT-H				
	Adaptation period	Alcohol exposure			D2	D4	D6	D8	D10	
Date	1~5 (5 days)	6~12 (1 wks)	13~33 (3 wks)	34~37 (4 days)	38~39	40~41	42~43	44~45	46~47	
Pretreatment period (37 days)					Treatment period (10 days)					

GHT-L: Galgunhaejutang low dose, GHT-H: Galgunhaejutang high dose.

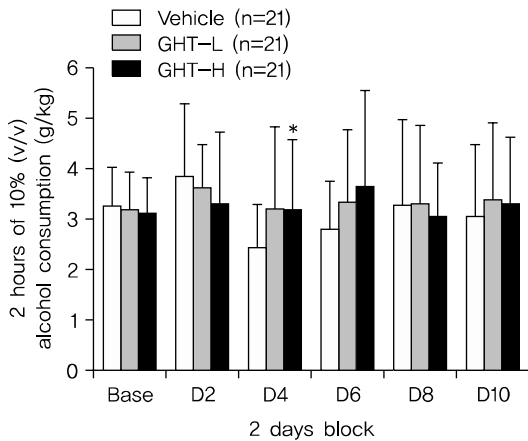


Fig. 1. Effect of GHT-L, GHT-H on alcohol consumption in C57BL/6 mice during 2-hr access to alcohol after GHT administration (g/kg). Value are mean±SD for 21 mice. Significant change was observed between vehicle D4 and high dose group D4 (p=0.043) with repeated measure ANOVA and independent t-test with vehicle group, *p<0.05. Base: mean of two days just before starting drug administration, D2, D4, D6, D8, D10: mean of two days when drug was administered, GHT-L: Galgunhaejutang low dose, GHT-H: Galgunhaejutang high dose, SD: Standard deviation.

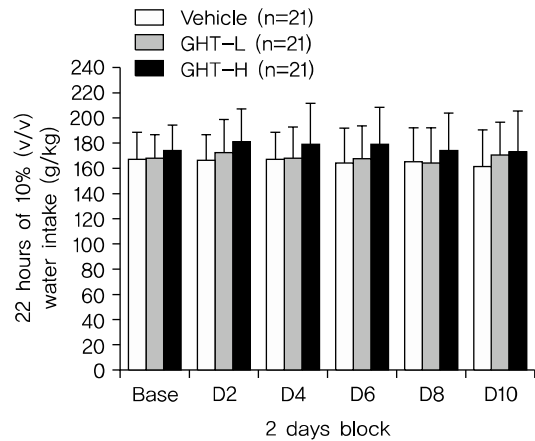


Fig. 2. Effect of GHT-L, GHT-H on water intake in C57BL/6 mice during 22-hr access to water after GHT administration (g/kg). Value are mean±SD for 21 mice. Non-significant with repeated measure ANOVA. Base: mean of two days just before starting drug administration, D2, D4, D6, D8, D10: mean of two days when drug was administered, GHT-L: Galgunhaejutang low dose, GHT-H: Galgunhaejutang high dose, SD: Standard deviation.

2. Animals

3-weeks old male C57BL/6 mice were used in this study. Mice were individually housed and maintained in a temperature-and humidity-controlled environment under a 12 hour light cycle (lights on 06:00). Food and water were provided *ad libitum*. Mice were acclimated to the environment for 5 days prior to the start of the experiment. Limited access procedure was employed to determine behavioral alcohol dependence⁸⁻¹¹. Inbrief, mice were forced to drink 10% (v/v) alcohol only (Sigma Inc. St, Louis, MO, USA) for 1 week, 24 hours a day and then were given 3 weeks of limited daily access to 10% (v/v) alcohol from 2:00 to 4:00 PM. Mice were acclimated to oral feeding by administration of 0.1 ml water for 4 days before treatment started.

3. Alcohol consumption and preference

After 3 weeks of limited daily access to alcohol, Galgunhaejutang was orally administered for 10 days during which weights of mice were measured every 2 days. Alcohol, food, and water intake were daily measured and taken the mean of two days when drug was administered (Table 2).

4. Statistical analysis

Statistical analysis was performed using independent *t*-test and repeated measure analysis of variance (ANOVA). The accepted level of significance was preset as *p*-value ($p < 0.05$). Data are presented as means \pm standard deviation. The SPSS Statistical Software Package was used.

III. Results

1. Alcohol consumption and water intake

Statistical significance was not observed between vehicle and low dose group. Although significant change was observed between vehicle D4 and high dose group D4 ($p=0.043$), no difference was observed between vehicle and high dose group (Fig. 1). Water intake among vehicle, low dose, and high dose groups was not significantly different (Fig. 2).

2. Alcohol preference analysis

Alcohol preference (alcohol intake/total water intake ratio) was not significantly different among vehicle, low dose, and high dose groups (Fig. 3).

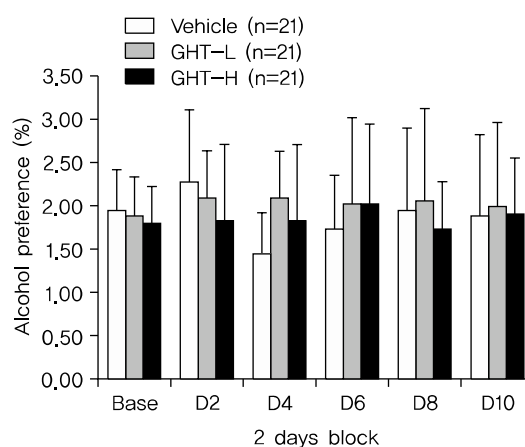


Fig. 3. Effect of GHT-L, GHT-H on alcohol preference (alcohol consumption/water + alcohol consumption) in C57BL/6 mice during limited access to alcohol and water after GHT administration (g/kg). Values are mean \pm SD for 21 mice. Non-significant with repeated measure ANOVA. Base: mean of two days just before starting drug administration, D2, D4, D6, D8, D10: mean of two days when drug was administered.

GHT-L: Galgunhaejutang low dose, GHT-H: Galgunhaejutang high dose, SD: Standard deviation.

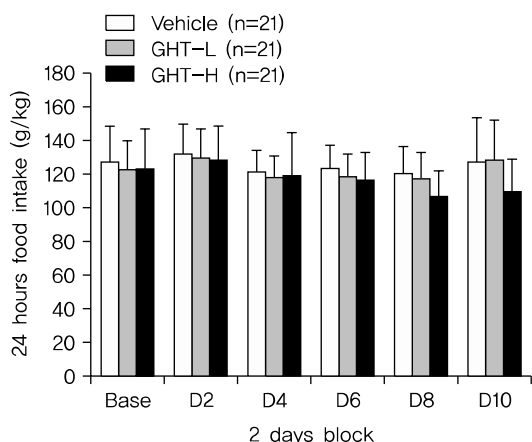


Fig. 4. Effect of GHT-L, GHT-H on food intake in C57BL/6 mice during 24-hr access to food after GHT administration (g/kg). Value are mean \pm SD for 21 mice. Non-significant with repeated measure ANOVA. Base: mean of two days just before starting drug administration, D2, D4, D6, D8, D10: mean of two days when drug was administered. GHT-L: Galgunhaejutang low dose, GHT-H: Galgunhaejutang high dose, SD: Standard deviation.

3. Food intake and weight change

Food intake was not significantly different among vehicle, low dose, and high dose groups (Fig. 4). Weight change was not significantly different among vehicle, low dose, and high dose groups (Fig. 5).

IV. Discussion

This study was preformed to evaluate the effects of Galgunhaejutang extract (GHT) on alcoholism in C57BL/6 mice. To evaluate this effect, we employed limited access procedure that is commonly used in alcohol dependence studies to determine alcohol preference and dependence⁸⁻¹¹⁾ but, that does not have been used in herbal studies. The availability of limited access procedure can facilitate investigations of various alcohol-related behaviors

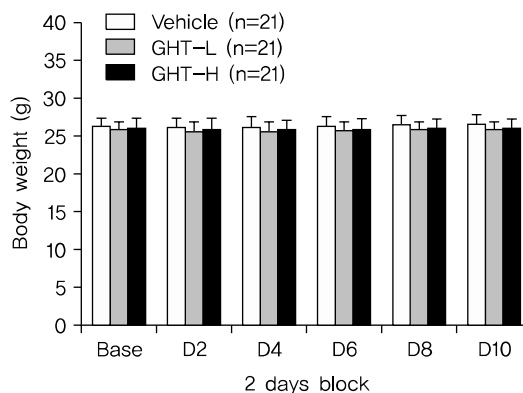


Fig. 5. Effect of GHT-L, GHT-H on body weight in C57BL/6 mice after GHT administration (g/kg). Value are mean \pm SD for 21 mice. Non-significant with repeated measure ANOVA. Base: mean of two days just before starting drug administration, D2, D4, D6, D8, D10: mean of two days when drug was administered.

GHT-L: Galgunhaejutang low dose, GHT-H: Galgunhaejutang high dose, SD: Standard deviation.

of mice that have been selected for difference. C57BL/6 mice are known as a alcohol-preference strain^{9,10)}. This study was a first study that used C57BL/6 mice and limited access procedure in herbal mixture study.

There is no significant difference in both alcohol intake and preference between 10-day-GHT-treated and control groups. We have observed that GHT (Puerariae Radix and other herbal mixture) is traditionally effective on alcoholism and the effects of Puerariae Radix (a main herb of GHT) on alcoholism are observed by many studies.

Keung et al. also observed daidzein and daizin, major components of Puerariae Radix extract, attenuated ethanol consumption in Syrian golden hamsters²⁾. Overstreet et al. reported that extracts of both Puerariae Radix plus Citrus Reticulata and Puerariae Radix suppressed alcohol intake in P and Fawn-Hooded rats (alcohol-preferring rats)³⁾.

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inhibited the desire for alcohol drinking in adult men who had recovered from acute alcohol intoxication or withdrawal symptoms⁴. Kim et al, proved that Puerariae Radix did not elevate blood alcohol levels in both alcoholics and non-alcoholics^{5,6}. Kang et al, Puerariae Radix suppressed desire for alcohol drinking and inhibited alcohol-induced increased alcohol craving⁷.

Our study was designed based on the traditional experience and the Puerariae Radix study but our results could have been due to drug dose and administration term.

We used low dose (688 mg/kg/day, GHT-L) and high dose (3,450 mg/kg, GHT-H) of GHT that were administered. We extracted GHT with water. So GHT could be lower dose than Puerariae Radix extract with methanol or butanol like other studies with intraperitoneal injection^{2,3}.

Since significant decrease in alcohol craving in patients with alcoholism was observed after 2-week-administration of Puerariae Radix in Yoon's clinical study⁴, further studies employing long term administration of GHT (more than 10 days) might be of benefit.

Further multi-dose and long term study of the effects of GHT on alcoholism in C57BL/6 mice is required. GHT appears safe, and is very effective in reducing craving in traditional experience. A study using a multi-dose and long term administration may have the statistical power to decipher a difference between GHT and vehicle.

Conclusion

There weren't significant differences in 2 hours of alcohol consumption, 22 hours of water consumption, 24 hours of food intake and body weight

for ten days between vehicle group and GHT-L or GHT-H group. Further studies employing multi-dose and long term administration of GHT (more than 10 days) might be of benefit.

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