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Clinical Benefits of *Antrodia Camphorata* Containing Antroquinonol on Daily Fatigue and Alcohol Syndrome

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ABSTRACT

Background: *Antroquinonol, which is contained in Antrodia camphorata, is recognized as having diverse biological activities, including liver protection. We conducted human clinical studies to evaluate the effects of Antrodia camphorate containing Antroquinonol on the metabolism of blood ethanol and acetaldehyde after alcohol consumption and on daily activities after work.*

Methods: *We conducted two studies. First, a randomized, double-blinded, placebo-controlled, crossover study evaluated the anti-alcohol effects of Antroquinonol-contained A. camphorata in healthy subjects before and after alcohol consumption. In this experiment, 40 healthy adult males and females ranging in age from 20 to 59 years ingested the equivalent of 0.5 g/kg of body weight (BW) of alcohol with either a placebo or Antroquinonol-contained A. camphorata. We then measured their post-ingestion blood ethanol and acetaldehyde levels as well as their antioxidative activity. The second was a randomized, double-blinded, placebo-controlled study of the effect of Antroquinonol-contained A. camphorata on the fatigue experienced by physical laborers. In this experiment, the subjects were 64 healthy adult males and females ranging in age from 40 to 64 who engaged in physical labor and who did not suffer from chronic fatigue syndrome. The subjects ingested either the Antroquinonol-contained A. camphorata or a placebo for a period of 12 weeks. We then measured their daily fatigue levels and their blood anti-oxidative function.*

Results: *The results of anti-alcohol study indicated that the ingestion of the Antroquinonol-contained A. camphorata caused significant decreases in blood ethanol and acetaldehyde levels after alcohol consumption as compared to the placebo. Examination using a subjective index also indicated that the Antroquinonol-contained A. camphorata significantly suppressed the mood-lifting effect of alcohol as well as uncomfortable symptoms associated with alcohol consumption such as reddening of the face compared to the placebo. The results of anti-fatigue study indicated that during the ingestion period, the "vigor-activity" score on the Profile of Mood States (POMS), which is a subjective index of mood, was significantly higher for those who ingested the Antroquinonol-contained A. camphorata as compared to the placebo food. In addition, investigation of changes in blood markers indicated that the γ-GTP and urea nitrogen levels, markers of liver and kidney function respectively, showed significant decreases among those who ingested the food containing antroquinonol as compared to those who ingested the placebo.*

Conclusion: *The ingestion of Antroquinonol-contained A. camphorata increased the metabolism of alcohol and acetaldehyde after alcohol consumption, decreased uncomfortable symptoms associated with alcohol consumption, and suppressed everyday feelings of fatigue.*

Keyword
Alcohol metabolism, Antroquinonol-contained *A. camphorate*, γ-GTP, Fatigue.

Introduction

Fatigue has a variety of causes. Typical examples include mental fatigue that results from the stress of everyday work and activities and physical fatigue that results from physical activities. Fatigue is classified by its type of manifestation as either primary fatigue or secondary fatigue. For example, the fatigue observed in patients who's primary (or, underlying) illness is diabetes or a malignant tumor is classified as secondary fatigue. This type of fatigue is resolved by focusing primarily on the treatment of the underlying illness. On the other hand, according to the Labor Health Survey conducted in 2002 by the Ministry of Health, Labour and Welfare of Japan, 72.2% of workers experienced "tiredness as a result of normal work [1]."

However, the majority of these workers were normal healthy individuals without any underlying illness whose fatigue manifest in the course of their daily activities. Aside from those who suffer from chronic fatigue syndrome, this type of fatigue can be clearly distinguished from any "illness." Thus, the preferred method of assisting healthy individuals to overcome their fatigue consists of adjustments to their diet and other normal daily activities rather than through the use of drugs. Specifically, alleviation and prevention of this type of stress is achieved by avoiding accumulation of the type of stress that leads to fatigue and by successfully dealing with the type of stress that temporarily accumulates over the course of normal daily activities. There are suggestions that there is a relation between stress and reactive oxygen species (ROS). This then suggests that the ingestion of foods that have antioxidative properties would be useful in reducing stress. Another method of dealing with accumulated stress is moderate alcohol consumption.

Moderate amounts of alcohol consumption helps the individual to experience a sense of freedom which releases stress. However, the acetaldehyde that is produced through the metabolism of alcohol causes uncomfortable symptoms associated with alcohol consumption. Approximately 30% of alcohol consumed orally is absorbed by the stomach and approximately 70% is absorbed by the upper small intestine, after which it is transported to the liver via the portal vein. It is then catalyzed into acetic acid by acetaldehyde dehydrogenase, which is subsequently broken down into water and carbon dioxide.

However, excessive alcohol consumption transports ethanol and acetaldehyde that could not be metabolized by the liver into the brain across the blood-brain barrier, which causes the symptoms of drunkenness. Symptoms resulting from alcohol consumption such as headache, increased heart rate (heart palpitations), and reddening of the face have been traced to the causative agent known as acetaldehyde [2,3].

Several species of medicinal mushroom such as *Antrodia camphorata, Ganoderma lucidum, Cordyceps sinensis,* and *Phellinus linteus* are known to be helpful in healing disease and maintaining health functions. *A. camphorata* is a well-known species native to Taiwan that has the effect of maintaining healthy functionality and have pharmacological effects. *A. camphorata* has been found to contain chemical compounds that have a variety of pharmacological effects. One of these chemical compounds is antroquinonol, which is known to have a protective effect on the liver. *A. camphorata* has long been known to be effective on a wide range of illnesses, including liver disease, hypertension, abdominal pain, and diarrhea [4].

Throughout the course of their long history, the indigenous inhabitants of Taiwan used *A. camphorata* for the purpose of improving the unpleasant symptoms and liver damage caused by alcohol consumption. However, today, it is difficult to collect fruiting bodies of wild-type *A. camphorata*. As a result, cultivation methods that utilized biotechnology were developed in order to ensure a stable supply of these medicinal mushrooms. Producing medicinal mushrooms using artificial cultivation technologies is useful because it allows humans to control the quality of the effective ingredients in the mushrooms and removes concern over the accumulation of environmental contaminants, which is a problem that is related to wild mushrooms. The antroquinonol contained in *A. camphorata* cultivated using these technologies and which has the effect of maintaining healthy functioning is now available in stable mass quantities.

Our previous research elucidated the mechanism of action by which *A. camphorata* improves liver damage [unpublished data].

The oxidative stress that is produced during the process of metabolizing alcohol causes damage to liver cells. This type of Alcoholic liver disease (ALD), which is caused by damage to liver cells, occurs in approximately 20% of all cases of alcohol dependency [5]. The major reason is that four by-products that are produced during the process of metabolizing alcohol cause damage to liver cells, which in turn leads to steatohepatitis, gangrene, cirrhosis of the liver, and/or hepatocarcinoma in some cases. In the United States ALD is a major health problem as it accounts for 15% of a total of seven types of medical costs and the mortality rate is 20% [6]. Alcohol consumption also causes overproduction of free radicals, which cause spontaneous depletion of hepatic glutathione [7]. The overproduction of free radicals not only causes liver damage, it also leads to vascular injury and peroxidation of lipids in the blood, which in turn becomes the causes of a variety of diseases. Antroquinonol is known as a compound that suppresses the generation of ROS, and its antioxidative properties are thought to be useful in the reduction of damage to liver cells [7].

However, these effects have only been verified at the cellular level and no human test has been conducted to verify these actions. Thus, we conducted human trials in order to investigate the antioxidative properties of Antroquinonol-contained *A. camphorata* by studying whether it reduced the unpleasantness associated with consumption of alcohol and improved the daily fatigue subjectively experienced by physical laborers.

Methods

Study 1

Study to investigate the effect of Antroquinonol-contained *A. camphorata* on blood ethanol levels, metabolism, and the unpleasant symptoms after alcohol consumption.

Study design

This study was a randomized, placebo-controlled, cross-over study. The subjects were 40 individuals aged 20 to 59 years. The inclusion criteria were as follows: individuals with no symptoms or history of liver disease or kidney disease who were able to ingest alcoholic beverages but who were selected via alcohol patch test to be liable to the alcohol flush reaction and hangover. In accordance with the Declaration of Helsinki, consent was obtained from the subjects prior to the start of the study. In addition, the Institutional Review Board of the Oriental Ueno Health Examination Center reviewed the study protocol and their approval was obtained prior to the start of the study. The study protocol was registered with UMIN-CTR (UMIN Clinical Trials Registry) prior to enrollment of the study participants (UMIN000022975).

The study was conducted between May 2016 and October 2016. During Phase I, selected subjects were instructed to continue fasting for a minimum of six hours. They then arrived at the hospital at 8 pm and consumed the evening meal (prescribed diet). At 9 pm they were subjected to a pre-load blood test, urine test, and they completed a Visual Analog Scale (VAS) questionnaire. At 9:15 pm (45 mins prior to alcohol load) they ingested either the test food or the placebo food (random selection). At 10 pm they consumed alcohol (alcohol load). At 60 mins, 90 mins, and 120 mins post-load blood samples were taken and they once again completed the VAS questionnaire, respectively. Then, lights were turned out and they went to sleep. Blood samples were taken at 180 mins and 240 mins post-load. They were awakened at 5:45 am the following morning. At 6:00 am (480 mins post-load) blood and urine samples were taken and they completed the VAS questionnaire. After a one-week washout period, the same procedure was repeated, but each subject was given a different type of food from the one they ingested during Phase I.

Test food and placebo food

The test food consisted of two tablets containing 0.45 mg of antroquinonol (Figure 1) each (total amount of antroquinonol ingested: 0.9 mg). The placebo food consisted of tablets that did not contain antroquinonol. The two types of foods were indistinguishable by appearance or taste. The composition of the test food is shown in Table 1.

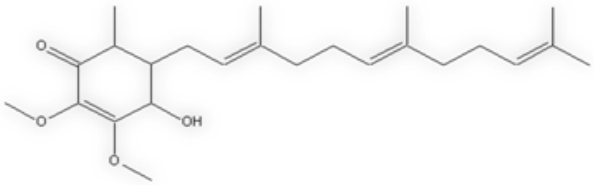

Figure 1. Structure of antroquinonol

Alcohol patch test

A patch test (Life Care Giken Inc., Toyama City, Toyoma, Japan) was conducted in order to identify and exclude individuals who had a complete lack of the ALDH2 gene and therefore were unable to metabolize alcohol. This method was developed by Makino et al. Specifically, a hydrous gel-patch containing 14% ethyl alcohol was left in place on a fleshy portion of the subjects' hypertrophic upper arms for 20 mins. Skin reddening was compared to a color chart in order to determine whether the individual had the heterozygous genotype of normal and inactive (ND) ALDH2 genes or normal (NN) ALDH2 genes. These two types were enrolled in the study, and were known to have a good correlation to normal genotype NN and partial defective genotype NM.

Amount of alcohol load

The amount of alcohol load was the equivalent of 40 proof whisky converted to 0.5 g/kg body weight (BW). The subjects were provided with a total of 200 ml of beverage, including water used as a mixer.

Measurement of blood ethyl alcohol, acetaldehyde, and antioxidative activity

Blood alcohol and aldehyde levels were measured using the headspace gas chromatography with flame ionization detection (HS-GC-FID) method. Preprocessing consisted of adding 0.05 ml of an internal standard fluid (methanol) to 0.5 ml of blood contained in a glass vial for use in the headspace method. This was sealed, agitated in a mixer, and stored frozen (-20°C) for 24 hrs. Then, 3 M perchloric acid (0.3 ml) was added and after agitating in a mixer the solution was heated to 50°C and left for 15 mins in a vial warmer. Using a gas-tight syringe, 1.0 ml of the gas in the headspace was injected.

The measurement protocol was as follows:
Column: TC-WAX 0.53mm×30m df 1.0, Column temperature: 40°C, inlet temperature: 100°C, FID temperature: 250°C, mobile phase gas: helium. Antioxidative activity in the blood was measured using the biological antioxidant potential (BAP) test and the d-ROMs test.

Visual Analogue Scale (VAS)

On the VAS, individuals were asked to indicate their responses along a 100 mm line in order to indicate their degree of sleepiness, heart pounding, headache, nausea, cheerfulness, facial reddening, body flush, and quickness of breath.

Study 2

Study to investigate the influence of Antroquinonol-contained *A. camphorata* on the fatigue experienced by individuals engaged in physical labor.

Study design

This study was a randomized, placebo-controlled parallel group study. The subjects were healthy individuals ranging in age from 40 to 64 years.
The inclusion criteria were as follows:

Individuals without psychological or chronic physical diseases who are engaged in physical labor and experience fatigue. In accordance with the Declaration of Helsinki, consent was obtained from the subjects prior to the start of the study. In addition, the Institutional Review Board of the Oriental Ueno Health Examination Center reviewed the study protocol and their approval was obtained prior to the start of the study.

The study protocol was registered with UMIN-CTR (UMIN Clinical Trials Registry) prior to enrollment of the study participants (UMIN000022974). Clinical study was conducted between May 2016 and December 2016. The selected subjects visited the hospital prior to the start of the study and 4, 8, and 12 weeks after the start of the study, respectively. During these visits, they completed the Profile of Mood States (POMS) questionnaire and underwent blood tests and an assessment of the quality of their sleep (OSA, Pittsburgh).

Test food and placebo food

The test food consisted of two tablets containing 0.45 mg of antroquinonol each (total amount of antroquinonol ingested: 0.9 mg) The placebo food consisted of tablets that did not contain antroquinonol. The two types of foods were indistinguishable by appearance or taste. The composition of the test food is shown in Table 1.

Components	Test	Placebo
Antroquinonol	0.45mg	0.000mg
Energy	0.92kcal	1.09kcal
Water	15.0mg	14.0mg
Protein	33.0mg	0.6mg
Fat	27.0mg	9.5mg
Carbohydrates	220.0mg	270.0mg
Sodium	0.052mg	0.221mg

Table 1: Composion of the test product and the placebo (one tablet).

POMS

POMS was completed by the participants prior to ingestion, and again 4, 8, and 12 weeks after ingestion, respectively. The Abbreviated POMS was utilized.

Statistical analysis

The results are indicated as mean ± standard deviation (SD). Statistical analysis was performed using SAS9.4 (SAS Institute Japan). The significance level was set at 5%.

Results

Study 1

Efficacy analysis set: Of the original 40 study participants, 7 who discontinued the study before its completion and 4 whose blood alcohol and acetaldehyde levels could not be measured were excluded from analysis. Thus, a total of 29 subjects comprised the efficacy analysis set. Background of participants were shown on Table 2.

Number	29
Sex (men/women)	16/13
Age (years)	37.2
Weight (kg)	59.18
BMI (kg/m^2)	21.58

Table 2: Participant background on Study 1. Mean ± SD.

Fluctuations in blood alcohol and acetaldehyde levels and in antioxidant activity

The fluctuations in blood alcohol, acetaldehyde levels, as well as in antioxidant activity are shown in Figure 2 and 3, Table 3 and 4, respectively. Blood alcohol levels reached a peak in both groups 60 to 90 mins after alcohol load followed by a decline, and measurements taken the following morning indicated that this level had returned to pre-ingestion baseline level. Comparison of the two groups indicated that the levels in the test food group decreased significantly at 60 mins after ingestion as compared to the placebo food group ($p<0.05$). This is almost perfectly consistent with the time at which peak blood ethanol levels were reached. In addition, peak blood acetaldehyde levels were gradually reached between 90 and 180 mins after alcohol ingestion in both groups, and then declined. By the following morning, they had returned to pre-ingestion baseline levels, as was observed with blood ethanol levels.

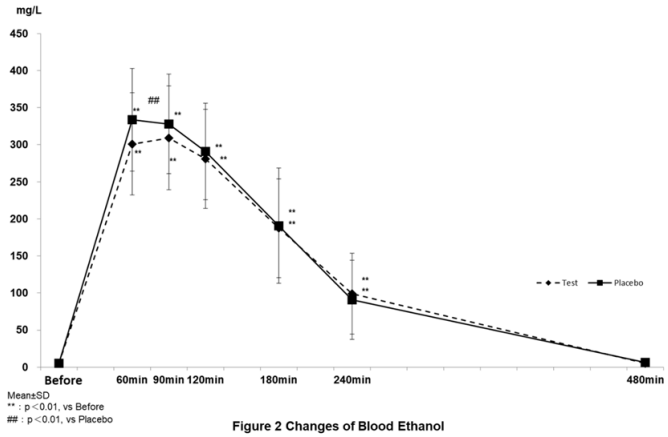

Figure 2: Structure of antroquinonol.

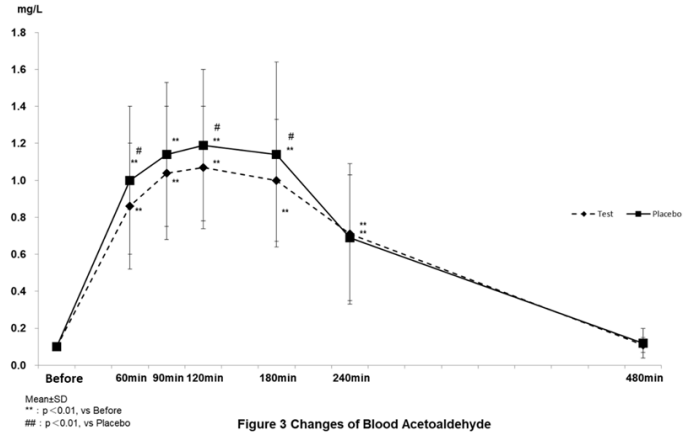

Figure 3: Changes of Blood Ethanol.

Item	Group	n	0–60 min	0–90 min	0–120 min	0–180 min	0–240 min	0–480 min
Ethanol	*A. camphorata* mycelia	29	9183.1 ± 2073.5 ##	18335.7 ± 4076.1 ##	27184.7 ± 5938.5 #	41232.9 ± 9514.4	49826.4 ± 12712.0	62310.5 ± 18612.2
	Placebo	29	10156.6 ± 2073.2	20081.4 ± 4029.2	29367.9 ± 5875.6	43817.6 ± 9605.6	52263.1 ± 12927.5	63894.8 ± 18505.1
Acetal-dehyde	*A. camphorata* mycelia	29	28.66 ± 10.29 #	57.10 ± 20.03 #	88.76 ± 29.17 #	150.72 ± 46.16 ##	202.03 ± 61.69 #	300.52 ± 99.42
	Placebo	29	33.10 ± 12.15	65.22 ± 22.93	100.14 ± 33.05	170.17 ± 54.64	225.31 ± 73.42	322.97 ± 108.71

Table 3: Area under the blood concentration-time curve of blood ethanol and acetaldehyde after alcohol consumption. **p < 0.01, paired t-test (vs. before).

Item	Unit	Group	n	Before	After 240 min	Next morning (after 480 min)
d- ROMs	U. CARR	*A. camphorata* mycelia	29	303.3 ± 71.5	290.1 ± 64.5**	304.9 ± 69.5
		Plaebo	29	297.4 ± 69.1	288.5 ± 63.5**	300.2 ± 62.5
BAP	µmol/L	*A. camphorata* mycelia	29	2059.5 ± 187.0	2163.0 ± 151.8*	2281.1 ± 133.7**
		Placebo	29	2098.0 ± 147.6	2144.1 ± 181.0	2267.5 ± 159.7**
BAP/d-ROMs ratio	µmol/L/U.CARR	*A. camphorata* mycelia	29	0.951 ± 0.235	1.038 ± 0.231**	1.040 ± 0.216**
		Placebo	29	0.985 ± 0.227	1.031 ± 0.227**	1.046 ± 0.226**

Table 4: Fluctuations in blood antioxidant activity (d-ROM test and BAP test). mean ± SD. **p < 0.01, paired t-test (vs. before).

Item	Group	n	After 60 min	After 90 min	After 120 min	Next morning (after 480 min)
Sleepiness	Test	29	3.06 ± 2.83**	3.22 ± 3.23**	3.54 ± 3.23**	1.89 ± 2.53**
	Placebo	29	3.14 ± 2.69**	3.34 ± 2.75**	3.62 ± 2.72**	2.17 ± 2.90**
Heart pounding	Test	29	3.67 ± 2.70**	2.51 ± 2.99**	1.64 ± 2.86**	-0.01 ± 1.97
	Placebo	29	3.19 ± 3.21**	2.32 ± 2.60**	1.20 ± 2.56*	0.36 ± 2.29
Headache	Test	29	1.15 ± 2.13**	1.15 ± 1.71**	1.24 ± 1.99**	1.03 ± 1.90**
	Placebo	29	0.99 ± 1.80**	0.99 ± 1.74**	0.79 ± 1.93*	1.58 ± 2.92**
Nausea	Test	29	0.57 ± 1.18*	0.56 ± 1.26*	0.75 ± 1.54*	0.88 ± 2.07*
	Placebo	29	0.56 ± 1.17*	0.46 ± 0.86**	0.47 ± 1.00*	1.27 ± 2.30**
Inebriated mood	Test	29	1.11 ± 1.82** ##	0.70 ± 1.96	0.09 ± 1.38	-0.46 ± 1.17*
	Placebo	29	1.96 ± 1.97**	1.06 ± 1.59**	0.48 ± 1.59	-0.01 ± 1.17
Facial flush	Test	29	4.89 ± 2.62**	3.21 ± 2.61**	2.31 ± 2.58**	0.08 ± 1.13 #
	Placebo	29	5.06 ± 2.73**	3.44 ± 2.56**	2.26 ± 2.20**	0.44 ± 1.37
Hot to the touch	Test	29	4.78 ± 2.22**	3.13 ± 2.80**	2.08 ± 2.19**	0.36 ± 1.88
	Placebo	29	4.61 ± 2.82**	2.90 ± 2.19**	1.87 ± 2.15**	0.51 ± 2.26
Increased respiratory rate	Test	29	3.03 ± 2.67**	1.83 ± 2.10**	1.23 ± 1.57** #	-0.02 ± 0.82
	Placebo	29	3.11 ± 2.41**	1.32 ± 2.14**	0.69 ± 1.29**	0.44 ± 1.91

Table 5: Changes in the VAS survey results. mean ± SD. *p < 0.05, **p < 0.01, paired t-test (vs. before). #p < 0.05, ##p < 0.01, Student's t-test (vs. placebo).

Comparisons of the two groups indicated that at 60, 180, and 240 mins after ingestion, levels in the test food group decreased significantly more than the placebo food group ($p<0.05$). Comparisons of Cmax up to 480 mins after ingestion indicated that the blood ethanol level was 321.3 ± 69.8 mg/l in the test food group and 344.2 ± 68.9 mg/l in the placebo food group. Comparisons of the blood acetaldehyde level indicated that it was 1.18 ± 0.34 mg/l in the test food group and 1.40 ± 0.46mg/l in the placebo food group. Both results of these groups indicate that levels were significantly lower in the test food group than in the placebo food group ($p<0.05$).

Changes in VAS

Table 5 shows the changes in the VAS questionnaire responses. Subjective assessments using the VAS questionnaire indicated that the mood and the unpleasant symptoms associated with alcohol ingestion, such as facial ingestion, were significantly improves in test food groups as compared to placebo food group.

Study 2

Efficacy analysis set: Of the original 32 participants, two were excluded from analysis, because of dropout. This left a total of 30 participants in the efficacy analysis. Background of participants were shown on Table 6.

Changes in Charder's Fatigue Scale

Charder's Fatigue Scale changes are shown in Table 7 and 8. In both groups, post-ingestion scores significantly decreased compared to

pre-ingestion scores. There was no significant difference between the two groups.

Group		Total	Male	Female
Test	Number	30	16	14
	Age	48.5 ± 0.9	49.8 ± 1.3	47.1 ± 1.3
Placebo	Number	30	17	13
	Age	48.9 ± 1.0	48.6 ± 1.4	49.2 ± 1.6

Table 6: Participant background on Study 2. Average ± SE.

Fluctuations in POMS results

POMS results are shown in Table 8 and 9. POMS results for the item "vigor and activity" were significantly higher in the test food group at 4 weeks, 8 weeks, and 12 weeks post-ingestion as compared to the placebo food group. No significant differences were observed between the groups for any other items.

Marker of liver function and urea nitrogen levels

γ-GTP, a marker of liver function and urea nitrogen levels are shown Table 10 and 11. Comparison of the two groups indicated that the levels in the test food group decreased significantly at 8 weeks and 12 weeks after ingestion as compared to the placebo food group ($p<0.05$).Urea nitrogen levels were also significantly decreased in the test group at 4weeks post-ingestion as compared to the placebo food group.

Discussion

We conducted two studies designed to investigate the effect of ingesting a food containing antroquinonol on the daily fatigue experienced by physical laborers and its effects after alcohol consumption. Analysis of the results indicated that the daily fatigue of physical laborers as measured by the Charder's Fatigue Scale showed no change but that the vigor and activity item on POMS was elevated.

	Group	n	Befire	1 week	4 week	8 week	12 week
CFS (Chalder's Fatigue Scale)	Test	30	23.6 ± 1.3	18.8 ± 1.2*	17.3 ± 1.1**	14.4 ± 1.2**	14.3 ± 1.2**
	Placebo	30	24.9 ± 1.4	17.8 ± 1.2**	16.0 ± 1.4**	15.6 ± 1.5**	14.9 ± 1.7**

Table 7: Chalder's Fatigue Scal. Average ± SE. *: $p<0.05$. **: $p<0.01$.

	Group	n	Befire	1 week	4 week	8 week	12 week
T-A	Test	30	55.1 ± 2.4	54.2 ± 2.5	52.2 ± 2.3	49.7 ± 2.1	50.3 ± 2.0
	Placebo	30	53.7 ± 2.8	51.2 ± 2.8	48.7 ± 2.3	49.5 ± 2.6	50.2 ± 2.7
D	Test	30	60.4 ± 2.8	61.4 ± 2.6	57.3 ± 2.5	53.7 ± 2.3	55.5 ± 2.6
	Placebo	30	57.5 ± 2.6	55.5 ± 2.6	54.6 ± 2.7	55.0 ± 2.7	53.1 ± 2.4
A-H	Test	30	57.1 ± 2.6	57.1 ± 2.6	53.2 ± 2.5	53.2 ± 2.3	51.6 ± 2.2
	Placebo	30	55.1 ± 2.7	51.2 ± 2.4	49.6 ± 2.2	51.4 ± 2.6	50.9 ± 2.4
V	Test	30	39.2 ± 1.6	42.9 ± 1.8	45.3 ± 2.1	45.1 ± 2.1	46.0 ± 2.2
	Placebo	30	39.9 ± 1.7	41.1 ± 1.8	41.3 ± 1.7	41.8 ± 2.0	42.4 ± 2.1
F	Test	30	66.2 ± 2.1	61.5 ± 2.0	57.4 ± 2.4*	56.2 ± 2.1**	55.8 ± 2.2**
	Placebo	30	67.7 ± 2.1	59.4 ± 2.4*	58.9 ± 2.3*	57.5 ± 2.6*	55.9 ± 2.2**
C	Test	30	56.4 ± 2.3	56.8 ± 2.5	54.2 ± 2.4	52.2 ± 2.1	52.6 ± 2.1
	Placebo	30	57.1 ± 2.8	56.6 ± 2.8	55.3 ± 2.8	55.4 ± 2.9	53.1 ± 2.6

Table 8: POMS (Profile of Mood States). Average ± SE. *:$p<0.05$. **:$p<0.01$.

	Group	n	Befire	1 week	4 week	8 week	12 week
T-A	Test	30	0.0 ± 0.0	-0.9 ± 1.9	-2.1 ± 1.8	-5.2 ± 1.5**	-4.8 ± 1.7*
	Placebo	30	0.0 ± 0.0	-2.5 ± 1.8	-4.5 ± 1.8*	-4.2 ± 1.7*	-3.5 ± 1.7*
D	Test	30	0.0 ± 0.0	1.0 ± 2.0	-2.6 ± 2.1	-6.0 ± 1.6**	-4.9 ± 2.0*
	Placebo	30	0.0 ± 0.0	-2.0 ± 1.9	-2.4 ± 2.2	-2.5 ± 2.3	-4.4 ± 1.9*
A-H	Test	30	0.0 ± 0.0	0.1 ± 1.9	-3.4 ± 2.2	-3.9 ± 1.8	-5.4 ± 2.1**
	Placebo	30	0.0 ± 0.0	-3.9 ± 1.6	-4.9 ± 1.6**	-3.7 ± 1.7	-4.2 ± 1.8
V	Test	30	0.0 ± 0.0	3.7 ± 1.1*	6.3 ± 1.5** #	5.9 ± 1.4** #	6.8 ± 1.5** #
	Placebo	30	0.0 ± 0.0	1.2 ± 1.1	1.5 ± 1.2	1.9 ± 1.5	2.6 ± 1.7
F	Test	30	0.0 ± 0.0	-4.7 ± 1.6*	-8.4 ± 2.3**	-10.2 ± 1.8**	-10.4 ± 2.0**
	Placebo	30	0.0 ± 0.0	-8.2 ± 1.6**	-8.6 ± 2.0**	-10.2 ± 1.9**	-11.7 ± 1.4**
C	Test	30	0.0 ± 0.0	0.4 ± 1.9	-1.7 ± 2.0	-4.0 ± 1.7**	-3.9 ± 1.7
	Placebo	30	0.0 ± 0.0	-0.5 ± 1.5	-1.5 ± 2.2	-1.7 ± 2.1	-4.0 ± 2.1

Table 9: POMS. Average ± SE. *: $p<0.05$. **:$p<0.01$.

Item	Unit	Group	n	Before	4 weeks	8 weeks	12 weeks
BUN, UN	mg/dL	Test	30	12.8 ± 0.7	12.6 ± 0.6	11.8 ± 0.6	13.1 ± 0.6
		Placebo	30	12.8 ± 0.5	14.0 ± 0.6*	12.4 ± 0.5	13.2 ± 0.6
CRE	mg/dL	Test	30	0.783 ± 0.032	0.749 ± 0.033*	0.779 ± 0.032	0.752 ± 0.032*
		Placebo	30	0.772 ± 0.024	0.757 ± 0.022	0.768 ± 0.021	0.742 ± 0.019*
UA	mg/dL	Test	30	5.09 ± 0.19	5.23 ± 0.21	5.01 ± 0.20	5.03 ± 0.22
		Placebo	30	5.00 ± 0.32	5.14 ± 0.30	5.18 ± 0.30	4.98 ± 0.28
AST, GOT	U/L	Test	30	19.4 ± 0.8	20.0 ± 0.8	17.3 ± 0.6**	18.6 ± 0.7
		Placebo	30	22.2 ± 1.6	20.9 ± 1.1	19.5 ± 1.2	20.9 ± 1.5
ALT, GPT	U/L	Test	30	18.7 ± 1.4	18.1 ± 1.1	16.4 ± 1.1*	17.1 ± 1.0
		Placebo	30	20.1 ± 1.8	20.6 ± 1.8	19.5 ± 1.6	21.3 ± 2.6
γ-GT, γ-GTP	U/L	Test	30	27.0 ± 3.4	27.4 ± 3.7	25.5 ± 3.1	27.0 ± 3.3
		Placebo	30	23.8 ± 2.5	27.2 ± 3.3	27.9 ± 3.6**	28.8 ± 3.7**
ALP	U/L	Test	30	194.8 ± 13.2	203.3 ± 14.6	190.7 ± 12.0	197.1 ± 11.9
		Placebo	30	212.3 ± 11.1	214.3 ± 9.2	213.2 ± 10.4	217.0 ± 10.4
LD	U/L	Test	30	168.2 ± 3.8	169.4 ± 4.4	166.2 ± 4.0	168.7 ± 4.7
		Placebo	30	176.5 ± 11.4	168.9 ± 4.9	166.3 ± 4.9	170.0 ± 4.2

Table 10: Markers of liver function and urea nitrogen levels. Average ± SE. Dunnett's test (VS before). *:p<0.05. **:p<0.01. Student's t-test #:p<0.05.

Item	Unit	Group	n	Before	4 weeks	8 weeks	12 weeks
BUN, UN	mg/dL	Test	30	0.0 ± 0.0	-0.2 ± 0.6] #	-1.2 ± 0.5	0.2 ± 0.5
		Placebo	30	0.0 ± 0.0	1.4 ± 0.5*] #	-0.4 ± 0.5	0.4 ± 0.5
CRE	mg/dL	Test	30	0.000 ± 0.000	-0.034 ± 0.013*	-0.010 ± 0.013	-0.031 ± 0.012*
		Placebo	30	0.000 ± 0.000	-0.019 ± 0.015	-0.004 ± 0.013	-0.030 ± 0.012*
UA	mg/dL	Test	30	0.00 ± 0.00	0.11 ± 0.12	-0.08 ± 0.10	-0.06 ± 0.10
		Placebo	30	0.00 ± 0.00	0.13 ± 0.12	0.18 ± 0.13	-0.02 ± 0.11
AST, GOT	U/L	Test	30	0.0 ± 0.0	0.4 ± 0.5	-2.1 ± 0.5**	-0.8 ± 0.7
		Placebo	30	0.0 ± 0.0	-1.9 ± 1.5	-2.7 ± 1.3	-1.3 ± 1.5
ALT, GPT	U/L	Test	30	0.0 ± 0.0	-1.0 ± 1.0	-2.6 ± 0.9*	-1.6 ± 1.1
		Placebo	30	0.0 ± 0.0	-0.1 ± 2.0	-0.7 ± 1.4	1.2 ± 2.4
γ-GT, γ-GTP	U/L	Test	30	0.0 ± 0.0	-0.2 ± 1.0	-2.1 ± 1.0] ##	-0.1 ± 1.4] #
		Placebo	30	0.0 ± 0.0	2.6 ± 1.5	4.2 ± 1.4**] ##	5.0 ± 1.8**] #
ALP	U/L	Test	30	0.0 ± 0.0	4.7 ± 5.4	-0.1 ± 4.9	2.3 ± 7.3
		Placebo	30	0.0 ± 0.0	-2.2 ± 5.4	0.9 ± 4.8	4.7 ± 5.2
LD	U/L	Test	30	0.0 ± 0.0	0.6 ± 2.9	-2.2 ± 2.6	0.4 ± 3.6
		Placebo	30	0.0 ± 0.0	-10.2 ± 9.7	-10.2 ± 10.0	-6.5 ± 10.2

Table 11: Changes markers of liver function and urea nitrogen levels. Average ± SE. Dunnett's test (VS before). *: p<0.05. **:p<0.01. Student's t-test. #:p<0.05.

The fact that both γ-GTP, which is a marker for liver function, and urea nitrogen, which is a marker for kidney function, decreased indicates that metabolism was affected, for example, by removal of ROS and by promotion of excretion of waste products, which in turn was the likely cause of increased vigor and activity reported by the participants. Analysis of the alleviation of unpleasant symptoms associated with alcohol consumption indicated that blood ethanol and acetaldehyde levels showed significant decreases in the test food group as compared to the placebo group, which indicates that the test food promoted the metabolism of alcohol.

As mentioned above, alcohol is transported to the bloodstream after ingestion, then metabolized in the liver by an enzyme known as alcohol dehydrogenase (ADH). ADH is an enzyme that plays an important catalyzing role in the conversion of the acetaldehyde contained in alcohol, which is converted into acetic acid by aldehyde dehydrogenase (ALDH). During the course of this process of alcohol breakdown in the liver, the by-product ROS is formed in large quantities. Antroquinonol is one of the main antioxidative chemical compounds found in the fungus *A. camphorate* [8].

Previous research has indicated that antroquinonol achieves the desired antioxidative activity through the Nrf2 pathway. The present clinical study indicated that the test food group had higher antioxidative activity after alcohol consumption than the placebo food group. Since liver cells play a role in health, this likely indicates that the metabolism of alcohol by liver cells could

be achieved more effectively. The daily fatigue that results from physical labor is one cause of increases in ROS levels. However, the results of the present clinical study indicated that γ-GTP, which is a marker for liver function, decreased significantly in the test food group as compared to the placebo food group. Urea nitrogen, which is a marker for kidney function, also decreased significantly in the test food group as compared to the placebo food group. These results indicate that antroquinonol protects the liver and kidneys by suppressing ROS production. In addition to changes in blood markers, subjective assessments by physical laborers indicated significant improvements in vigor and activity and suppression of unpleasant symptoms associated with alcohol consumption such as elevated mood and facial reddening. These results were likely caused by antroquinonol's antioxidative activity and protective effect on liver cells.

Conclusion

We conducted clinical studies of healthy men and women in order to determine the effect of a food containing antroquinonol has on the alleviation of the daily fatigue experienced by physical laborers and its ability to protect the liver after alcohol consumption. The results of anti-fatigue clinical study indicated that both the liver and kidney function of physical laborers were improved after antroquinonol-contained *A. camphorate* consumption. The results of anti-alcohol clinical study also revealed that blood alcohol and blood acetaldehyde levels were decreased significantly in the test food group as compared to the placebo food group. These results indicate that the test substance increases the daily vigor and activity of physical laborers, improves the unpleasant symptoms associated with alcohol consumption, and maintain the liver healthy and functioning.

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