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Hangover relieving and antioxidant effects of *Gynostemma pentaphyllum* (Thunb.) Makino and/or *Hovenia dulcis Thunb.* extracts

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ABSTRACT

The present study attempts to study alcohol metabolizing and antioxidant properties of *Gynostemma pentaphyllum* (Thunb.) Makino distillate (GPD) and combination effects with *Hovenia dulcis Thunb*. extract (HDE) on these activities. The alcohol-metabolizing activity of GPD with/without HDE was determined by assessing alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities. To define the effect of GPD with/without HDE on alcohol metabolism, antioxidant activities and total phenolic content of GPD with/without HD extract were evaluated using 2-diphenyl-1-picrylhydrazyl free radical scavenging, ferrous chelating assays, and the Folin–Ciocalteu method. Cytotoxicity against human normal liver CHANG cells was also evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. GPD treatment alone or in combination with HDE significantly increased ADH and ALDH activities; combined treatment was most effective. Total phenolic and flavonoid contents were greater in combination than the level found in GPD alone. GPD revealed a synergistic antioxidant effect when combined with HDE. GPD and/or HDE had no antiproliferative activity against the normal liver cell line. These results suggest that GPD-HDE combination is the possible natural resource for the management of alcohol-induced liver injury.

INTRODUCTION

Alcohol use is widespread throughout the world. Many previous works have focused on addressing alcoholism and relieving hangover because heavy alcohol drinking is associated with many social problems (Bourogaa *et al*., 2013). In the liver, alcohol dehydrogenase (ADH) metabolizes alcohol to acetaldehyde and then converts to acetate and water by aldehyde dehydrogenase (ALDH) (Bourogaa *et al*., 2013). Acetaldehyde, the metabolic intermediate of alcohol oxidation, is further oxidized into acetic acid and water by ALDH. Acetaldehyde could cause toxic effects including lightheadedness, a rapid pulse, sweating, nausea, vomiting, and functional modulation of proteins (Bourogaa *et al*., 2013; Lee and Park, 1999). Alcohol toxicity is mainly caused by reactive oxygen species and free radicals resulting from ethanol metabolism in the liver (Lieber, 1997). Conventional or synthetic drugs for enhancing hepatic antioxidant abilities and the treatment of liver disease have been used widely (Okaiyeto *et al*., 2018). However, it needs to find beneficial natural hepatoprotective drugs for alcohol consumers to avoid alcoholism and alcohol-induced diseases because of side-effects of synthetic medicines.

Gynostemma pentaphyllum (Thunb.) Makino, a perennial herb distributed mostly in Korea, Japan, China, and Southeast Asia, has been reported to have a wide range of health benefits, including immunomodulatory, antitumor, nephroprotective, hepatoprotective, antimicrobial, and anti-inflammatory activities (Long, 2010; Wang *et al*., 2002). Furthermore, both basic and clinical studies have suggested that *G. pentaphyllum* (Thunb.) Makino may have modest benefits in the treatment of fatty liver disease (Hong *et al*., 2015). However, its effect on alcohol oxidation has yet to be reported. The present study was therefore designed to investigate the effect of *G. pentaphyllum* (Thunb.) Makino distillate (GPD) on hangover relief, which would be

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linked to the elimination of alcohol from the body through sweat, breath, and urine.

In traditional medicine system, mixtures of plants are used rather than one species because the mixture of the two or more species gives a better activity than either species on its own. *Hovenia dulcis Thunb* is a well-known natural hepatoprotective agent which has been used for the treatment of alcohol-related liver diseases and alcoholism (Hyun *et al*., 2010; Liang and Olsen, 2014). Even the antioxidant and alcohol relieving activity of *Hovenia dulcis Thunb.* has been extensively reported (Hyun *et al*., 2010; Liang and Olsen, 2014), there was only the action of the individual compound. In this study, the enhancing effects of *Hovenia dulcis Thunb.* extract (HDE) with GPD on antioxidant effect as well as ADH and ALDH activities were explored.

MATERIALS AND METHODS

Plant sample preparation

Gynostemma pentaphyllum (Thunb.) Makino used in this study was harvested in Jeju-do Province, South Korea in 2016. GPD sample was provided by Youngmul company (Jeju, Korea). Briefly, samples were washed, rinsed carefully, and then dried at room temperature for 1 week. Dried *G. pentaphyllum* (Thunb.) Makino (2.8 kg) were soaked in water (18 l) and then distilled at 116°C for 190 minutes using a high-speed, low-temperature vacuum extractor (DM-3000, Daehan median co., LTD, Seoul, Korea). The primary distillate was filtered to remove particles and stored at 4°C for later use. HDE sample was prepared using a previously described protocol (Kim, 2017).

ADH and ALDH activities

Enzyme-linked immunosorbent assay kits were used to measure EtOH metabolites: ethanol and acetaldehyde. ADH and ALDH activities were measured by using an ethanol and acetaldehyde quantification assay kit (Megazyme, Wicklow, Ireland) following the manufacturer's instructions. The enzyme activity of ADH and ALDH in the control was set to 100% and the treated groups were measured in comparison to them.

Antioxidant activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was determined as described in our recently published paper (Kim, 2017). All tests were performed in triplicate. The ferrous ion chelating ability was also determined as described earlier (Kim, 2017). Briefly, 250 µl of the GPD and/or HDE was mixed with 5 µl of 2 mM ferrous chloride ($FeCl_2$). The reaction was initiated by the addition of 10 µl of 5 mM ferrozine and then incubated at 25ºC for 10 minutes. The absorbance of the reaction mixtures was measured at 562 nm against blank samples.

Total phenolic and flavonoid contents

Total soluble phenolics were spectrophotometrically determined with Folin–Ciocalteu reagent (Sigma-Aldrich, MO) using gallic acid as the standard as reported previously (Kim, 2017). Total phenolic content was calculated as gallic acid equivalents (GAE) per liter of sample on the basis by comparison with a standard curve of gallic acid.

Total flavonoid content was determined by the spectrophotometric method of rutin based on procedures described previously (Kim, 2017). Total flavonoid content was calculated from a calibration curve of ruin and the results were expressed as the mg of rutin equivalents (RE) per liter of sample.

In vitro cytotoxic activity

CHANG cells were maintained in Dulbecco's modified Eagle's medium (Lonza, Walkersville, MD) supplemented with L-glutamine, antibiotics, and FBS. The *in vitro* cytotoxic activity was determined from the mitochondrial activity of cells which represent the number of viable cells after the treatment, by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Boehringer Mannheim, Indianapolis, IN, USA) cytotoxic assay on human CHANG normal liver cells in tissue culture.

Statistical analysis

Comparisons of all results between GPD-HDE combination and GPD alone were made by using a nonparametric test (Mann–Whitney U test) with $p < 0.05$ (SPSS, ver. 12.0; SPSS Inc., Chicago, IL). Three replicate measurements were performed.

RESULTS AND DISCUSSION

Alcohol metabolism is usually considered to be one of the major causes of alcohol-induced liver injury (Zakhari and Li, 2007). The liver is the main site of alcohol metabolism in the body. More than 85%–90% of ingested alcohol is metabolized in the liver (Zakhari and Li, 2007). Alcohol is metabolized to acetaldehyde by ADH and, subsequently, to acetic acid by ALDH. Therefore, the toxicity of alcohol is associated with the activities of ADH and ALDH (Yoo *et al*., 2011). Herbal hangover remedy has been commonly used for centuries. Numerous studies show that natural products have beneficial effects on alcohol metabolism in animal and human studies (Wang *et al*., 2016). It increased the activity of ADH and ALDH in the serum and liver along with the decreased risk of the liver (Wang *et al*., 2016). In this study, the ADH or ALDH activation ability was measured in GPD alone or the mixture of GPD and HDE *in vitro*. ADH activity was slightly increased up to 106% by GPD alone with no observed significance, whereas the mixture of GPD with HDE revealed the extremely higher ADH activity (157%) than that of GPD alone (Table 1). However, ALDH activities were similarly increased in both individual GPD (320%) and combination with HDE (307%) (Table 1). Combination of GPD with HDE did not change the ALDH activity suggesting that GPD had no interaction with HDE.

Many medicinal herbs have great antioxidant potential and exhibit a wide range of biological effects such as free radical scavenging (Li *et al*., 2013). Previous studies described that

Table 1. ADH and ALDH activity of GPD with/without HDE.

	ADH activity (%)	ALDH activity (%)
GPD	105.9 ± 6.93	319.7 ± 13.01
GPD+HDE	157.3 ± 2.91*	306.9 ± 22.95

GPD = *G. pentaphyllum* (Thunb.) Makino distillate; HDE = *Hovenia dulcis Thunb.* extract; GAE = Gallic acid equivalent; RE =Rutin equivalent. All values are expressed as mean ± S.D ($n = 3$). *Data were statistically different from the value of GPD alone ($p < 0.05$).

Table 2. Antioxidant activity and total phenolic and flavonoid contents of GPD with/without HDE.

	DPPH free radical scavenging activity (%)	Ferrous ion chelating activity (%)	Total phenolic contents (mg GAE/l)	Total flavonoid contents (mg RE/l)
GPD	5.5 ± 1.74	3.1 ± 0.81	16.3 ± 0.46	2.7 ± 0.28
GPD + HDE	101.4 ± 3.45*	89.1 ± 1.81*	1,721.4 ± 15.20*	1,070.6 ± 22.94*

GPD = *G. pentaphyllum* (Thunb.) Makino distillate; HDE = *Hovenia dulcis Thunb.* extract; GAE = Gallic acid equivalent; RE =Rutin equivalent. All values are expressed as mean ± S.D (n = 3). *Data were statistically different from the value of GPD alone ($p < 0.05$).

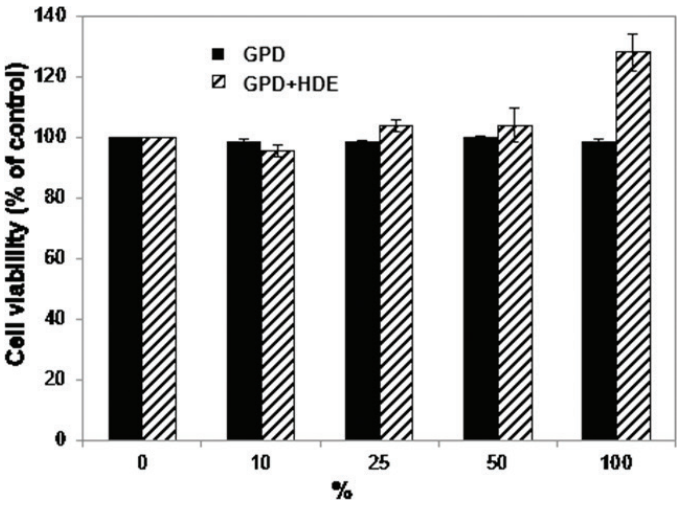

Figure 1. Cytotoxicity of GPD with/without HDE in CHANG normal liver cell lines. Values are the means ± S.D (n = 3).

oxidative stress involved in alcohol metabolism causes alcohol-related hangovers and suggests that antioxidants could alleviate hangover symptoms (Marino *et al*., 2004). Several natural herbs have a beneficial effect on alcohol-induced hangovers in animal and human studies (Huang *et al*., 2005; Simic, 1988). Due to their excellent antioxidant activity, considering the complexity of the composition of herbs, their combination is needed for prevention and treatment of alcohol-induced hangover symptoms (Huang *et al*., 2005). Herein, both DPPH scavenging test and the ferrous ion chelating ability were determined to evaluate the antioxidant property of the individual GPD or GPD-HDE combination. In the present study, a binary combination of GPD and HDE has demonstrated considerable strong antioxidant effects (89 and 101%) (Table 2) indicating that concurrent usage of these plants has greatly increased the antioxidant activity, which may attenuating withdrawal syndromes of alcohol use disorder.

As is well known, flavonoids and other polyphenols lead to powerful antioxidant effects (Stevenson and Hurs, 2007). Therefore, we measured total phenolic and flavonoid contents in GPD alone or GPD-HDE combination (Table 2). Result clearly shows that the mixture of GPD and HDE (1,721 mg GAE/l and 1,071 mg RE/l, respectively) significantly increased ($p < 0.05$) total phenolic and flavonoid contents compared to single GPD (Table 2). We also found that the phenolic content had a positive correlation with the antioxidant capacity to scavenge the DPPH radical and chelate ferrous ion (Table 2), which is similar to previous research (Stevenson and Hurs, 2007).

The toxicological potential of a compound is analyzed through the affectation of vital functions of normal cell lines. It is important that such selection could be done at the very beginning of the developmental process, at the stage of *in-vitro* studies (Yahima *et al*., 2014). Thus, the cytotoxic effect of GPD alone or GPD-HDE combination on normal cells (human CHANG normal liver cells) was tested. We observed no antiproliferative effects of GPD alone or GPD-HDE combination on CHANG cells (Fig. 1).

The results presented in this work indicated that the GPD-HDE combination enhanced alcohol metabolizing enzyme activities and exerting antioxidant effects. Thus, GPD-HDE combination could be a new candidate therapeutics for alcohol-related liver disease and alcohol use disorders.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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