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Original Research Article

Protective effect of purple sweet potato leaf (*Ipomoea batatas* Linn Convolvulaceae) against alcohol-induced liver damage in mice

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Abstract

Purpose: *To investigate the hepatoprotective effect of Ipomoea batatas extract against alcohol-induced liver damage in mice.*
Methods: *Male C57BL/6 mice were randomly divided into 4 experimental groups (n = 10). Normal Group: The animals received distilled water 5 ml/kg for 7 days; Alcohol Group: The animals received alcohol 5 ml/kg of 40 % w/v alcohol for 7 days; Alcohol + Purple sweet potato leaf extract (PSPE) Group: PSPE 400 mg/kg was for 7 days. The animals received alcohol 5 ml/kg of 40 % w/v alcohol for 7 days; Alcohol + Hovenia dulcis Thunb extract (HDE) Group: HDE 400 mg/kg was for 7 days. To confirmed to the liver protection effect of PSPE, it was calculated, and the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), and total cholesterol (TC) in serum were detected. To evaluate changes of histological in alcohol-fed mice, liver tissue was determined by H&E staining.*
Results: *Blood alcohol concentration in purple sweet potato leaf extract (PSPE) 200 mg/kg and Hovenia dulcis (H. dulcis) extract (HDE) 200 mg/kg treated group significantly decreased compared to - alcohol with water treated group ($p < 0.05$). Serum ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were markedly reduced. Liver sections in mice stained with H&E (hematoxylin and eosin) stain to displayed the physiological changes in the liver tissue. Furthermore, the results showed that inflammatory cells increased in the alcohol group compared to the normal group, but spontaneously decreased in the PSPE or HDE-treated group.*
Conclusion: *These results demonstrate that Ipomoea batatas may be therapeutically effective in protecting the liver from alcohol-induced hepatotoxicity and fatty liver.*

Keywords: *Ipomoea batatas, alcohol, Hepatoprotective, AST (aspartate aminotransferase) ALT (alanine aminotransferase)*

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INTRODUCTION

Alcoholic liver disease (ALD) is a leading cause of liver disease and associated with significant disease and mortality worldwide, including Korea [1]. Alcohol consumption is also a major cause of liver disease because it could cause serious liver injuries and lead to liver fibrosis and cirrhosis [2]. Despite the insightful economic and influence impact of ALD, the underlying mechanisms of

alcohol-induced liverdamage are ambiguous and targeted treatments are inaccessible. Therefore, there is a need to develop novel, safe and pathophysiological treatments for liver damage caused by alcohol.

Alcohol is mainly metabolized in the liver through three enzymatic paths: alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH) and cytochrome p450 2E1 (CYP2E1). Excessive alcohol consumption leads to an increase in highly toxic acetaldehyde, which can damage membrane lipids and alter enzyme activity [3]. Acetaldehyde, the first metabolite of ethanol, is formed by alcohol oxidation through the action of ADH. Since the liver serves as a major site for ethanol oxidation, there is ample evidence that acetaldehyde acts as a major cause of liver damage after chronic alcohol consumption [4].

Natural plant products are attracting a lot of attention as potential therapeutics in the prevention and treatment of ALD due to their low toxic side effects [5]. Purple sweet potato scientifically known as *Ipomoea batatas* Linn. from the family Convolvulaceae is a herbaceous perennial vine with white and purple flowers, large nutritious storage roots and heart-shaped, lobed leaves [6]. *I. batatas* mainly known as sweet potato has played an essential role as an energy source in human diet. The plant has significant medicinal importance and many parts of the plant are used in traditional medicine [7]. From the leaf of sweet potato, the caffeoylquinic acid derivatives such as 3-mono-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, 3,4,5-tri-O-caffeoylquinic acid, and Caffeic acid have been isolated [8]. These compounds were known to prevent proliferation of human cancer cells cause to colon cancer, stomach cancer, and promyelocytic leukemia cell [9].

However, few studies have been conducted on the hepatoprotective effect of purple sweet potato leaf. The present study was performed to investigate the possible effect of purple sweet potato leaf on alcohol induced-liver damage in mice.

EXPERIMENTAL

Preparation of PSPE and HDE extracts

In this study, purple sweet - potato leaf was collected at the end August to about the middle of September at Goesan-gun (Chungcheongbuk-do, Korea). The raw materials were dried, ground to a fine powder (~ 20 mesh) using a milling machine, and then extracted with 70% ethanol three times by stirring for 24 h at RT. After filtration the solvent was removed using a rotary vacuum evaporator, and the freeze-dried extract stored deep freezer (-80℃) until used for further analysis. *Hovenia dulcis* Thunb extract (HD) were obtained from Natural F&P Co. (Chungcheongbuk-do, Korea).

Blood alcohol analysis

Twenty male Sprague Dawley rats (age 6 weeks old) were obtained from DBL Inc. (Eumseong-gun, Korea). All the experimental animals were divided in a group (n = 5) and fed water and feed, under 12 h light and dark conditions. After a week, each of 5 animals were divided into alcohol group, alcohol + PSPE 100 mg/kg, 200 mg/kg, 300 mg/kg group, and alcohol + HDE 200 mg/kg group. Rats were fasted for 18 h before the experiment, and water was supplied without restriction. The PSPE and HDE groups were orally administered PSPE and HDE 30 min before alcohol administration, and the alcohol group was orally administered distilled water (DW) instead of the sample. For alcohol administration, 30% alcohol was orally administered once at a level of 3 mL/kg. To measure the alcohol concentration, blood was collected through the tail vein at 0 min, 30 min, and 2 h after alcohol administration. The collected blood was centrifuged at 4℃, 3,000 rpm for 15 min to obtain serum, and immediately placed in a -80℃ deep freezer. In order to measure blood alcohol concentration, it was measured using an ethanol assay kit (BioVision Inc, Milpitas, CA, USA).

Animals and experimental design

Five-week-old male C57BL/6 mice were obtained from Daehan Bio Link Co. (Eumseong, Korea), and were maintained in an animal house at 22 ± 2℃ with a 12 h light/dark cycle. After a 1-week adaption period, the mice were divided in to 4 experimental groups (n = 10). The experimental group were divided as follows: Normal Group: The animals received DW 5 ml/kg for 7 days; Alcohol Group: The animals received alcohol 5 ml/kg of 40% w/v alcohol for 7 days; Alcohol + Purple sweet potato leaf extract (PSPE) Group: PSPE 400 mg/kg was for 7 days; Alcohol + *Hovenia dulcis* Thunb extract (HD) Group: HDE 400 mg/kg. PSPE and HD were administered 30 min before alcohol administration.

Assessment of body weight change and feed and water intake

Diets of the mice were fed a pellet diet (Rodent diet 2918C; Harlan Teklad, Indianapolis, IN,

USA) with the water. During the 7 days experimental period, all groups measured body weight, diet and water intake before drug administration every day.

Analysis of AST, ALT, TC, and TG profiles in serum

At the end of treatment (7 days for alcohol treatment), the mice were sacrificed and the blood collected. The serum was obtained by centrifugation (3,000 rpm for 15 min at 4 ℃) using a serum separator tube at -70 ℃ to estimate serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), and total cholesterol (TC). The concentration of serum ALT, AST, TC, and TG were measured using a Konelab20XT automatic blood analyzer.

Histopathological analysis

Liver tissue was cut into small pieces at the time of sacrifice for histopathological analysis. The tissue blocks were then fixed in a 10 % formaldehyde solution neutralized to pH 7.4 for 24 h, embedded into a paraffin block, successively cut into 4 µm-thick slices, placed on normal glass, deparaffinized in xylene twice for 5 min, twice for 5 min, and rehydrated with a series of graded alcohol. Each tissue slide was stained with Mayer's hematoxylin and eosin (H&E) and observed under a microscope at magnification of 200.

Determination of ADH activity

Liver samples were grinded in cold PBS for homogenization and centrifuged at 3000 rpm for 10 min to get supernatant. The levels of ADH were detected by ADH activity assay kit (BioVision Inc, Milpitas, CA, USA) based on the manufacturer's instructions. All results were standardized to total protein measured handling BCA protein assay Kit (ThermoFisher, MA, USA) based on the manufacturer's instructions.

Chlorogenic acid contents analysis

The method of analyzing the chlorogenic acid of PSPE was carried out by applying the method of European Pharmacopoeia 6.6. For pretreatment of the sample, 50 ml of methanol was added to 0.5 g of PSPE and extracted with a reflux extractor at 70℃ for 1 h, and the supernatant obtained through centrifugation was placed in a 200 ml quantitative flask and then filled up with distilled water (DW). As a standard solvent, 5.0 mg of chlorogenic acid was dissolved in 50 ml methanol, and 5 ml of this solution was placed in a 20 ml quantitative flask, 5 ml of methanol was added, and then filled up with DW. The HPLC analysis method was performed as shown in Table 1.

Table 1. HPLC analysis condition of chlorogenic acid

Item	Condition		
Column	- Size: l = 0.250 m, φ = 4.6 mm - Stationary phase: octadecylsilyl silica gel for chromatography R (5 µm)		
Oven temperature	40℃		
Mobile phase	- mobile phase A: phosphoric acid, water (0.5 : 99.5, v/v) - mobile phase B: phosphoric acid, acetonitrile (0.5 : 99.5 v/v)		
	Time (min)	Mobile phase A (percent v/v)	Mobile phase B (percent v/v)
	0-1	92	8
	1-20	92→75	8→25
	20-33	75	25
	33-35	75→0	25→100
Flow rate	1.2 ml/min		
Detection	spectrophotometer at 330 nm		
Injection volume	25 µl		

Statistical analysis

All data are presented as mean ± SEM. Statistical significance ($p < 0.05$ for all analyses) was assessed by ANOVA with Instat 3.05 (GraphPad, San Diego, CA), followed by Student–Newman–Keuls analysis

RESULTS

Alcohol in blood concentration

Alcohol concentration after oral administration of alcohol gotten a peak level of 11.06 nmole/mM at 30 min and declined gradually. The alcohol group only showed higher alcohol concentration at 30 min when compared to sample treated group. At 120 min, however, alcohol concentration in purple sweet potato leaf extract (PSPE) 300 mg/kg and *Hovenia dulcis* (*H. dulcis*) extract (HDE) 200 mg/kg treated group significantly decreased when compared to - alcohol only treated group (Table 1). This result showed that the elimination of alcohol in -rat treated with PSPE 300 mg/kg and HDE 300 mg/kg was faster than - alcohol only treated group.

Table 2. Blood alcohol concentration in rat serum after alcohol administration

Group	Blood alcohol concentration (nmol/mM)		
	0 min	*30 min*	*120 min*
Alcohol	2.19±0.03	11.06±0.13	2.26±0.52
Alcohol + PSPE 100 mg/kg	1.98±0.03	7.26±0.33*	6.90±0.46
Alcohol + PSPE 200 mg/kg	2.00±0.17	6.96±0.73*	5.32±0.71
Alcohol + PSPE 300 mg/kg	1.99±0.17	5.38±1.62**	2.86±0.46*
Alcohol + HDE 200 mg/kg	2.23±0.48	4.6±0.55**	2.84±0.76*

Data are presented as mean ± SEM (n = 3) for three independent experiments. *P < 0.05 and **p < 0.01, compared with alcohol group

Effect of PSPE on body weight, feed intake, and water intake

Body weight, feed intake, and water intake were compared in Figure -1. The body weight gains during 7 days of experimental periods were decreased significantly (p < 0.05) in alcohol group when compared with intact normal group. But increases of body weights were observed in 7th treatment day of test material in PSPE and HDE treated mice when compared with - alcohol group (Figure 1 A). Daily feed intake and water intake was decreased significantly (p < 0.05) in alcohol group when compared with intact - normal group. An enhanced feed intake and water intake were observed in test material in PSPE and HDE treated mice when compared with alcohol group, respectively (Figure 1 B and C).

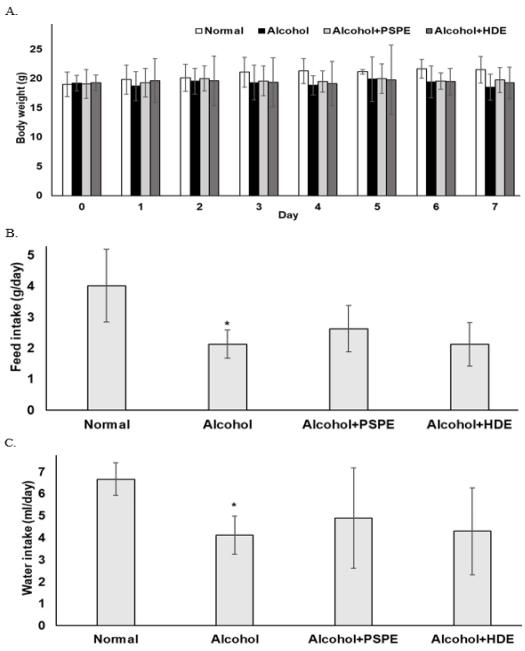

Figure 1: Effect of PSPE on body weight changes (A), feed intake (B), and water intake (C) in alcohol-fed mice. Data are showed as mean ± SEM for three independent experiments; *p < 0.05, compared with normal group by Student t-test

Effects of PSPE on serum ALT and AST activity

Alanine aminotransferase (ALT) activity in the alcohol treated group was significantly increased by alcohol administration compared to the untreated group However, PSPE and HDE diet group exhibited the significantly decreased the ALT activities (Figure 2A). The AST activity was alike to the ALT activity. However, there were no significant differences between the experimental groups (Figure 2B).

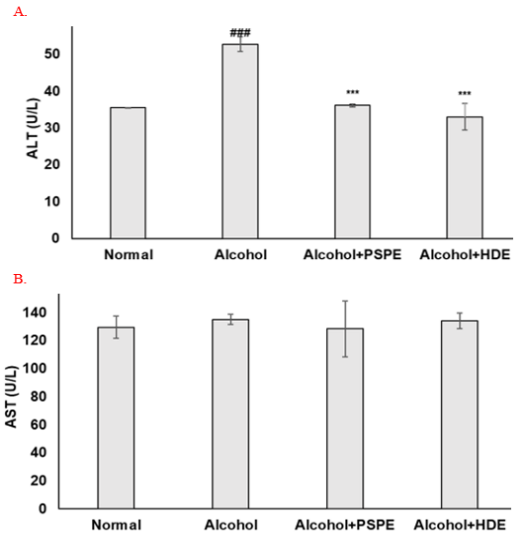

Figure 2: Effects of PSPE on serum ALT (A) and AST (B) activities in alcohol-fed mice. Data are showed as mean ± SEM for three independent experiments; ###p < 0.001, when compared to normal group; ***p < 0.001 when compared to alcohol group by Student t-test

Effect of PSPE on serum lipid profile

Figure 3 shows changes in serum total cholesterol (TC) and triglycerides (TG) in the different experimental groups. The alcohol treated group significantly increased in the levels of serum TC and TG (###P < 0.001) in compared to normal group. Whereas, PSPE and HDE groups, serum TG and TC levels decreased (** P<0.01) significantly compared to alcohol group.

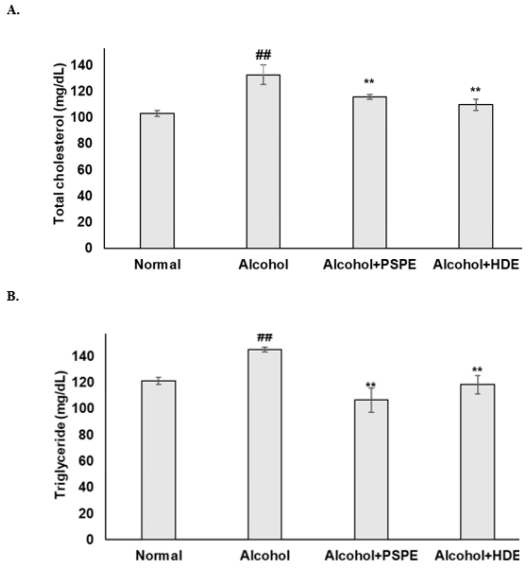

Figure 3: Effect of PSPE on serum total cholesterol (A) and triglyceride (B) in alcohol-fed mice. Data are showed as mean ± SEM for three independent experiments; ## $p < 0.01$; compared to control group. ** $p < 0.01$; compared to ethanol group

Effect of PSPE on histological changes in the liver

To determine whether liver protection effect of PSPE in alcohol-fed mice, liver sections were stained with hematoxylin and eosin. Our results showed that inflammatory cells were increased in the alcohol group compared to the normal group, but spontaneously decreased in the PSPE or HDE-treated group.

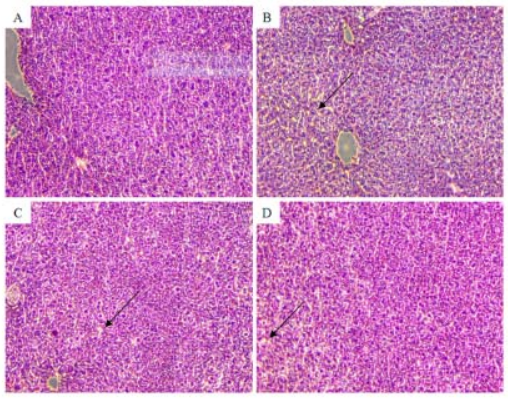

Figure 4: Effect of PSPE on morphology H & E in alcohol-fed mice (200X). (A) Normal, (B) Alcohol, (C) Alcohol + PSPE, (D) Alcohol + HDE

Effect of PSPE on hepatic alcohol metabolizing enzyme activities

Figure 5 shows the results of measuring alcohol dehydrogenase (ADH) enzyme activity in liver tissue to investigate the effect of PSPE on alcohol metabolism enzymes activity. The ADH activity in the normal group was 2.90±0.91 U/g protein, and the alcohol group showed statistically low activity as 1.34±0.39 U/g protein. The PSPE and HDE group showed statistically higher ADH activity than the alcohol group, with 1.88±0.23 U/g protein and 2.12±0.20 U/g protein, respectively.

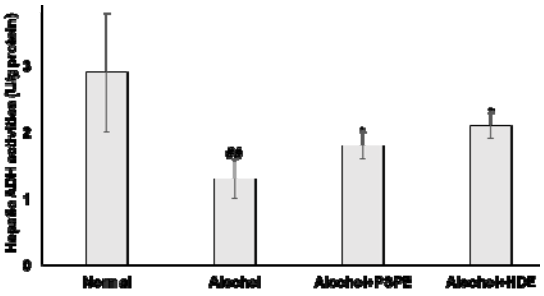

Figure 5: Effects of PSPE on hepatic alcohol metabolizing enzyme activites in alcohol-fed mice Data are presented as mean ± SEM (n = 3) for three independent experiments; $^{\#\#}p < 0.01$; when compared to normal group; $^{*}p < 0.05$, compared to alcohol group

Contents of chlorogenic acid in PSPE

The analytical High-Performance Liquid Chromatography (HPLC) chromatograms of PSPE containing chlorogenic acid were presented in figure 6. The calibration curves for the chlorogenic acid standards have been established. Content of chlorogenic acid in the PSPE was 5.16±0.05 μg/mg.

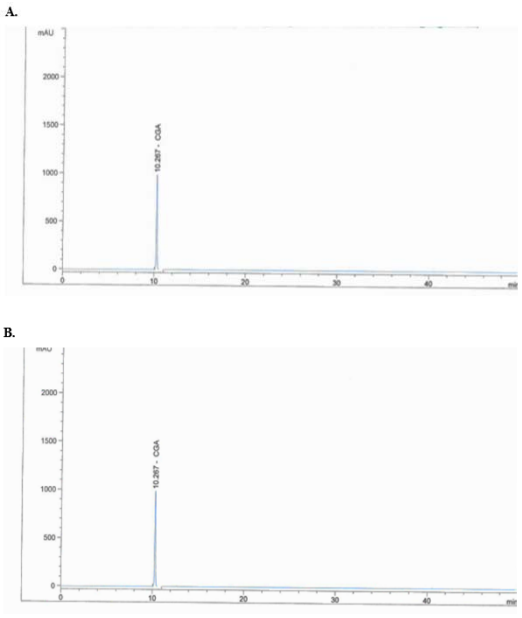

Figure 6: HPLC chromatograms of chlorogenic acid standard (A), PSPE (B)

DISCUSSION

Ipomoea batatas Linn. from the family Convolvulaceae is a herbaceous perennial vine that has white and purple flowers, large nutritious storage roots and heart-shaped, lobed leaves [10]. It is consumed as vegetable in tropical areas, especially Southeast Asia, used as a folk medicine in Brazil and commonly eaten as root crop in Japan, Korea and other Asian countries [10,11]. Nutritionists at the Center for Science in the Public Interest (CSPI) reported that *I. batatas* is the single most important dietary crops that would replace fatty foods [12].

Traditionally, the leaves and roots of *I. batatas* have been used in treating urinary infections, reducing fever, skin diseases, diabetes, curing boils and acnes [13]. A review of pharmacological studies on *I. batatas* indicated that it possesses anti-diabetic, hypoglycemic, neuroprotective, antiproliferative, antioxidant, antiulcer, antitumor, anti-inflammatory, wound healing, antimutagenic and hepatoprotective properties [14]. Previous study reported that IBE extract significantly inhibited production of NO, suppressed the expression of iNOS and COX-2 protein levels and attenuated increased TNF-α production in LPS-simulated BV-2 microglial cells. The IBE extract also exhibited significant antioxidant activity as evaluated by DPPH free radical scavenging assay [15].

Reports also reveal that *I. batatas* possess strong antioxidant compounds [16,17]. In the present study, the IBE extract also exhibited significant free radical-scavenging effect indicating that IBE extract might contain potential antioxidant agents. Previous studies on 3T3-L1 adipocytes *in vitro* revealed that *I. batatas* extract suppressed the inflammatory response [18]. The antioxidant glycosides and anthocyanin present in *I. batatas* have also been reported to exhibit anti-inflammatory effects [19,20].

In the PSPE group, the serum level of AST and ALT were significantly attenuated compared to the HDE treatment group, suggesting that *I. batatas* has potential for use as HDE intervention. Choi *et al* [10] reported that *H. dulcis* decreased about 20 % of blood alcohol concentration at 3 h. In this study, after 30 min, 300 mg/kg of PSPE group decreased the blood alcohol concentration as much as HDE 300 mg/kg group.

Serum ALT and AST activities are reliable markers of liver function [21]. It is established that AST is present in liver, lung, kidney, skeletal muscle, pancreas, brain, cardiac muscle, erythrocytes, and leukocytes, whereas ALT is present in liver [22]. The increased levels of serum enzymes such as AST and ALT indicate increased permeability and injury and/or necrosis of hepatocytes [23]. In the current study, the levels of serum ALT and AST, as biomarkers of liver function, were significantly decreased in the group treated with PSPE-alone. Alanine aminotransferase and aspartate aminotransferase are simple and widely accepted biomarkers for hepatic dysfunction, indicating that the APAP-induced liver injury model has been successfully developed in mice [24].

In the early stage of alcohol liver disease, triglycerides accumulate in hepatocytes, resulting in a reversible condition fatty liver (steatosis). Alcoholic fatty liver (AFL) is one of the earliest and most common consequences of chronic and excess alcohol consumption and can lead to more serious forms of liver damage such as cirrhosis, steatohepatitis, and hepatic fibrosis in humans [25].

Levels of serum triglyceride and cholesterol increased from alcohol absorption. It might be due to several processes, such as increased availability of L-glycerophosphate and free fatty acids, decreased secretion of very low density [26]. These results showed that alcohol treatment group significantly elevated serum triglyceride level compared to alcohol untreated group. While PSPE-alone group significantly decreased serum TG level, suggesting that PSPE prevent hyperlipidemia. In addition, total cholesterol in serum also slightly decreased in PSPE-alone and HDE group.

Chlorogenic acid is a kind of phenolic compound that exists in most plants and is known as a compound that has strong antioxidant activity [27], and Shi *et al* [28] found that chlorogenic acid reduces inflammation caused by liver toxicity It has been reported to improve fibrosis. In addition, it has been reported that chlorogenic acid inhibits the biosynthesis of fatty acids and cholesterol by participating in the process of fat metabolism [29]. In this study, chlorogenic acid content of PSPE confirmed that it contained a content of 5.16±0.05 μg/mg. Based on the above results, it is considered that the protective effect of PSPE against alcoholic liver damage is due to phenolic compounds such as chlorogenic acid derived from PSPE.

CONCLUSION

Alcohol administration significantly decreases liver and body weight. And PSPE administration

significantly inhibits the increase in serum ALT and AST levels caused by alcohol. These results demonstrate that PSPE and HDE experimental group is a promising agent to protect the liver from hepatotoxicity and fatty liver induced by alcohol intake.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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