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Protective Effects of SAPP, a Novel Herbal Complex, in Acute Hepatotoxic Mouse Model

Geum Seon Lee[1], Ki Man Lee[1], Seung Hyun Kim[1], Nam-Joo Jeong[2], Young-Jung Kim[3], Ju-Young Jung[3], and Tae Jin Kang[1,*]

[1]College of Pharmacy and Institute of Chronic Disease, Sahmyook University, Seoul 139-742, Korea
[2]Pharmaceutical Research Lab. PharmaKing Co. Ltd. Eumseong-gun, Chungbuk 369-852, Korea
[3]Department of Veterinary Medicine & Institute of Veterinary Science, Chungnam National University, Daejeon 305-764, Korea

Abstract – The protective effect of SAPP, an extract from a novel herbal complex, on acute liver injury was investigated using mouse animal model in this study. The content of total phenol in SAPP was increased at dose dependent manner. Consistent with the content of total phenol, SAPP showed the significant anti-oxidative effects on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. Acute liver injury was induced by D-galactosamine (D-GalN) in mouse. Treatment with SAPP significantly reduced the level of alanine transaminase (ALT) and aspartate transaminase (AST) in serum. Histological observation revealed that whereas D-GalN treated mouse showed vacuolization of hepatocytes, sinusoidal dilation and congestion, loss of cell boundaries and ballooning degeneration, loss of architecture and cell necrosis, treatment with SAPP improved D-GalN-induced liver injury. These results suggest that SAPP shows protective effects against D-GalN-induced hepatotoxicity *in vivo* acute mouse model.
Keywords – Hepatoprotective, D-GalN, Herbal complex

Introduction

The liver plays a pivotal role in many critical functions within the body from protein production and blood clotting to cholesterol, glucose, and iron metabolism. And should it become diseased or injured, the loss of those functions can cause significant damage to the body. Liver problems include a wide range of diseases such as fatty liver, hepatitis, cirrhosis, and so on. Some liver problems are temporary and the liver may be able to recover and resume its normal functions, while other liver problems can last for a long time and lead to serious complications. Acute or chronic liver diseases constitute a global concern and the concern is worsened by the lack of reliable hepatoprotective medicines, despite the increasing need for agents to protect the liver from damage. Therefore, alternative medicines for the treatment of liver diseases have been receiving considerable interest. Therapeutically effective agents from natural products may reduce the risk of clinical toxicity.

*Author for correspondence
Tae Jin Kang, Ph.D. College of Pharmacy, Sahmyook University 26-21, Kongnung 2-dong, Nowon-gu, Seoul, 139-742, Korea
Tel: +82-2-3399-1608; E-mail: kangtj@syu.ac.kr

A lot of natural products and plants have been used as medicinal materials for treatment of liver diseases in oriental medicine or as foodstuffs (Seeff *et al*., 2001; Kim *et al*., 2012). Artemisiae Capillaris Herba and Patriniae Radix are well known and commonly prescribed herbal medicine in oriental country, and have been used as an analgesic and a remedy for the treatment of hepatitis and biliary diseases (Choi *et al*., 2011; He *et al*., 2012; Wang *et al*., 2012; Yuan *et al*., 2012). However, it is important to investigate the active principles of the herbal medicines for quality control and to determine their therapeutic value in modern pharmacology. In this study, we report that newly formulated herbal medicine SAPP, which consists of Schisandrae Fructus, Artemisiae Capillaris Herba, Patriniae Radix, and Phellodendri Cortex, has strong hepatoprotective activity and SAAP exerts more potent inhibitory effect on acute liver injury.

Experimental

Reagents – Silibinin and D-(+)-galactosamine hydrochloride (D-GalN) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and thiopental sodium was from Choongwae Pharma Co Ltd. (Seoul, Korea). AM

101-K for measurement of ALT and AST was purchased from Asan Pharmaceutical Co. Ltd. (Seoul, Korea). Water extract of SAPP was kindly supplied by PharmaKing Co. Ltd (Chungbuk, Korea).

Preparation of SAPP – SAPP consists of four plants (Schisandrae Fructus, Artemisiae Capillaris Herba, Patriniae Radix, and Phellodendri Cortex). All plant materials were purchased from Kyoungdong oriental drug store (Seoul, Korea). Water extract was then prepared by steeping finely crushed herbs containing the SAPP. Briefly, 600 g of Schisandrae Fructus, 600 g of Artemisiae Capillaris Herba, 1200 g of Patriniae Radix, and 1200 g of Phellodendri Cortex were placed in distilled water and boiled for about four hours at 115 °C. After concentration, the extract was spray-dried, powdered, and stored in an airtight container at 25 ± 4 °C until use.

Total phenol content – Total phenol content in SAPP was determined with the Folin-Dennis method (Whang *et al*., 2001) using tannic acid as a standard. In brief, 50 μl of the SAPP were added to 50 μl of 1 N FC reagent in 96 well plates. After 3 min of shaking incubation, 50 μl of 10% Na_2CO_3 reagent was added and the mixture was then allowed to stand for 1 h in the dark. The absorbance was measured at 700 nm using an ELISA reader.

DPPH free radical scavenging capacity – Free radical scavenging activity was investigated as the degree of coloration using reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH, Sigma Co., USA) (Gordon *et al*., 2001). DPPH solution (2×10^{-4} M) was prepared in 50% EtOH, and 180 μl of this solution was mixed with 20 μl of 0.25, 0.5, 1, and 2 μg/ml SAPP in 96 well plates, respectively. The mixtures were then incubated at room temperature for 30 min in the dark and the absorbance of each sample was measured at 515 nm against 50% EtOH as blank. Ascorbic acid (Vitamin C) was used as a control antioxidant. The capacity was calculated as the percentage of the absorbance value of the SAPP solution and control.

Animals – Four-week-old male ICR mice were purchased from Hanlim Laboratory Animal Inc. (Whasung, Korea) and maintained in conventional condition at ambient temperature (23 ± 2 °C) and humidity (55 ± 10%) with free access to chow pellets and water for one week before the start of the experiments. The experimental groups consisted of 5 - 7 animals per drug and dose. Animal treatment and maintenance were conducted in accordance with the Animal Care and Use Guidelines of Sahmyook University, Korea.

Animals were divided into six groups; control, D-GalN, SAPP treatment + D-GalN, and silibinin + D-GalN. Animals were orally administered daily for 3 days with 5, 10, and 20 mg of SAPP/kg of body weight. On the last day of oral treatment of the SAPP, mice were injected *i.p.* with D-GalN (800 mg/kg) (Marzouk *et al*., 2002; Zhao *et al*., 2012) to induce acute hepatotoxicity. At 24 h after the injection of D-GalN, the mice were sacrificed under anesthesia with thiopental sodium (50 mg/kg) by *i.p.* injection. Silibinin (50 mg/kg) was used as a positive control (Zhao *et al*., 2012). After the animals were sacrificed, livers were immediately removed and weighed.

Assay of serum ALT and AST – As a marker of acute liver damage in animals, serum alanin aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by using a commercial enzyme assay kit AM 101-K based on the method of Reitman and Frankel (Choi *et al*., 2006).

Histological examination – Liver tissue for histopathological analysis was fixed in 10% buffered formalin, subsequently dehydrated and embedded in paraffin. The tissue paraffin was cut into 5 μm sections. Fixed sections were then stained with both hematoxylin and eosin (HE). The histopathological characters were observed and recorded under Nikon-80i, image analyzer.

Statistical analysis – Experimental values are given as the mean ± SEM. The significance of the data was examined by one-way analysis of variance. The statistical differences between the groups were considered significant at $p < 0.05$ with Newman-Keul's test as a post-hoc test.

Results and Discussion

The total phenol content was measured spectrophotometrically as in the Folin-Dennis method, Prussian blue method, and Vanillin HCl method. The total phenol content of SAPP was determined using the Folin-Dennis method and the standard tannic acid calibration curve. The results showed that the total phenol content of SAPP was increased in a dose-dependent manner (Fig. 1). The anti-oxidative activity is important as a target of study concerning the physiological activities of herbs. The DPPH scavenging is important indicators of the potential antioxidant activity. Therefore the anti-oxidative activity was measured by DPPH. The results showed that the anti-oxidative activity of SAPP was also increased in a dose-dependent manner (Fig. 2) and were consistent with the results for total phenol content (Fig. 1). It seems that the anti-oxidative activity resulted from the content of phenolic compounds. The results of this study suggest that components of SAPP have anti-oxidative activity and their effects will be further analyzed.

In acute liver injury animal model, SAPP treatment

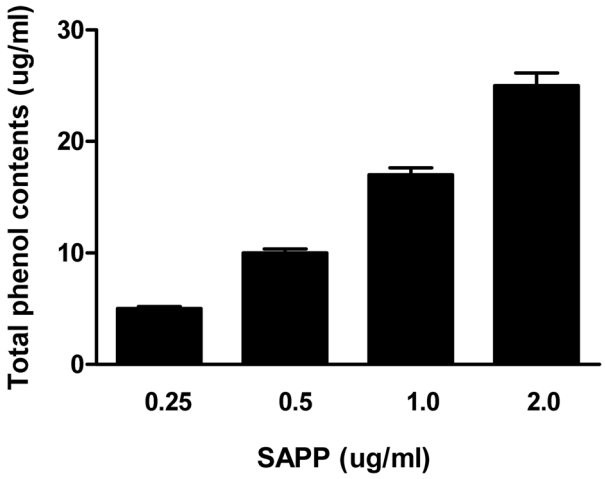

Fig. 1. Total polyphenol content of SAPP. Data are representative of at least two independent experiments in duplicate. Data are expressed as mean ± S.E.M.

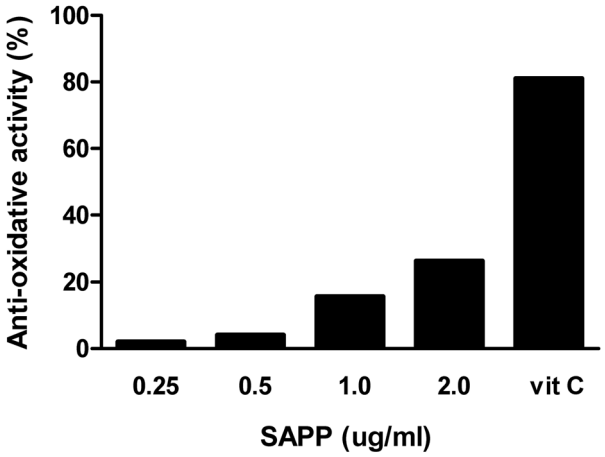

Fig. 2. Anti-oxidative activity of SAPP by DPPH free radical scavenging assay. Vitamin C (Vit C, 10 μg/ml) was used as a control. Data are representative of at least two independent experiments in duplicate.

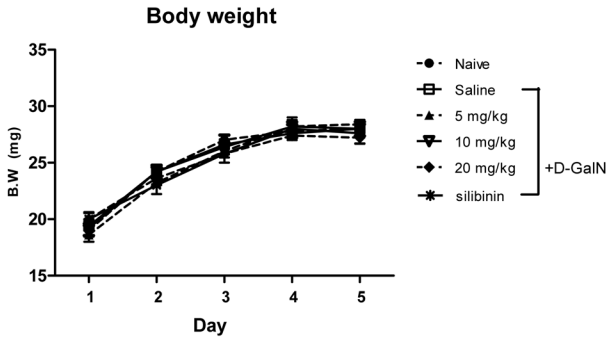

Fig. 3. Body weight of D-GalN-induced hepatotoxic mice treated with SAPP. Mice were treated with SPAA by oral administration at dose dependent manner (5, 10, and 20 mg/kg) for 3 days and then acute liver injury was induced by treatment with D-GalN (800 mg/kg). The saline and the silibinin (50 mg/kg) were used as control for SAPP. Each symbol represents the mean ± S.E.M of body weight for 10 days (n = 5~8).

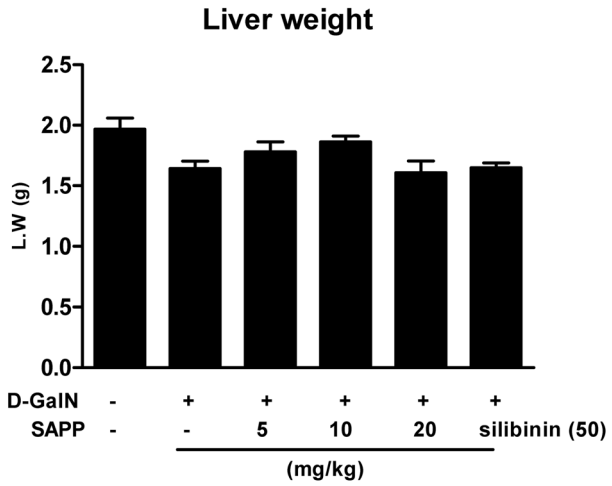

Fig. 4. Effect of SAPP on liver weight of D-GalN-induced acute hepatic damage mice. Mice were treated with SAPP by oral administration at dose dependent manner (5, 10, and 20 mg/kg) for 3 days and then acute liver injury was induced by treatment with D-GalN (800 mg/kg). The saline and the silibinin (50 mg/kg) were used as control for SAPP. Each symbol represents the mean ± S.E.M of body weight for 10 days (n = 5~8). Each bar represents the mean ± S.E.M of liver weight for 10 days.

resulted in slightly decreased gains in body weights and relative liver weights. However, the body weights and organ weights were rapidly recovered during the study and there is no significant difference in body weight and organ weight (Fig. 3 and 4).

Treatment with 800 mg/kg D-GalN induced liver injury as indicated by the rise in serum ALT and AST (Fig. 5). The rise in these enzymes was reduced significantly by oral administration with SAPP for 3 days prior to the *i.p.* injection of D-GalN. Interestingly the hepatoprotective effect of the SAPP was more potent at the dose of 5 mg/kg than high doses (10 and 20 mg/kg) we used. In addition, SAPP exhibited much stronger reducing effect on hepatic enzymes compared to silibinin, which has hepatoprotective property that protect liver injury (Jayaraj *et al*., 2007; Al-Anati *et al*., 2009).

Although SAPP has hepatoprotective activity, at a dose 10 mg/kg showed comparatively weaker effect in the reduction of serum ALT and AST level compared to doses of 5 mg/kg (low dose) and 20 mg/kg (high dose). It seemed that these results were due to individual variation of mice. To reduce the variation, it is necessary that the mouse population should be increased in our future study.

Histopathological observations further confirmed the

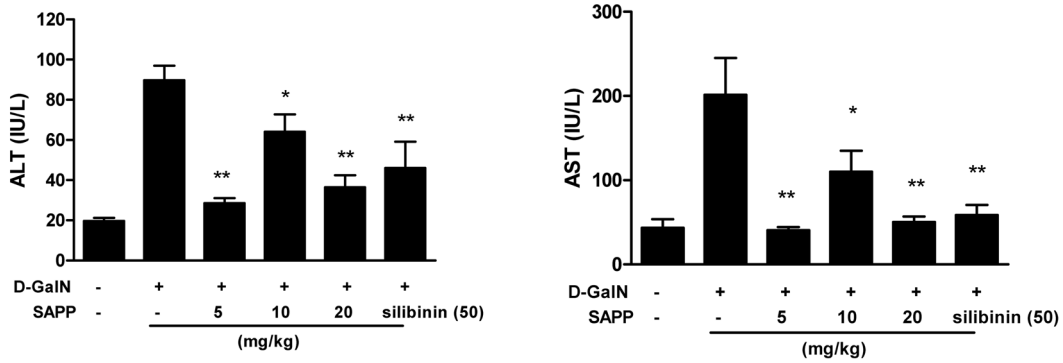

Fig. 5. Effects of SAPP on serum aminotransferase activities. Mice were treated with SAPP by oral administration at dose dependent manner (5, 10, and 20 mg/kg) for 3 days and then acute liver injury was induced by treatment with D-GalN (800 mg/kg). The saline and the silibinin (50 mg/kg) were used as control for SAPP. Serum was obtained 24 h after D-GalN injection. Each symbol represents the mean ± S.E.M of body weight for 10 days (n = 5~8). $^{*}P < 0.05$, $^{**}P < 0.01$ were significantly different from D-GalN alone.

effect of SAPP on liver injury induced by D-GalN. Hepatocyte necrosis induced by D-GalN was largely prevented by pretreatment of SAPP. The changes of histopathological appearance from D-GalN-treated mice were significant compared to normal control group. Histopathological analysis of the liver from non-treated normal mouse showed a normal central vein and hepatocytes with prominent nuclei and uniform cytoplasm. Whereas D-GalN treated mouse showed vacuolization of hepatocytes, sinusoidal dilation and congestion, loss of cell boundaries and ballooning degeneration, loss of architecture and cell necrosis, the mouse treated with SAPP prior to D-GalN treatment revealed obvious improvement of liver damage. The changes from SPAA pretreated mice showed significant hepatoprotective effects of SAPP against D-GalN-induced liver injury in mice. The histopathological appearance from SAPP (20 mg/kg) pretreated mice were close to normal group. Silibinin also showed the improvement of liver damage, however, it less showed than the histopathological change of SAPP treated mouse liver.

Many reports have shown that Schisandra has the anti-hepatotoxic effects through the properties of anti-oxidation and lipid peroxidation inhibition (Hikino *et al*., 1984; Ip *et al*., 1995; Mak *et al*., 1996). Recently, it is reported that aqueous extract of *schisandra chinensis* suppressed generation of ROS in the colonic epithelial cell line HT-29 (Lee *et al*., 2009). Therefore schisandra is considered to be a source of the anti-oxidant activity of SAPP. Phellodendri Cortex has been used in traditional medicine to treat dysentery, meningitis, pneumonia, tuberculosis, and liver cirrhosis (Li *et al*., 2009). Plants of the genus Phellodendron are rich sources of phenolic compound and contain flavonoid such as phellodensin A, B, C, amurensin,

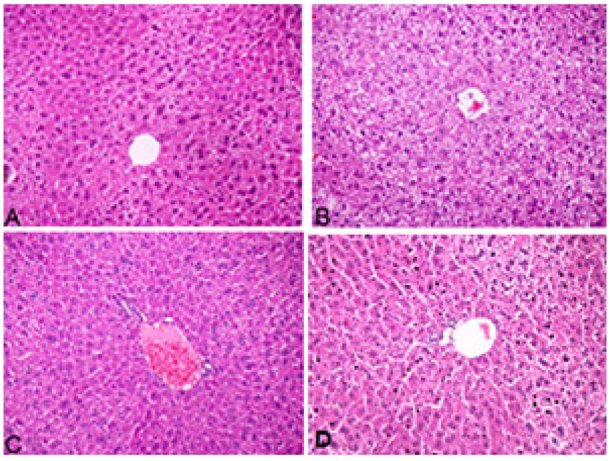

Fig. 6. Effect of SAPP on liver histopathological change of D-GalN-induced acute hepatic damage mice (H&E staining, X 200). (A) Non-treated normal mouse, (B) D-GalN-treated mouse, (C) SAPP (20 mg/kg) + D-GalN-treated mouse, (D) silibinin (50 mg/kg) + D-GalN-treated mouse.

quercetin, and kaempferol. They also have phenolic constituents such as syringin, osmanthuside H, and Kelampayoside A (Li *et al*., 2012). Gomisin A from Schisandra Fructus has an anti-hepatotoxic activity in *in vitro* assays and reduces the release of the intracellular enzyme ALT from the hepatocytes treated with toxic agents, such as CCl_4 and D-GalN (Hikino *et al*., 1984). Furthermore, *in vivo* gomisin A treatment also reduces the release of intracellular enzymes, including ALT and LDH, from the injured livers of CCl_4-treated rats (Maeda *et al*., 1982). *Artemisia iwayomogi* is also a medicinal plant with hepatoprotective effect and is used in traditional medicine in Korea and China. The pharmacological activities of *A. iwayomogi* and its major compound, scopoletin, have been previously shown to have anti-oxidant (Kim *et al*.,

2004; Seo and Yun, 2008) and hepatoprotective effects (Choi *et al*., 2005; Wang *et al*., 2012). Two studies have proposed that *A. iwayomogi* has antifibrotic effects in carbon tetrachloride (CCl_4) injury rat models (Park *et al*., 2000; Wang *et al*., 2012). Capillarisin from Artemisiae Capillaris Herba also has scavenging activity of free radical and protect hepatocyte from oxidative injury (Lee *et al*., 2009). Treatment with esculetin from Artemisiae promoted bile secretion *in vivo* study (Tsai *et al*., 1999).

In our study the effect of SAPP on acute liver injury was compared with that of silibinin, suggesting that the SAPP may be an alternative substance for the protection of acute liver damage. Understanding the relation between each step involved in hepatoprotective activities and natural materials was pivotal for better studying of the molecular mechanism underlying the cellular response. Our results suggest that SAPP has some bioactive component which can be a new candidate material for the treatment of liver injury. Our future study will be to analyze and probe each of the chemical constituents of SAPP and thereafter establish the biochemical and molecular mechanism of their anti-hepatotoxic activity. Nevertheless, our findings suggest that SAPP could be applicable to develop potent therapeutic reagent for the treatment of liver injury.

Acknowledgments

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