ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF HYPERICUM SAMPSONII

Nguyen Viet Dung[1], Nguyen Duy Thuan[2], Dang Thi Thu Hien[3] and Pham Thi Van Anh[3,✉]

[1]Department of Pharmacology, Military Institute of Traditional Medicine
[2]Vietnam University of Traditional Medicine
[3]Hanoi Medical University

Hypericum sampsonii or Sampson's St John's Wort is a species of flowering plant in the Hypericaceae family. Our previous study revealed the diverse and bioactive chemical constituents of the plant. This study investigates the antioxidant activity and potential hepatoprotective of Hypericum sampsonii extract (BLD1 extract) in vitro and in vivo. The in vitro experiment was carried out with DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay to assess the antioxidant properties of BLD1 extract. In the in vivo experiment, the hepatoprotective activity of BLD1 extract was evaluated on an acetaminophen-induced hepatotoxicity mouse model. The in vitro assay demonstrated that the extract exhibited antioxidant activity in a dose-dependent manner. In the in vivo experiments, BLD1 extract at both doses (3.6 g/kg b.w day and 10.8 g/kg b.w day) reflected the decrease in serum ALT, AST and MDA level. Hepatic histology was also investigated post-mortem to assess the degree of liver injury. The overall findings of this study demonstrate the potential hepatoprotective effect of Hypericum sampsonii in acetaminophen-induced oxidative stress and liver toxicity in mice. This study has established the efficacy of Hypericum sampsonii, which should warrant further clinical trials and applications.

Keywords: Hypericum sampsonii, BLD1 extract, hepatoprotective, antioxidant, acetaminophen, experimental animals.

I. INTRODUCTION

Hypericum sampsonii (common name Sampson's St John's Wort) is a species of flowering plant in the St. John's Wort family, Hypericaceae. It is an important medicinal plant which traditionally used for diseases such as backache, burns, diarrhea and swelling, and has recently been investigated as an anti-hepatitis drug.[1,2] The chemical constituents of *Hypericum* species have attracted attention due to their biological activities and structural diversity; different kinds of compounds such as xanthones, benzophenones, bisanthraquinones, and polyprenylated phloroglucinols have since been isolated. In the previous study, by various chromatographic separations, we revealed the presence of five compounds, including two xanthones, and three benzophenones which were isolated from the aerial parts of the herb; these compounds were found to have potent antioxidant and anti-inflammatory effects based on *in vitro* experiment.[2]

Oxidative stress influences the progression of liver diseases and consequently induces hepatotoxicity.[3] Oxidative stress can stem from toxic exposure, viral infections, or genetic diseases such as muscular dystrophy. By attenuating oxidative stress, cellular

Corresponding author: Pham Thi Van Anh
Hanoi Medical University
Email: phamthivananh.hmu@gmail.com
Received: 21/08/2023
Accepted: 01/10/2023

antioxidant systems prevent liver diseases. Currently, available synthetic antioxidants are associated with several adverse effects and are not recommended for long-term use.[4] Therefore, antioxidants of herbal origin are being considered as they are generally safer and possess a wider range of clinical usage. We conduct this study to further investigate the hepatoprotective activity of *Hypericum sampsonii* (BLD1 extract) *in vitro,* and *in vivo* on acetaminophen-induced liver damage mice.

II. MATERIAL AND METHOD

1. Plant materials

Hypericum sampsonii was collected from Vi Ha, Bach Thong district, Bac Kan province, Vietnam in 2006. The name BLD1 has been coined by Ms. Nguyen Quynh Nga (Department of Natural Resources - National Institute of Medicinal Materials, Vietnam). The specimens (BLD1) have been registered at the herbarium of the Vietnam Academy of Science and Technology – VAST.

Preparation of *Hypericum sampsonii* extract

In order to prepare the required extract, dried *Hypericum sampsonii* aerial parts (300 g) were ground and extracted with water at 60^{o}C, 1h for three times (each 3L). After that, these extracts are combined and concentrated under temperature (60^{o}C) to obtain liquid extract (100 mL). BLD1 extract with a concentration of 3mg/mL was prepared in the Department of Pharmacy – Military Institute of Traditional Medicine.

The quality control of raw materials and extraction procedures of the extract were followed by Vietnamese Pharmacopoeia V.

2. Animals

Studies were carried out using adult *Swiss* albino mice (*Mus musculus*) of both sexes, the average weight was 30 ± 2 g. The mice were obtained from the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam. The animals were grouped and housed in cages with not more than ten animals per cage and maintained under standard laboratory conditions of humidity (50 ± 5%), and temperature (25 ± 2^{0}C) with dark and light cycles (12/12 h). The animals were fed with a standard pellet diet supplied by the National Institute of Hygiene and Epidemiology and fresh water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before the commencement of the experiment

3. Methods

In Vitro Antioxidant Activity[5]

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant potential of BLD1 extract at different concentrations (100 and 500 µg/mL). Freshly prepared purple-colored DPPH solution turned yellow after incubating with test samples for 30 min at room temperature, and changes were measured using a spectrophotometer at 514 nm. The results were calculated and expressed as the inhibition percentage of DPPH radical formation.

In Vivo Antioxidant and Hepatoprotective Studies [6–8]

Mice were randomly divided into five groups with 10 animals in each. The animals were orally administrated for ten successive days as follows: groups I and II received distilled water (20 mL/kg) and served as the normal control and acetaminophen-only control groups, respectively. Group III received silymarin (140 mg/kg/day) whereas groups IV and V received 3.6 and 10.8 g/kg BLD1 extract, respectively. On the 9th day (i.e., one day before the last administration) animals of all groups fasted for 18 hours. On the 10th day, 1h after the last oral dosing, all mice except those in

Group I was given acetaminophen (400 mg/kg; p.o). All animals were sacrificed 48 h after acetaminophen administration. Blood samples were collected from each animal to assess serum AST (alanine transaminase) and ALT (aspartate transaminase) levels. The liver was rapidly isolated and homogenized using cold saline to prepare a 10% homogenate aliquot, and later used for estimation of malondialdehyde (MDA) level. Part of the liver was also preserved in 10% formalin for histological assessment.

4. Statistical analysis

Statistical analyses were carried out using SPSS ver. 20.0. The data were expressed as the mean ± standard deviation (SD); Student's t-test was used to show the scavenging effects of test samples compare means. The p-values of <0.05 were considered to be statistically significant.

III. RESULTS

1. In Vitro Antioxidant Activity

Table 1 on DPPH radical. Percent inhibition of DPPH radical of the BLD1 extract at the concentrations of 100 µg /mL and 500 µg / mL were 57.21 ± 2.16 and 86.30 ± 2.49%, respectively. The experiment demonstrated the antioxidant activity of BLD1 extract *in vitro* was dose-dependent and comparable with the standard control ascorbic acid.

Table 1. DPPH radical scavenging activities of BLD1 extract at different concentrations

Plant extracts/chemical	Concentrations (µg/mL)	% Inhibition (mean ± SD)
BLD1	100	57.21 ± 2.16
	500	86.30 ± 2.49
Ascorbic acid*	10	26.84 ± 1.18
	50	93.69 ± 0.29

*Ascorbic acid as the standard substance. All values represent mean ± SD. Student's t-test.*p < 0.05 (Statistically significant compared with normal control)

2. In Vivo Antioxidant and Hepatoprotective Studies

Effect of *Hypericum sampsonii* extract on serum transaminase (AST, ALT)

Results of biochemical parameters indicative of liver damages are summarized in **Table 2.** Acetaminophen administration caused severe hepatotoxicity in mice as evidenced by significantly elevated serum levels of ALT and AST compared to normal water-administered control. The elevation in ALT and AST levels was significantly alleviated by silymarin pretreatment (140 mg/kg). Pretreatment with BLD1 extract significantly reduced these levels at both doses (3.6 and 10.8 g/kg) compared to the acetaminophen-only control group.

Table 2. Effect of BLD1 extract on serum transaminase levels of control and treated mice

Treatments	Dose	AST (UI/L)	ALT (UI/L)
Normal		140,91 ± 25,82	76,45 ± 11,93
Acetaminophen		409,58 ± 98,75***	288,08 ± 65,56***
Acetaminophen + Silymarin	140 mg/kg	296,75 ± 75,83++	186,17 ± 36,99+++
Acetaminophen + BLD1	3.6 g/kg	152,08 ± 34,99+++	84,92 ± 17,03+++
Acetaminophen + BLD1	10.8 g/kg	142,18 ± 31,70+++	90,82 ± 22,48+++

All values represent mean ± SD. Student's t-test.

***$p < 0.001$ (Statistically significant compared with normal control)

$^{++}p < 0.01$; $^{+++}p < 0.001$ (Statistically significant compared with acetaminophen control).

Lipid peroxidation (MDA) activity

Acetaminophen exposure without any treatment significantly ($p<0.05$) increased the MDA level compared to the normal control group (**Table 3**). Acetaminophen-induced mice pretreated with silymarin or BLD1 extract at both doses (3.6 and 10.8 g/kg) decreased MDA levels compared to mice exposed to acetaminophen alone, however, the difference was statistically insignificant.

Table 3. Effect of BLD1 extract on MDA level in liver tissue

Groups	Dose	MDA (nmol/100mg)
Control		10,75 ± 2,90
Acetaminophen		13,66 ± 2,04*
Acetaminophen + Silymarin	140 mg/kg	12,50 ± 2,52
Acetaminophen + BLD1	3.6 g/kg	12,70 ± 1,84
Acetaminophen + BLD1	10.8 g/kg	12,04 ± 2,77

All values represent mean ± SD. Student's t-test.

***$p < 0.001$ (Statistically significant compared with normal control) $^{++}p < 0.01$;

$^{+++}p < 0.001$ (Statistically significant compared with acetaminophen control).

Histopathological assessment

Histopathological assessment of mouse liver tissue (**Figure 1**) from Group I animals shows normal hepatic cells with no significant histological abnormality. In the acetaminophen-induced group (Group II), severe hepatotoxicity was observed by severe necrosis with the disappearance of nucleic. Group III which was pretreated with silymarin showed the absence of cell necrosis and minimal inflammatory conditions with near-normal liver architecture. The liver taken from Group IV animals pretreated with BLD1 extract at the dose of 3.6 g/kg/day showed normal hepatic cells with near normal portal vein and hepatic artery. In Group V, at the dose of 10.8 g/kg/day of BLD1 extract, moderate degenerative hepatocytes and mild inflammation in the portal area was observed.

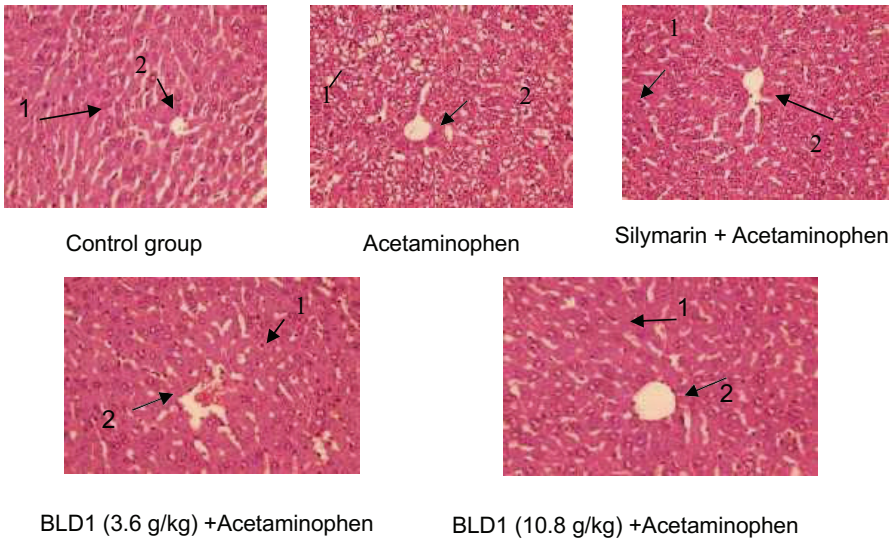

Figure 1: Liver histopathology in acetaminophen-induced mice after treatment with BLD1 extract at the indicated doses (1)hepatocyte (2)portal venule (Selected microphotographs HE staining magnification × 400)

IV. DISCUSSION

Antioxidant compounds in fresh aerial tissues of *Hypericum sampsonii* (Sampson's St John's Wort) were first investigated by Chen et al. (2009). In this study, among the three tested Hypericum species, *Hypericum sampsonii* contained more ascorbate, thiol, and phenolics, the potent antioxidant compounds, than *Hypericum japonicum* (Japanese St John's Wort) and *Hypericum perforatum* (St John's Wort).[9] In addition, *Hypericum sampsonii* also has high Superoxide dismutase (SOD) activity, the enzyme responsible for catalyzing the conversion of superoxide to elemental oxygen and hydrogen peroxide.[9] Further study by our group revealed five notable compounds in which the xanthones mangiferin showed the most potent antioxidant effect, and together with 3 other benzo-phenone compounds sampsine A, sampsine B, and petiolin F showed potent inhibition of nitric oxide production *in vitro.*

Acetaminophen overdose is the most common cause of Drug-induced liver injury and acute liver failure. Lipid peroxidation and subsequent oxygen- reactive species formation have been suggested as the main cause of hepatic apoptosis in Acetaminophen poisoning.[10] In this study, the administration of acetaminophen (400 mg/kg, p.o.) to fasting mice resulted in acute hepatic injury which is reflected by the massive increases in both serum ALT and AST levels. Consistent with the biochemical changes, the histopathological observations in acetaminophen-induced mice revealed severe degenerative changes in most of the hepatic lobules. It was established that oxidative stress constitutes a major mechanism underlying the pathogenesis of acetaminophen-induced liver damage and many other liver diseases.[11] Thus if *Hypericum japonicum* can alleviate acetaminophen-induced liver injury, it can also be applied to many other clinical circumstances.

The pretreatment with silymarin or BLD1 extract, at both doses of 3.6 g/kg and 10.8 g/kg,

significantly attenuated the elevated levels of the serum markers. The normal level of serum ALT and AST in the BLD1 extract-conditioned group suggests that it is able to increase the membrane integrity of hepatocytes to protect it from acetaminophen-induced apoptosis. The histopathology examination in mice treated with either silymarin or BLD1 extract also exhibited significant liver protection against acetaminophen-induced liver damage, as evident by the presence of normal hepatic cells and absence of necrosis. BLD1 pretreatment at 3.6 g/kg was found to be as effective as silymarin with negligible liver toxicity. We also monitored the liver's malondialdehyde (MDA), a marker of oxidative stress, in the mice model to assess the hepatoprotective effect of BLD1.[12] However, the reduction in MDA level was not statistically significant in either the silymarin or the BLD1 pretreated group. The reason might be the time (48 hours) after acetaminophen administration that we sacrifice the mice, which would leave little time for MDA to drop to the normal value.

V. CONCLUSION

The overall findings of this study demonstrate a potential hepatoprotective effect of *Hypericum sampsonii* in acetaminophen-induced oxidative stress and liver toxicity in mice. Such effects can be correlated directly with its ability to reduce lipid peroxidation and enhance the antioxidant capability of the liver. This study has established the efficacy of *Hypericum sampsonii* and such should warrant additional research as possible candidates for further clinical trials and applications.

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