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HEPATOPROTECTIVE EFFECTS AND SAFETY EVALUATION OF METHANOLIC EXTRACT FROM *SEDUM SARMENTOSUM* BUNGE

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Summary

Hepatoprotective Effects and Safety Evaluation of Methanolic Extract from *Sedum sarmentosum* Bunge

The aim of this study was first to investigate the hepatoprotective activity of methanol extract from *Sedum sarmentosum* Bunge (SSE) in *Swiss albino* mice submitted to paracetamol-induced liver injury. Secondly, acute and subchronic oral toxicity assays of SSE were carried out in *Swiss albino* mice and *Wistar* rats. The results showed that SSE (at the doses of 0.5 and 1.0 g/kg; p.o) attenuated both acute and chronic paracetamol-induced liver intoxication as indicated by reduced level of serum liver enzymes, aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT). Additionally, the LD_{50} of SSE in mice was 25.56 g/kg body weight for single dose oral administration. In the subacute toxicity study, rats were administered SSE doses of 275 and 825 mg/kg (p.o) for 30 days. After 15 and 30 days, blood and tissue samples were taken for determination of hematological, biochemical and histopathological parameters. All parameters in SSE groups did not significantly change as compared to respective control.

Keywords: *Sedum sarmentosum, Acute and subacute toxicities, Paracetamol-induced liver injury, Hepatoprotective effect.*

1. Introduction

Sedum sarmentosum Bunge (Crassulaceae) is a perennial plant distributed throughout mountainous areas in China, Korea, Vietnam and Thailand where it has been used for hepatoprotection and other benefits [1],[2]. Principal chemical components of *Sedum sarmentosum* (*S. sarmentosum*) with hepatoprotective effects and inhibition of lipid accumulation in the liver are megastigman and flavonoids [2],[3]. Few studies of the chemical composition and biological activities of *S. sarmentosum* have been made in Vietnam [4], for which the age-adjusted death rate due to liver disease is 23.34 per 100,000 population [5]. The objective of this study was to evaluate the acute, subacute toxicity and hepatoprotective effects of *S. sarmentosum* collected in Vietnam.

2. Materials and Methods

2.1. Materials

✓ Preparation of *S. sarmentosum* Bunge extract:

Whole plant parts of *S. sarmentosum* Bunge were collected at Sa Pa, Lao Cai province, Vietnam in June 2015 and identified by Department of Resource Medicinal Materials, National Institute of Medicinal Materials, Hanoi, Vietnam (NIMM). The voucher specimens have been deposited in the Herbarium of Military Institute of Traditional Medicine (voucher specimens DL-300615). *S. sarmentosum* extract (SSE) was prepared by cutting aerial parts into small pieces and drying at 55°C. Plant material (9 kg) was extracted with methanol (3 times x 15 L). The organic layer was evaporated under vacuum to yield the dried crude extract (400 gr).

✓ Chemicals and reference drug:

- Paracetamol (Efferalgan® 500 mg), Bristol-Myers Squibb, French.
- Silymarin (Legalon® 54 mg), Madaus, Germany.

- Quantitative kits for ALT, AST, urea, creatinine, total protein, total bilirubin were supplied by Erba, Czech Republic.

- The analysis kit for red blood cells, white blood cells, platelets, hematocrit, hemoglobin, lymphocytes was provided by Diatron, Hungary.

✓ Animals:

Healthy *Swiss albino* mice of both sexs, aged 5-6 weeks, weighing on average 23 - 25 g and 18-20 g (to assess the hepatoprotective effect and acute toxicity, respectively) were supplied by the National Institute of Hygiene and Epidemiology, Vietnam. Healthy male and female *Wistar* rats, weighing between (180 - 250) g, were provided by Military Medical Academy, Vietnam, and used for evaluting subacute toxicity. Animals were fed standard diet pellets and allowed food and water had free access for an acclimation period of 4 days. Animals were kept under controlled conditions (temperature 22 ± 1°C; 12 h light/dark cycle, lights on at 7:00 a.m).

2.2. Methods

2.2.1. Acute toxicity assessment:

Acute oral toxicity of SSE was determined by previously described methods [6],[7]. Before administration, all mice were fasted overnight (12 h) with free access to water. Animals were randomly divided into one control group and 5 treatment groups (n = 10, male: female = 1:1 per group). Each group of mice was given different oral doses of SSE (20.0 g/kg; 25.0 g/kg; 28.0 g/kg; 31.3 g/kg; and 35.0 g/kg). The changes in general behavior, fur change, eating, urination and mortality of the mice within 72 hours were observed and recorded. Dead mice were necropsied . Mice without signs of abnormalities were monitored for an additional 7 days. The lethal dose (LD_{50}) was estimated using Behrens-Karber method [6].

2.2.2. Subacute oral toxicity test:

A subacute toxicity study was designed and performed according to the Ministry of Health, Vietnam [8]: *Wistar* rats were andomized into 3 groups (10 rats/group): Group 1: control group received 0,5% cacboxymethylcellulose (CMC) and 2 treatment groups (SSE treatment at the doses of 275 and 825 mg/kg) a for 30 consecutive days using oral gavage. The body weight of each rat was measured at the initiation of SSE administration and weekly thereafter. Hematological and serum biochemical evaluations were conducted at 3 different times, including before, and after 15, 30 days of SSE treatment. At the end of the SSE administration, rats were sacrificed for analysis of organ weights and histopathology.

2.2.3. Evaluation method for evaluating the effect of SSE on paracetamol-induced acute liver injury [9],[10]:

For paracetamol-induced acute hepatotoxicity, mice were randomly divided into 5 groups:

Group 1 (normal control): orally received vehicle (0.5% CMC)

Group 2 (acute hepatotoxic control): orally received vehicle (0.5% CMC)

Group 3 (standard drug control): orally received silymarin (100 mg/kg)

Group 4 and 5: orally received SSE (0.5 and 1.0 g/kg, respecially)

Animals were given either vehicle or study samples continuously for 8 days. At the end of day 6, the mice were fasted, drinking water freely. One hour after the administration of pre-treatment drugs on day 7^{th}, mice in groups 2 to 5 were administered with paracetamol (220 mg/kg; p.o) to induce liver damage. On day 8, one hour after treatment, all animals were sacrificed, blood was collected from the common carotid artery, and serum was separated to quantify serum liver enzymes of ALT and AST.

2.2.4. Evaluation method for evaluating the effect of SSE on paracetamol-induced chronic liver injury [11]:

Mice were randomized into 5 groups and were treated as described below:

Group 1 (normal control): mice were given orally 0.5% CMC.

Group 2 (long-term hepatotoxicity control): mice were given orally 0.5% CMC and paracetamol.

Group 3: mice were given orally silymarin (200 mg/kg) and paracetamol.

Group 4 and 5: mice were given orally SSE (0.5 and 1.0 g/kg, respecially) and paracetamol.

Animals were given either 0.5% CMC or silymarin SSE of continuously for 24 days. On the third day after pre-treatment for one hour, mice in groups 2 to 5 were administered with paracetamol (200 mg/kg; p.o). Paracetamol was not given on day 4. Starting on day 5, paracetamol was given orally (400 mg/kg; p.o) twice daily for the rest of the experiment. After taking the last dose of paracetamol for 1 day, animals were sacrificed, blood was collected from the common carotid artery, and serum was separated to quantify serum liver enzymes of ALT and AST.

2.2.5. Data analysis:

Data were analyzed using SPSS 23.0 software. All data were expressed as mean ± SE (M: mean, SE: standard error). Statistical analysis was performed with the t-test or ANOVA test and post-hoc to compared mean among groups if the data was normal distributions. For non-normal-distributed samples, Kruskal-Wallis test was used to compare the differences between groups, and the Wilcoxon test was used to compare differences within the same groups. $P < 0.05$ was considered to be statistically significant difference.

3. Results and discussion

3.1. Result of acute toxicity study of SSE

The experiments were conducted on 5 groups of mice given SSE oral doses of 20.0 g/kg; 25.0 g/kg; 28.0 g/kg; 31.3 g/kg; and 35.0 g/kg, and the results are described as follows:

- Group 1: animals were given an oral dose of 20.0 g/kg, mice did not exhibit any abnormality and moved normally. After 72 hours of follow-up, all mice of the experimental group were alive and healthy.

- Group 2: animals were given an oral dose of 25.0 g/kg. After treatment, the majority of mice still functioned normally, and after about 30 minutes, 4 mice showed slowness, shortness of breath, and convulsions, and died within one hours. One mouse died after about 10 hours. Necropsy of the dead mouse did not detect any abnormalities in the heart, liver, kidneys, but the stomach was filled with a black sample (the color of SSE). After 72 hours of follow-up, there were 3 mice that died in total, the rest of them survived and were healthy. After another week of follow-up, the surviving mice are still healthy.

- Group 3: animals were given an oral dose of 28.0 g/kg. After treatment for about 10-20 minutes, some mice showed signs of being sedentary; 5 mice died 15-30 minutes after drinking; 4 more mice died 3-5 hours after. The abdominal dissection of the dead mice did not detect any abnormalities in the heart, liver, or kidneys. After 72 hours of follow-up, there were 9 dead mice and 1 healthy survivor. After another week of follow-up, the one surviving mouse was still healthy.

- Group 4: animals were given an oral dose of 31.25 g/kg. 5-10 minutes after treatment, some mice showed sedentary expression, convulsed, and died: 2 mice died 10-15 minutes after treatment, 5 mice died 20-40 minutes after treatment, 1 mouse died 70 minutes after treatment, and 1 mouse died about 10 hours after drinking. The abdominal dissection of the dead mouse did not detect any abnormalities in the heart, liver, kidneys, and stomach which was filled with a black sample. After 72 hours of follow-up, there were 9 dead mice and 1 healthy survivor. After another week of follow-up, one surviving mouse was still healthy.

- Group 5: animals were given an oral dose of 35.0 g/kg body. After treatment, all mice showed signs of sedentary behavior and convulsions and died within 10-20 minutes. The abdominal dissection of the dead mouse did not detect any abnormalities in the heart, liver, or kidneys.

The results of acute toxicity assay are presented in Table 1.

Table 1. Acute toxicity test data of SSE

Groups	Dose (g /kg)	n	Number of dead mice	d	z	d x z
1	20.0	10	0			
2	25.0	10	4	5.00	2.0	10.0
3	28.0	10	9	3.00	6.5	19.5
4	31.3	10	9	3.25	9.0	29.3
5	35.0	10	10	3.75	9.5	35.6
Σ (d x z)						94.4

The results of Table 1 show that the LD_0 of the SSE is 20.0 g/kg, and the LD_{100} dose is 35.0 g/kg. Calculated LD_{50} value = 25.56 g/kg. According to the calculation of the safe dose, the effective dose of the drug should be at least 10 times less than the LD_{50} dose, the greater this coefficient, the higher the safety of the drug. Therefore, a dose of 1.0 g/kg SSE is relatively safe in terms of acute toxicity when used to test paracetamol induced liver damage in mice.

3.2. Result of subacute toxicity

3.2.1. Effects of SSE on on body weight, food and water consumption:

SSE did not produce any obvious symptoms of

toxicity or mortality in all the treated rats. Besides, no significant change occurred in food and water consumption in rats treated with repeated oral doses of SSE (275 mg/kg and 825 mg/kg). The change of rat body weight during the experimental period was shown in Table 2. There was no statistically significant weight difference between the treated groups and the control group ($P > 0.05$).

Table 2. Effect of SSE on body weight of rats (n=10)

Group	Day 0	Day 15	Day 30
Physiological	218.1 ± 10.4	226.5 ± 9.8	248.2 ± 13.4*
SSE (275 mg/kg)	228.9 ± 7.7	235.0 ± 8.2	247.8 ± 9.9*
SSE (825 mg/kg)	228.0 ± 4.7	238.9 ± 5.4	260.7 ± 11.0*

** $P < 0.05$ compared to the first time in the same group.*

3.2.2. The effect of SSE on hematological parameters:

Hematological parameters presented in Table 3 showed that after 15 days, 30 days of SSE treatment, all hematological parameters in all 3 groups were not significantly different from the control group at the same period, as well as compared with themselves before the experiment ($P > 0.05$).

Table 3. Effect of SSE in hematopoietic function (n=10)

Parameters	Groups	Day 0	Day 15	Day 30
Number of white blood cells ($10^3/mm^3$)	Control	8.6 ± 0.2	8.2 ± 0.3	8.8 ± 0.3
	SSE (275 mg/kg)	8.4 ± 0.3	7.9 ± 0.3	8.7 ± 0.4
	SSE (825 mg/kg)	687.1 ± 56.6	706.1 ± 48.5	674.1 ± 61.7
Number of red blood cells ($10^6/mm^3$)	Control	691.6 ± 38.7	714.2 ± 46	693.9 ± 39.6
	SSE (275 mg/kg)	787.8 ± 42.2	750.8 ± 79.3	711.0 ± 95.4
	SSE (825 mg/kg)	14.1 ± 0.3	13.9 ± 0.4	15.1 ± 0.4
Number of Platelet ($10^3/mm^3$)	Control	13.9 ± 0.3	14.2 ± 0.5	14.6 ± 0.4
	SSE (275 mg/kg)	13.7 ± 0.5	13.6 ± 0.4	14.4 ± 0.3
	SSE (825 mg/kg)	46.9 ± 1.0	43.1 ± 1.7	49.2 ± 0.8
Hemoglobin (g/dL)	Control	46.2 ± 0.9	43.9 ± 1.4	47.2 ± 1.1
	SSE (275 mg/kg)	45.3 ± 1.4	41.5 ± 2.2	47.9 ± 1.1
	SSE (825 mg/kg)	76.9 ± 4.4	81.1 ± 3.6	77.6 ± 3.8
Hematocrit (%)	Control	77.0 ± 4.2	84.5 ± 5.0	79.6 ± 4.6
	SSE (275 mg/kg)	74.8 ± 4.7	73.6 ± 6.2	81.1 ± 4.1
	SSE (825 mg/kg)	8.8 ± 0.7	9.5 ± 0.6	9.0 ± 0.7
Lymphocytes ($10^3/mm^3$)	Control	8.7 ± 0.6	9.4 ± 0.6	9.4 ± 0.7
	SSE (275 mg/kg)	7.3 ± 0.7	8.5 ± 1.0	9.1 ± 0.5
	SSE (825 mg/kg)	8.6 ± 0.2	8.2 ± 0.3	8.8 ± 0.3
Lymphocytes (%)	Control	8.4 ± 0.3	7.9 ± 0.3	8.7 ± 0.4
	SSE (275 mg/kg)	687.1 ± 56.6	706.1 ± 48.5	674.1 ± 61.7
	SSE (825 mg/kg)	691.6 ± 38.7	714.2 ± 46	693.9 ± 39.6

P (control-treatment) in all groups is > 0.05; P (before-after) in all groups > 0.05.

3.2.3. The effect of SSE on liver and kidney function:

The biochemical parameters of liver and kidney of rats after 3 times of testing were presented in Table 4.

Table 4. Effect of SSE on liver and kidney function (n=10)

Parameters	Groups	Day 0	Day 15	Day 30
ALT (U/L)	Control	66.9 ± 3.7	79.6 ± 4.6	70.6 ± 4.2
	SSE (275 mg/kg)	73.9 ± 2.7	72.9 ± 3.6	67.4 ± 2.6
	SSE (825 mg/kg)	64.7 ± 3.3	73.0 ± 6.0	62.0 ± 5.5
AST (U/L)	Control	166.2 ± 6.0	165.2 ± 5.7	166.1 ± 6.0
	SSE (275 mg/kg)	167.3 ± 5.4	160.1 ± 8.2	163.7 ± 4.0
	SSE (825 mg/kg)	164.0 ± 5.5	167.9 ± 5.6	162.7 ± 7.2
Creatinin (µmol/L)	Control	78.3 ± 5.3	82.0 ± 3.3	72.0 ± 4.5
	SSE (275 mg/kg)	79.2 ± 3.9	85.4 ± 2.6	73.9 ± 6.2
	SSE (825 mg/kg)	76.1 ± 2.7	80.0 ± 2.0	69.9 ± 5.6

Parameters	Groups	Day 0	Day 15	Day 30
Total protein *(g/L)*	Control	76.2 ± 3.4	80.5 ± 2.5	78.1 ± 1.6
	SSE (275 mg/kg)	75.3 ± 2.8	80.4 ± 1.8	78.1 ± 1.7
	SSE (825 mg/kg)	76.5 ± 2.9	79.6 ± 3.3	74.4 ± 1.5
Ure *(mg/dL)*	Control	5.0 ± 0.3	5.7 ± 0.4	5.0 ± 0.6
	SSE (275 mg/kg)	5.5 ± 0.3	5.5 ± 0.3	5.2 ± 0.6
	SSE (825 mg/kg)	5.6 ± 0.4	5.4 ± 0.4	5.6 ± 0.7
Bilirubin *(μmol/L)*	Control	16.1 ± 1.4	16.1 ± 1.8	19.4 ± 1.9
	SSE (275 mg/kg)	15.9 ± 1.4	16.3 ± 1.0	18.7 ± 1.2
	SSE (825 mg/kg)	16.0 ± 1.9	18.8 ± 1.5	19.5 ± 0.9

Note: P (control-test) in all groups is > 0.05, P (before-after) in all groups > 0.05.

The results from table 4 showed that, after 15 days, 30 days of SSE treatment, the liver function (bilirubin, total protein, serum AST and ALT enzyme activities) and kidney function (serum levels of urea and creatinine) in both SSE groups did not have significant differences compared with the control group and compared to themselves before treatment ($P > 0.05$).

3.2.4. Histopathological examination:

All the rats in 3 groups did not see any gross pathological changes in the organs of the liver, kidney, spleen, and heart. The organ mass to body weight ratio was presented in Table 5.

Table 5. Weight of rat organs after 30 days SSE treatment (n=10)

Groups	Liver (g/100 g body weight)	Kidney (g/100 g body weight)	Heart (g/100 g body weight)
Control	2.94 ± 0.13	0.29 ± 0.02	0.24 ± 0.01
SSE (275 mg/kg)	2.77 ± 0.11	0.29 ± 0.03	0.26 ± 0.02
SSE (825 mg/kg)	2.88 ± 0.11	0.31 ± 0.03	0.26 ± 0.02

The results in Table 5 showed that the ratio of heart, liver, kidney, spleen weight compared to the body weight of rats in the SSE and control groups was not different (P > 0.05).

At the microscopic level, after 30 days of SSE treatment at the doses of 275 mg/kg and 825 mg/kg liver tissues had normal hepatocytes, regular size, and veins. The central and venous sinuses are not congested, nor was there degeneration and necrosis, and the portal space was not inflamed. The results of the microscopic examination of the kidneys also showed that all the experimental rats had regular-sized glomeruli, non-inflammatory interstitial tissue, and no damage to the renal tubules. There was no difference in the microstructure of the liver and kidney between the SSE treatment groups and the control group. (Because there was no difference in liver and kidney histopathology between the SSE groups and the control group, illustrations are not provided in this publication, but will be provided upon request.)

3.3. SSE improves paracetamol-induced acute hepatic injury in mice

3.3.1. Liver body weight of mice:

The effect of SSE on mouse liver weight in paracetamol-induced acute hepatic injury was shown in Table 6.

Table 6. Effect of SSE on mouse liver weight in paracetamol-induced acute hepatic injury

Groups	n	Liver *(g/100 g body weight)*	% Reduction compared to (2)	P compared to (2)	P compared to (1)
Normal control (1)	10	**5.2 ± 0.3**		< 0.05	
Paracetamol control (2)	11	6.1 ± 0.3			< 0.05
Silymarin (0.2 g/kg) (3)	12	**6.1 ± 0.2**	0.2	> 0.05	< 0.05
SSE (0.5 g/kg) (4)	11	**6.3 ± 0.1**	-2.9	> 0.05	< 0.05
SSE (1.0 g/kg) (5)	12	**6.0 ± 0.3**	2.4	> 0.05	< 0.05

The results of Table 6 showed that paracetamol increased markerly liver weight of the mice in all groups compared with the normal group (P < 0.05). There was no significant

difference about liver weight of the mice in SSE groups (0.5 g/kg and 1.0 g/kg) and silymarin (0.2 g/kg) compared to paracetamol-treated group. Thus, SSE at both doses did not have the effect on paracetamol-induced increase in mouse liver weight.

3.3.2. Levels of transaminases:

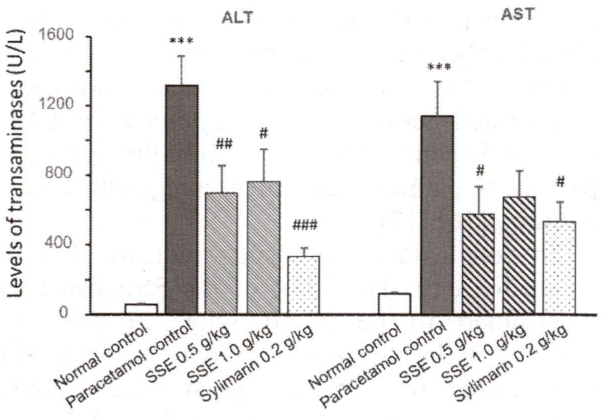

Fig. 1. Effect of SSE on alanine aminotransferase (ALT), aspartate aminotransferase (AST) in mice pretreated SSE prior to paracetamol-induced acute hepatic injury.
**** $P < 0.001$ compared to normal control; # $P < 0.05$; ## $P < 0.01$; ### $P < 0.01$ compared to paracetamol control. n = 10-12 mice/group.*

The results of Fig. 1 showed that the serum levels of ALT and AST in the of paracetamol group increased significantly compared to the normal control group ($P < 0.001$). The serum levels of ALT and AST in silymarin group (0.2 mg/kg) decreased significantly compared to the paracetamol control, percentage reduced was 56.4% and 53.7% respectively, ($P < 0.001$ and $P < 0.05$%). Mice with acute liver injury caused by paracetamol were treated with 0.5 g/kg SSE had a significant inhibitory effect on the increase of ALT and AST enzyme activities ($P < 0.01$ and 0.05, respectively) compared to the control group. The percentage decreased, compared to the control group, was 47.5% and 49.6%, respectively. Mice with acute liver injury caused by paracetamol were treated with 1.0 g/kg SSE also inhibited the increase in the serum ALT and AST (percentage reduced was 42.7% and 41.3%, respectively). However, only the level of ALT enzyme reached statistical significance ($P < 0.05$).

3.4. SSE improves paracetamol-induced chronic hepatic injury in mice

3.4.1. Levels of transaminases:

The effect of SSE on the weight of paracetamol-chronic hepatic injury in mice was shown in Table 7.

Table 7. Effects of SSE on mouse liver weight in paracetamol-induced chronic hepatic injury

Groups	n	Liver *(g/100 g body weight)*	% reduction compared to (2)	P compared to (2)	P compared to (1)
Normal control (1)	10	4.73 ± 0.3		<0.01	
Paracetamol control (2)	13	6.74 ± 0.5			< 0.01
Silymarin (0.2 g/kg) (3)	13	6.46 ± 0.2	4.2	> 0.05	< 0.001
SSE (0.5 g/kg) (4)	12	6.67 ± 0.3	1.1	> 0.05	< 0.001
SSE (1.0 g/kg) (5)	11	6.85 ± 0.3	-1.6	> 0.05	< 0.001

The results of Table 7 showed that paracetamol increased the liver weight of mice in all groups compared with the normal group ($P < 0.01$), the SSE (0.5 and 1.0 g/kg) and silymarin (0.2 g/kg) all had liver weight similar to the paracetamol groups. Thus, SSE at both dose levels did not inhibit the increase in liver weight of mice caused by long-term paracetamol.

3.4.2. Levels of transaminases:

The results of Fig. 2 showed that serum level of ALT and AST in the group of paracetamol mice increased significantly compared with the normal control group ($P < 0.001$). The serum level of ALT and AST in the silymarin group (0.2 mg/kg) decreased significantly compared with the paracetamol control, the percentage decreased was 53.7% and 70.4%, respectively, reaching statistical significance with $P < 0.05$ and 0.01, respectively. Mice with chronic liver injury caused by paracetamol were treated with 0.5 g/kg SSE exhibited the decrease of levels of ALT and AST enzyme with a percentage reduction of 52.5 % and 43.6% compared to the control group, respectively. However, only the decrease in ALT enzyme level reached statistical significance ($P < 0.05$), while at the dose of 1.0 g/kg SSE, only the decrease in AST enzyme level reached the statistical significance ($P < 0.05$).

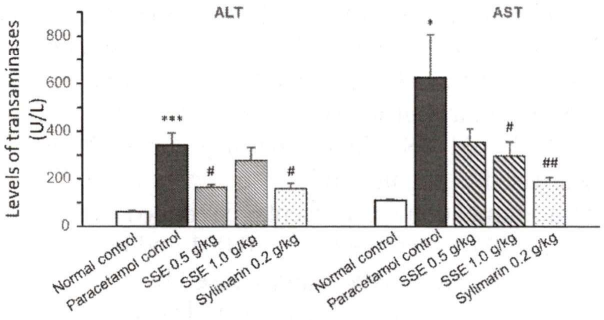

Fig. 2. Effect of SSE on the serum ALT and AST in paracetamol-induced chronic hepatic injury in mice. *** $P < 0.001$; * $P < 0.05$ compared to normal control; # $P < 0.05$; ## $P < 0.01$ compared to paracetamol control; $n = 10$-13 mice/group.

Paracetamol is the most commonly used as mild analgesic and antipyretic drug worldwide [12]. When administered in high doses or repeated doses, paracetamol can cause severe liver injury, liver necrosis, and kidney damage in human beings and animals [13],[14]. Therefore, paracetamol-induced liver damage in animal has been used as a hepatotoxicity model for testing phytotherapeutic and other hepatoprotective interventions [10],[11]. In this study, acute and long-term paracetamol treatment was found to cause liver damage as indicated by the high level of serum liver enzymes of ALT and AST. SSE or sylimarin treatment was able to reduce serum liver enzymes level indicating that liver injury caused by paracetamol was improving after treatment with SSE or sylimarin. The results of our study are also consistent with other publications in recent years.

It has been found that *S. sarmentosum* can improve liver injury caused by LPS/D-GalN in mice via by the mechanism related to the increase in miR-124 expression, decrease in Hedgehog and HMGB1 signaling pathway activities, and reduction in inflammatory responses in the liver [15]. Furthermore, some lines of evidence have reported *S. sarmentosum* total flavanones ameliorated *Schistosomiasis japonica*- or CCl_4-induced hepatic fibrosis by inhibiting the TGF-β1/Smad7 pathway and intervening with Smads pathway [16],[17].

In conclusion, our results indicate that: 1) Acute toxicity: The LD_{50} of the SSE has been determined to be 25.56 g/kg. 2) Subacute toxicity: It has been determined that the SSE with doses of 275 mg/kg and 825 mg/kg administered orally continuously for 30 days in rats did not affect the systemic condition, body mass development, hematopoietic function, liver function, kidney function, gross organ and histopathology. 3) Hepatoprotective effect: SSE has a protective effect on liver injury in both acute and chronic models of paracetamol-induced damage in mice, demonstrating its ability to reduce the serum level liver activity ALT and/or AST at the experimental doses of 0.5 g/kg and 1.0 g/kg.

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ANTIOXIDANT, ANTI-HYPERGLYCEMIC AND ANTI-INFLAMMATORY EFFECTS OF *VIBURNUM LUTESCENS* BLUME

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Summary

Antioxidant, Anti-Hyperglycemic and Anti-Inflammatory Effects of *Viburnum lutescens* Blume

Viburnum lutescens Blume, a Caprifoliaceae family member epidemic to Vietnam is a scarce medication used in traditional medicine to treat ailments such as rheumatism, injuries, measles, and ovarian obstruction. However, there are not many research papers on the properties and chemical components of this plant. Hence, our study is the first to assess the pharmacological capabilities of *Viburnum lutescens* including: anti-inflammation, anti-hyperglycemia and antioxidant that could be used to prevent some chronic illnesses. In our study, 3 extracts of this plants including methanol (MeOH), ethylacetate (EtOAc), and dichloromethane (CH_2Cl_2) were evaluated. The results show that the EtOAc extract performed distinguished properties. We also found that 6α-hydroxybetulinic (VL2), a triterpene acid isolated from *V. lutescens* exhibited a high anti-inflammatory activity against the inhibition of NO production in LPS-activated RAW264.7 cells with IC_{50} value of 56.09 ± 4.76 μM.

Keywords: *Viburnum lutescens, Bioactivity, Antioxidant, Antihyperglycemic, Anti-inflammatory.*

1. Introduction

Inflammation is a pivotal physiological response that aids in the defense of the body against dangerous external agents such as physical or chemical agents, pathogens, and viruses. This kind of response is classified into two main stages: acute and chronic inflammation [1]. The visible manifestations of inflammation are redness, heat, swelling and pain. These symptoms result from the dilation of blood vessels that increases blood flow to the damaged site as well as the massive recruitment of immune cells [1]. Nitric oxide (NO) is an omnipresent signaling molecule in the body and is synthesized by a wide range of NO synthase (NOS) from endothelial cells, and neurons through the breakdown of arginine to citrulline and NO [2],[3]. Moreover, NO is considered an anti-inflammatory agent in the normal condition. On the other hand, when its production is impaired, NO acts as a pro-inflammatory mediator that triggers inflammatory [4].

Many diseases were proved as the repercussion of inflammation including diabetic complications. Type 2 diabetes mellitus (T2DM) is the most common endocrine disease in the world and in Vietnam. T2DM is associated with low levels of peripheral glucose uptake and increased endogenous glucose production as the result of insulin resistance, which leads to numerous complications [5].

In recent time, there is a huge effort to find the active compounds that relent the pathogenesis of inflammation and diabetes mellitus. According to a research, the majority of world's population employs traditional medicine, in another word, herbal extracts to treat acute or chronic ailments [5],[6]. Vietnam is a tropical country with many untapped medicinal resources. *Viburnum lutescens* (Vót vàng nhạt or Giáng cua), a Caprifoliaceae family member endemic to Vietnam has been traditionally used to treat rheumatism, injuries, measles, and ovarian obstruction [7]. Based on our knowledge, there is no study about the anti-inflammatory and anti-hyperglycemic activities of this plant. Hence, this work aims to provide a general view of these properties via investigating antioxidant, anti-hyperglycemic assessment, and anti-inflammatory effects of this plant.

2. Materials and methods

2.1. Sample collection and preparation