EVALUATION OF ACUTE TOXICITY AND HEPATOPROTECTIVE EFFECTS OF CAPSULES FROM MEDICINAL HERBS ON A MODEL OF CHRONIC LIVER INJURY INDUCED BY ETHANOL

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Received: 2 May 2025; Revised: 19 May 2025; Accepted: 31 May 2025

ABSTRACT

The present study attempts to explore the hepatoprotective effects of capsules containing dry extracts of Turmeric, Bitter melon, *Phyllanthus urinaria* L., *Psidium guajava* L. leaves, *Kaempferia parviflora* Wall. Ex Baker, and *Curcuma aromatica* Salisb. on a Swiss white mouse of chronic liver damage caused by ethanol with increasing concentrations (10%, 20%, 30%, 40%). The results showed that the capsule had a protective effect on the mice's liver against ethanol-induced damage and did not show acute toxicity on any experimental mice organs. Specifically, after 2 and 4 weeks of using the capsule at oral doses of 1 capsule/kg and 2 capsules/kg, the increase in AST and ALT activities in mouse plasma was inhibited. The capsule also reduced the MDA content and restored the endogenous GSH content in the mouse liver to near normal levels.

Keywords: Hepatoprotective activity, acute toxicity, ethanol-induced liver injury, AST, ALT, MDA, GSH.

1. INTRODUCTION

Alcoholic liver disease (ALD) is the leading cause of alcohol-related deaths worldwide [1]. More than 95% of the alcohol absorbed into the body will be metabolized by the liver, the rest will be excreted through sweat and urine. During the process of alcohol metabolism, free radicals are created that cause peroxidation, which damages liver cells, leading to a number of alcoholic liver diseases such as fatty liver, liver fibrosis, hepatitis, liver cancer, etc. Currently, among the methods of treating liver diseases, research and development of new drugs from medicinal herbs is a potential approach and attracts much attention from scientists around the world. Many medicinal herbs and active ingredients extracted from them are reported to be effective in treating hepatitis due to many different causes [2-4]. Research on the development of drugs from medicinal herbs with liver-protecting effects is increasingly being studied. Polyphenols found in plants such as beta-carotene, curcumin, ellagic acid, quercetin, etc. have a liver-protective effect by reducing the toxic effects of alcohol on liver metabolism through antioxidant, free radical scavenging, anti-inflammatory, anti-fibrotic, anti-mutagenic and antimutagenic and lipotropic actions, all of which are of significant use in elderly people [5]. In traditional medicine, turmeric, Thai black ginger (*Kaempferia parviflora* Wall. Ex Baker), Curcuma aromatica (*Curcuma aromatica* Salisb.), Chamber bitter (*Phyllanthus urinaria* L.), bitter melon (*Momordica charantia* L.), and guava leaves (*Psidium guajava* L.) are all polyphenol-rich medicinal herbs that have long been used to support the treatment of liver

damage. This study developed a hard capsule formula from these medicinal herbs and evaluated the liver-protective, liver-damage-recovery and antioxidant effects of the hard capsule in an experimental model

2. MATERIALS AND METHODS

2.1. Materials

Experimental animals: White Swiss albino mice (20 ± 2 g) were provided by Pasteur Institute of Ho Chi Minh City. Mice were fed pellet food, ad libitum water drinking, and allowed to settle in the pharmacology laboratory for at least one week prior to testing.

Chemicals: DPPH (2,2-Diphenyl-1-picrylhydrazyl, China), methanol (China), ascorbic acid (China), distilled water (Vietnam), ethanol (China), HAMEGA (Nam Duoc JSC), AST, ALT quantitative kit (Human - Germany), 1.15% KCl buffer solution, Tris-HCl buffer (Merck, Germany), trichloroacetic acid (TCA), Thiobarbituric acid (TBA) (Merck - Germany), Ellman reagent [5,5'-dithiobis - (2-nitrobenzoic acid)] (Sigma, USA).

Equipments: Analytical balance, technical balance, Elisa plate reader, UV-Vis spectrophotometer, Semi-automatic biochemistry analyzer, centrifuge, refrigerator.

Research object: Hard capsules containing 370 mg of herbal extracts including *Curcuma aromatica* extract, Thai black ginger extract and dried extracts of Turmeric, bitter melon, Chamber bitter, Guava leaves provided by Biogreen Biotechnology and Pharmaceutical Chemistry Joint Stock Company. The capsule manufacturing process is shown in Figure 1.

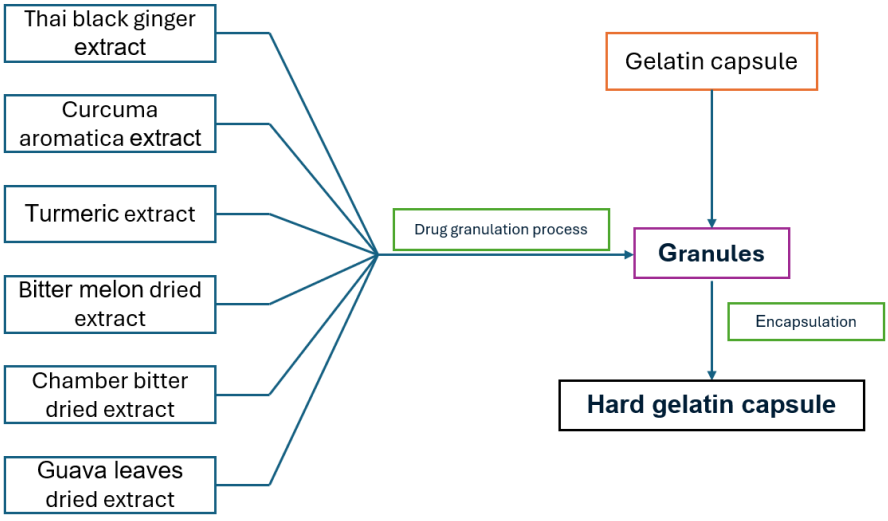

Figure 1. Experimental hard capsule preparation process

2.2. Methods

2.2.1. Acute toxicity studies in experimental animal models

Swiss albino mice (20 ± 2g) were randomly divided into groups of 6 mice (3 males and 3 females) each. The mice were given the drug powder in capsules orally at increasing doses (0.2 mL/10 g body weight of a solution) to determine the lowest dose that killed 100% of the mice and the highest dose that did not kill the mice. The mice were fasted for 12 hours before being given the test sample. All movements, expressions, and deaths of the mice were

monitored and recorded within the first 72 hours and continued to be monitored for up to 14 days. The number of surviving mice was recorded after 14 days of observation, and dead mice were dissected and observed grossly [6, 7].

2.2.2. Evaluation of antioxidant activity

The powder in the capsules was tested for antioxidant capacity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging method [8]. The powder was dissolved in MeOH and then centrifuged at 2000 rpm for 5 min to remove the insoluble excipients. The clear solution was diluted to 6 concentrations of 1000, 500, 250, 125, 62.5 and 31.25 µg/mL. Ascorbic acid was used as a positive control. The test procedure was presented in Table 1.

Table 1. Procedure for evaluating DPPH free radical scavenging activity

Ingredients	Test sample	Blank test sample	Control sample	Blank control
Capsule/ acid ascorbic (µL)	100	100		
DPPH 0.2 mM (µL)	100		100	
Methanol (µL)		100	100	200
Incubate for 20 minutes at 37 ^{o}C → Measured spectrophotometrically at 517 nm				

2.2.3. Ethanol-induced liver damage model

The experimental mice were randomly divided into 5 groups (n = 8), the experiment lasted 5 weeks: normal group (given distilled water), model group (given distilled water and ethanol), treated group I (given 1 capsule/kg test capsule and ethanol), treated group II (given 2 capsules/kg test capsule and ethanol) and control group (given 1 capsule/kg HAMEGA and ethanol). After 1 hour of giving the mice the capsule or HAMEGA capsule, the mice continued to drink ethanol with increasing concentrations every week (10%, 20%, 30%, 40%) with a drinking volume of 10 mL/kg mouse body weight, within 4 weeks. At week 5, the mice in the treatment and control groups continued to be treated, but were not given ethanol. Venous blood was collected from mice to test AST and ALT activities after 2 weeks and after 4 weeks in the experimental group (according to the AST and ALT quantification kit) [9].

Determination of malondialdehyde(MDA) and glutathione (GSH) levels in the liver of mice: After 5 weeks, the livers of mice in each group (1 hour after drug administration) were isolated to determine the malonyl dialdehyde (MDA) and glutathione (GSH) content (Fang-Ping Liu, 2016). The mouse livers were ground in 1.15% KCl buffer solution and centrifuged at 13,000 rpm for 1 minute. 2 mL of mouse liver homogenate was taken to quantify MDA levels and 1 mL of homogenate was taken to quantify GSH levels; Tris buffer solution (pH = 7.4) was added to make 3 mL. The mixture was incubated at 37 ^{o}C for 60 minutes and the reaction was terminated with 1 mL of 10% trichloroacetic acid (TCA). Then, the mixture was centrifuged at 10,000 rpm for 10 minutes at 5 ^{o}C [9].

MDA quantification: Take 2 mL of the centrifuged solution and react with 1 mL of 0.8% thiobarbituric acid at 100 ^{o}C for 15 minutes and measure the optical density at λ = 532 nm. MDA content (nM/g protein) is calculated by the linear regression equation of the MDA standard [10, 11]. MDA concentration in the homogeneous solution is calculated by the formula:

$$C_{MDA\ (nM/ml)} = (C_{MDA\ (nM/ml)} * 1000) / C_{protein}$$

In there: Cprotein = 36,629 ($mg_{protein}$ /$mL_{protein}$) [12].

GSH quantification: After centrifugation, take 1 mL of the homogeneous solution and react with 0.2 mL of Ellman's reagent, which is 5,5'- dithiobis-(2-nitrobenzoic acid) and add phosphate buffer - EDTA (pH 7.4) to make 3 mL. Leave for 3 minutes at room temperature and then measure the optical density at a wavelength of λ = 412 nm. GSH content (nM/g protein) is calculated according to the linear regression equation of the GSH standard [11].

2.2.4. Statistical analysis

The collected data were processed using biomedical statistics according to T test in SPSS 25.0 software. The results are presented in the form of $\overline{X} \pm$ SD. The difference is statistically significant when $p \leq 0.05$

3. RESULTS

3.1. Acute toxicity studies in experimental animal models

Mice were given capsules at a dose of 100.500 g/kg with a volume of 0.2 mL/10 g of body weight. After the first 24 hours, all mice in all groups were active, agile, ate well, had dry feces, smooth fur, and no mice died or showed any abnormal signs. Continued observation for 48 and 72 hours did not show any toxic symptoms and there were no deaths. After 14 days, all mice were still living normally and healthy. Mice were dissected to observe the organs in the body. The results showed no abnormal changes. The liver, kidney, heart, lung, and digestive system were similar in color and size, and showed no signs of congestion or damage compared to the normal group that did not use the drug.

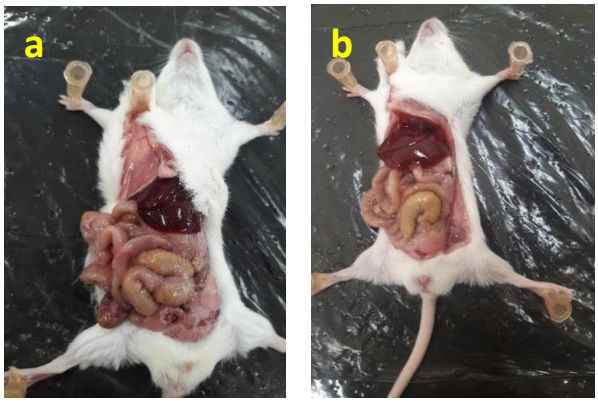

Figure 2. Gross appearance of (a) male and (b) female mice after 14 days

3.2. Antioxidant activity of capsules

Ascorbic acid and phytochemicals with strong antioxidant effects will help protect liver cells from free radicals, thereby reducing inflammation and enhancing liver health. The antioxidant capacity of the capsule was compared with ascorbic acid, using the DPPH free radical scavenging model, the antioxidant activity results of the capsule and ascorbic acid are shown in Table 2, Table 3 and Figure 2, respectively.

Table 2. Results of antioxidant activity survey of capsules

Concentration (µg/mL)	32.25	62.5	125	250	500	1000
Free radical scavenging percentage (%)	47.79 ± 0.71	51.13 ± 2.73	53.07 ± 2.02	59.07 ± 5.63	63.65 ± 3.34	76.32 ± 1.02

The free radical scavenging ability of the capsule was directly proportional to the concentration. The antioxidant capacity of the capsule was expressed by the regression equation y = 0.0277x + 49.403; R^2 = 0.9733. At a concentration of 21.55 µg/mL, the capsule was able to scavenge 50% of the DPPH free radicals (IC_{50} = 21.55 µg/mL).

Table 3. Results of antioxidant activity of ascorbic acid

Concentration (µg/mL)	0.625	1.25	2.5	5	10
Free radical scavenging percentage (%)	4.99 ± 0.42	8.29 ± 1.15	17.66 ± 0.34	52.04 ± 0.29	85.19 ± 0.78

The antioxidant capacity of ascorbic acid was expressed through the linear regression equation y = 8.9131x – 0.9044; R^2 = 0.9794. The results of the study showed that the capsule had good antioxidant activity when compared with ascorbic acid (IC_{50} = 5.71 µg/mL).

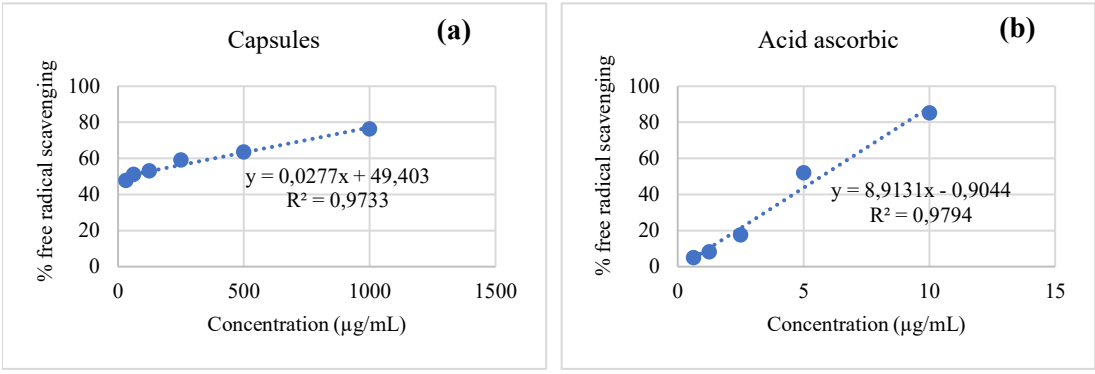

Figure 3. Regression equations showing the antioxidant activity of (a) capsules, (b) ascorbic acid

3.3. Hepatoprotective effects of capsules in experimental animal models

3.3.1. AST and ALT activities in plasma of ethanol-induced liver injury in mouse

Table 4. AST-ALT activities in plasma of experimental mouse groups

Groups	AST activity (U/L)		ALT activity (U/L)	
	2 weeks	4 weeks	2 weeks	4 weeks
Model group	51.67 ± 3.06	71.07 ± 5.18	65.10 ± 2.13	69.83 ± 2.15
Normal group	43.33 ± 1.53*	42.10 ± 2.71**	45.13 ± 1.11***	43.50 ± 1.32***
Control group	44.23 1.67*	47.43 ± 3.11$^{*\#}$	49.17 ± 2.47*	53.67 ± 1.53$^{***\#}$
Treated group I	45.37 ± 3.20$^{*\#}$	46.40 ± 1.44$^{**\#}$	48.60 ± 2.16**	50.13 ± 3.44$^{**\#}$
Treated group II	44.00 ± 2.65*	43.07 ± 2.57$^{**@}$	43.80 ± 1.21$^{***@\wedge}$	47.20 ± 1.73$^{***\#@@}$

*, **, ***: *Differences from model group with $p < 0.05$; $p < 0.01$, $p < 0.001$*
$^{\#}$: *Differences from normal group with $p < 0.05$*
$^{@}$, $^{@@}$: *Differences from control group with $p < 0.05$; $p < 0.01$*
$^{\wedge}$: *Differences between treated group I and treated group II with $p < 0.05$*

The results of Table 4 show that the AST activity in plasma after 2 and 4 weeks of testing of the model group both increased significantly with values of $p < 0.05$ and $p < 0.01$, respectively, compared to the normal group at the same time of the survey. The diseased mice were given

HAMEGA and the drug tested at both doses, which showed a decrease in AST activity. Of which, after 2 weeks, the treated group I had a statistically significant effect on reducing the activity of this enzyme compared to the model group and normal group ($p< 0.05$). After 4 weeks, all 3 groups: treated group I, treated group II and control group had statistically significant effects in reducing AST activity compared to the model group at the same time of survey. At the same time, both experimental doses of the capsule showed the same effect of reducing AST-ALT liver enzyme activity as HAMEGA at a dose of 1 capsule/kg, bringing the plasma AST activity value of the liver-damaged mice group close to the value of the normal group.

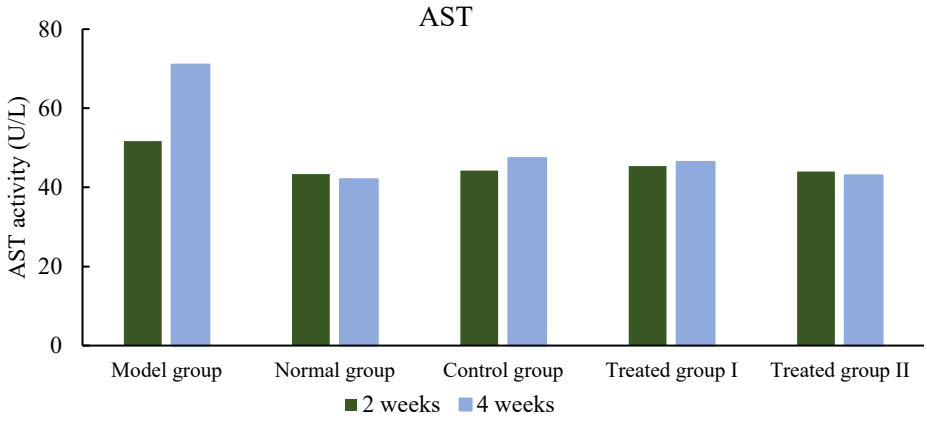

Figure 4. AST activity in mouse plasma of experimental groups

In addition, the ALT activity in the liver of mice given the experimental capsule also decreased significantly in the 2nd and 4th weeks. At a dose of 1 capsule/kg, the capsule reduced the ALT activity in the liver of mice by 25.35% and 28.21% after 2 and 4 weeks of testing, respectively. At a dose of 2 capsules/kg, the enzyme activity in the liver of mice decreased by 32.72% and 32.41%, respectively, compared to the model group. The above results showed that both the group given the experimental capsule and the control drug (HAMEGA) had the effect of reducing liver enzymes to near normal levels when compared to the normal group, the result was statistically significant with $p< 0.05$ (Table 4). Thus, the experimental capsules at all tested doses had the effect of reducing the activity of liver enzymes AST-ALT in plasma in mice with chronic liver damage caused by ethanol.

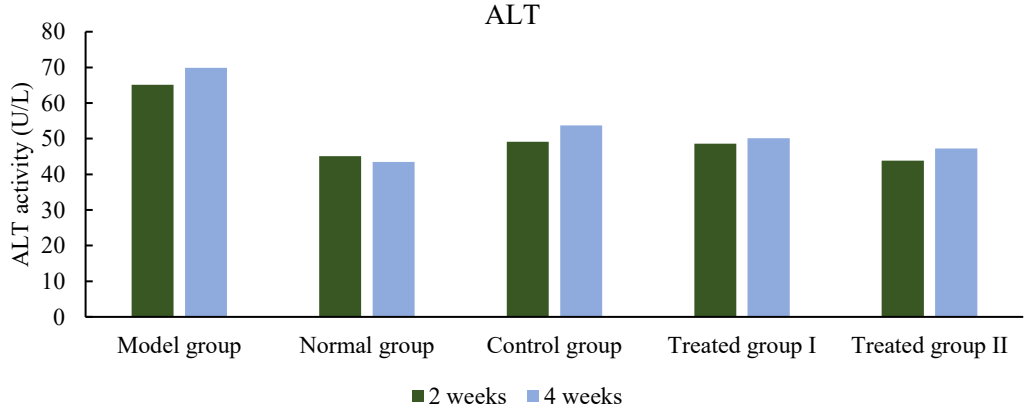

Figure 5. ALT activity in mouse plasma of experimental groups

3.3.2. MDA and GSH levels in the livers of mice with ethanol induced liver injury

Table 5. MDA and GSH concentrations in the liver of mice with ethanol-induced liver injury

Group (n= 8)	MDA content (nM/g protein)	GSH content (nM/g protein)
Model group	69.51 ± 3.33	5973.73 ± 304.04
Normal group	40.87 ± 1.07***	8159.77± 105.56***
Control group	43.87 ± 2.11***	7793.78 ± 211.75
Treated group I	44.20 ± 1.26***, #	7639.47 ± 307.02**
Treated group II	42.43 ± 3.23**	7934.24 ± 356.11**

, **, *: Differences from the model group with p < 0.05; p < 0.01 and p < 0.001 respectively*
#, ##, ###: Differences from the normal group with p < 0.05; p < 0.01 and p < 0.001 respectively

Long-term regular use of ethanol increases the enzyme P450 (CYP)2E1, which participates in the metabolism of ethanol to create free radicals in lipids. This is the cause of a series of cell lipid peroxidation reactions that cause destruction of the cell membrane structure, leading to increased MDA levels.

The results of giving ethanol to mice daily (model group) with increasing doses over the week increased the MDA content in the liver homogenate of mice with statistical significance compared to the normal group ($p < 0.001$), and at the same time reduced the GSH content with statistical significance compared to the normal group ($p < 0.001$).

The group of mice given HAMEGA capsules (liver protection, liver detoxification due to alcohol) at a dose of 1 capsule/kg had a statistically significant decrease in MDA content compared to the model group ($p < 0.001$). The group of mice given capsules tested at both experimental doses of 1 capsule/kg ($p < 0.001$) and 2 capsules/kg ($p < 0.01$) both showed the effect of reducing the content in the liver, statistically different from the model group. There was no difference when comparing the MDA concentration in the liver homogenate between the treated group I and treated group II ($p > 0.05$).

GSH is an endogenous tripeptide produced by the liver and is considered a reserve of antioxidants and slows down the aging process through its participation in the neutralization of free radicals in the body. A study by Italian scientists in 1995 reported that GSH was most effective when given intravenously to people with fatty liver disease at high doses, significantly improving the rates of some liver tests after several months of treatment [13].

The results in Table 5 also show that the GSH concentration decreased significantly in the model group compared to the other 4 groups. There was no difference when comparing the GSH concentration in the liver homogenate between the treated group I and treated group II ($p > 0.05$). The experimental capsule at both study dose levels tended to increase the GSH concentration in the liver homogenate and there was a statistically significant difference when compared to the model group ($p > 0.01$).

4. CONCLUSION

The research results have demonstrated that the experimental medicinal capsule has no abnormal toxicity in experimental mice. At the same time, at the two experimental doses, it has a liver-protecting effect, reducing the activity of liver enzymes AST-ALT in plasma and reducing the content of MDA, inhibiting the process of lipid peroxidation in liver cells in the model of chronic liver damage in mice by ethanol and showing the effect of restoring the content of GSH in the liver. Thus, through the research results, it is shown that the capsule derived from

medicinal herbs (including extracts of Curcuma aromatica, Thai black ginger, Turmeric, Bitter melon, Chamber bitter, Guava leaves) is safe with the effect of protecting the liver.

Acknowledgements: This research was financially supported by Life Gift Vietnam Co.,Ltd and the Science and Technology Development Fund - Nguyen Tat Thanh University. The authors gratefully acknowledge their support, which made this research possible.

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TÓM TẮT

ĐÁNH GIÁ ĐỘC TÍNH CẤP VÀ TÁC DỤNG BẢO VỆ GAN CỦA VIÊN NANG TỪ THẢO DƯỢC TRÊN MÔ HÌNH TỔN THƯƠNG GAN MÃN TÍNH DO ETHANOL

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Nghiên cứu đánh giá tác dụng bảo vệ gan của viên nang chứa chiết xuất khô của Nghệ, Mướp đắng, Diệp hạ châu, lá Ổi, Ngải đen và Nghệ trắng trên mô hình chuột nhắt trắng Thụy Sĩ bị tổn thương gan mãn tính do ethanol với nồng độ tăng dần (10%, 20%, 30%, 40%). Kết quả cho thấy viên nang có tác dụng bảo vệ gan chuột chống lại các tổn thương do ethanol gây ra và không biểu hiện độc tính cấp tính trên bất kỳ cơ quan nào của chuột thí nghiệm. Cụ thể, sau 2 và 4 tuần sử dụng viên nang với liều uống 1 viên/kg và 2 viên/kg đã cho thấy sự giảm hoạt động của enzym AST và ALT trong huyết tương chuột. Ngoài ra, viên nang cũng làm giảm hàm lượng MDA và khôi phục hàm lượng GSH nội sinh trong gan chuột về gần mức bình thường.

Từ khóa: Hoạt động bảo vệ gan, độc tính cấp tính, tổn thương gan do ethanol, AST, ALT, MDA, GSH.