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Hepatitis treatment potential of *Echeveria elegans* extract in *Mus musculus* var. *albino* with CCl_4-induced liver injury

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Abstract

This study investigated the potential hepatoprotective effects of *Echeveria elegans* extract against CCl_4-induced liver injury in albino mice (*Mus musculus* var. *albino*). Mice were divided into 6 groups: control, *E. elegans* extract 100% (SD100), CCl4, silymarin (standard drug), CCl_4 + *E. elegans* extract 25% (CCl_4.SD25), and CCl_4 + *E. elegans* extract 50% (CCl_4.SD50). After 6 weeks, growth performance parameters like weight gain, average daily gain, and specific growth rate were measured. Serum levels of AST, ALT, and bilirubin were analyzed as biochemical indicators of liver function. Histological examination of liver tissues was also performed. The CCl_4 group showed signs of liver injury like increased liver size, pale color, and white spots. However, the *E. elegans* extract groups, especially CCl_4.SD50 demonstrated significant improvement in gross liver morphology and histological architecture, comparable to the silymarin group. While serum enzyme levels did not differ significantly between groups.The CCl_4.SD50 group showed a greater reduction in bilirubin levels than silymarin. The findings suggest that *E. elegans* extract, particularly at 50% concentration, exhibits promising hepatoprotective potential against CCl_4-induced chronic liver injury, warranting further investigation into its therapeutic efficacy and mechanism of action.

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Introduction

In the human body, the liver is the largest internal organ in the body, an essential organ of life because it performs many metabolic activities necessary for homeostasis, nutrition, detoxification, and excretion of many endogenous and exogenous compounds (Nguyen*et al.*, 2012). In liver diseases, hepatitis can be considered the most common disease caused by many causes such as bacteria, viruses, parasites, alcohol, drugs, or toxic chemicals... (Nguyen*et al.*, 2005). In particular, the CCl_4 hepatotoxicity model used as a model for inducing liver damage in animals is common in studies evaluating the protection and recovery of liver damage by drugs with symptoms similar to acute viral hepatitis and alcohol use (Crespo Yanguas *et al.*, 2016; Lieber, 1990; Nguyen*et al.*, 2012). Flavonoids and tannins in many plant species have been rated beneficial in treating and restoring the liver(Elmasry and Moawad, 2021; López-Angulo *et al.*, 2022; Phan*et al.*, 2016, 2019).

E. elegans belongs to the Crassulaceae family with ingredients such as alkaloids, flavonoids, glycosides, saponins, steroids, and tannins that have effects such as analgesic, anti-hyperglycemia, anti-inflammatory and so on(Nair *et al.*, 2016). Although the extract of *E. elegans* has good components for health, there have not been many studies on its ability to treat certain diseases, especially its ability to treat hepatitis.

The study aimed to assess the ability of *E. elegans* extract to protect against and treat chronic liver injury induced by carbon tetrachloride (CCl_4) in an albino mouse model. It examined the extract's impact on various growth, biochemical, and histological markers to determine its potential as a hepatoprotective agent.

Material and methods

Extract preparation

E. elegans was purchased in Da Lat City (Vietnam). Puree 240g of fresh plant leaves, shake them gently for a few minutes and filter through two layers (coarse cloth and filter paper) to obtain the extract. This extract is stored at a temperature of 0-5°C and used during the day. This extract is mixed into 3 concentrations of 25%, a concentration of 50%, and a concentration of 100%. *E. elegans* extractfor oral administration corresponded to the weight of mice of 1mL/10g of body weight, up to 0.5 mL.

Laboratory animals

Albinomouse (*Mus musculus* var. *albino*) 4 weeks old purchased from the Pasteur Institute Ho Chi Minh City, Vietnam. Micewere stably raised at the Human and Animal Anatomy and Physiology Laboratory (Ho Chi Minh City University of Education) for 3 weeks to reach 20-32g corresponding to 7 weeks of age with a 12-hour light-evening cycle (light on at 6:30am and light off at 4:30pm) at room temperature from 27-29°C, humidity above 78%.Mice were fed a standard rodent chow diet and had free access to tap water.

Experimental procedures and sampling

Mice meeting the experimental criteria (20-32 g) were divided into 6 experiments, of which group 1 was controlled: Mice drank only dechlorinated tap water; group 2 (SD100) mice were given *E. elegans* extract100%; group 3 (CCl_4): mice were given CCl_4 at a concentration of 2.5 mg/kg body weight/day (once a week) with olive oil in a ratio of 1:4; group 4 (Silymarin): mice given CCl_4 + olive and silymarin at doses of 20 mg/kg body weight/day; group 5 (CCl_4.SD25): mice were given CCl4 + olive and *E. elegans* extract 25%; group 6 (CCl_4.SD50): mice were given CCl_4 + olive and *E. elegans* extract 50%.

Mice were given a solution of CCl_4(CAS: 56-23-5, Merck), Silymarin, and *E. elegans* extract by using a 1mL cylinder attached to a specialized needle tip injected directly into the esophagus at the survey dose. Through calculations, the volume of solution for mice to drink should not exceed 0.5mL/1 time. The time for mice to drink the CCl_4 solution at 6 a.m. to 7 a.m. every Monday takes place 30 minutes before feeding. Time for mice to drink Silymarin (for group 3) or *E. elegans* extract (for groups 5 and 6) at 5:30-6:00 p.m. every day, which takes place 30 minutes after feeding. Feeding time for mice each day is 6:30-7:00 am and 5:00-5:30 p.m. After giving the mice a

solution of CCl_4, silymarin, or *E. elegans* extract, monitor and record the mice's abnormal signs daily in a diary.

After 6 weeks, mice were given serum blood collection to determine biochemical parameters (AST, ALT, and total bilirubin). The mice were then sacrificed, and their livers were harvested for gross examination and histological analysis.

Growth performance

To assess the growth performance of mice, their body weight gain, average daily gain, and specific gain ratio were measured. The body weight of each mouse was weighed before the start of the experiment (week 0) and then twice a week in the morning before eating. The weight of each mouse corresponded to the concentration of solutions used in each experiment. Parameters of the growth performance of each mouse at the time of the survey were calculated as follows (Tanquilut *et al.*, 2015):

Weight gain (WG, g) = final weight (g) – initial weight (g);

Average daily gain (ADG, g d^{-1}) = weight gain (g) / time (days);

Specific growth rate (SGR, % d^{-1}) = 100 ×(Ln final weight (g) – Ln initial weight(g))/time (days).

Examining the histological structure of the liver

After 6 weeks of the experiment, mice were dissected by stretching the cervical vertebra (euthanasia), the abdominal cavity was opened, and the liver of each experimental group was collected. Samples fixed in 10% formaldehyde solution supplemented with KH_2PO_4 (4 g/1000 mL), and Na_2HPO_4 (6.5 g/1000 mL) and stained with H&E. Histological structure, the level of damage of each sample was assessed through inverted microscope images (TiU, Nikon).

Biochemical analysis

Quantification of AST, ALT, total bilirubin indices in rat serum: taking mouse blood, centrifuging 10000 rounds in 10 minutes, collecting serum, reading results on Beckman Coulter's AU680 system.

Data analysis

The data was analyzed using ANOVA one way and the significant difference between treatments was determined by the Turkey test ($p<0.05$). Mean values were reported as X±SD (means±standard deviation).

Results and discussion

Growth performance and some biochemical indicators of white mice causing chronic hepatitis on the CCl_4model

Growth performance in groups of experimentedmice is shown in Table 1. The results showed that most groups of mice after 6 weeks of the experiment had an increase in weight.

The weight of mice in the control group (5.96±5.38 g) showed a steady and steady increase in weight over the course of the experiment. Meanwhile, mouse weight increased significantly in the groups of mice taking CCl_4 (9.11±2.87 g), silymarin (8.68±3.91 g), and CCl_4.SD25 (9.81±4.08 g) and CCl_4.SD50 (9.77±4.65 g). At first, due to the toxic effects of CCl4, the rats were lethargic and inactive, but after 1 week, the rats were active normally and ate more, leading to a sharp increase in the weight of the mice. However, the increase in weight in these groups was not statistically significant.

Table 1. Growth performance of groups of mice after the experiment.

Experiment	WG (g)	ADG (g.d^{-1})	SGR (%.d^{-1})
Control	$5.96±5.38^A$	0.153±0.128	0.565
SD100	$-2.20±3.61^B$	-0.052±0.086	0.100
CCl_4	$9.11±2.87^A$	0.216±0.068	0.633
Silymarin	$8.68±3.91^A$	0.207±0.093	0.451
CCl_4.SD25	$9.81±4.08^A$	0.234±0.097	0.674
CCl_4.SD50	$9.77±4.65^A$	0.233±0.111	0.189

A, B – The difference is statistically significant ($p<0.05$).

There was a significant drop in mouse weight in the SD100 group (-2.20±3.61 g), a difference that was statistically significant. The cause may be that the extract at 100% concentration caused the mice to show symptoms of anorexia, drink more water, and increase activity than usual. Therefore, the mass of mice had an increase but not significantly compared to a decrease in weight.

Table 1 shows the growth performance of different groups of mice after a 6-week experiment. The experiment found that most groups of mice had an increase in weight over this period. The control group showed a steady increase in weight throughout the experiment, starting from 5.96±5.38 g. Meanwhile, the groups of mice taking CCl_4 (9.11±2.87 g), silymarin (8.68±3.91 g), and CCl_4.SD25 (9.81±4.08 g) and CCl4.SD50 (9.77±4.65 g) showed a significant increase in weight. At first, due to the toxic effects of CCl4, the rats were lethargic and inactive, but after one week they became active and ate more, leading to a sharp increase in their weight. However, this increase in weight was not statistically significant.

One group of mice, however, showed a significant drop in weight of -2.20±3.61 g. This group was exposed to the extract at 100% concentration, which caused the mice to exhibit symptoms of anorexia, drink more water, and be more active than usual. Thus, while the mice showed an increase in mass, it was not significant compared to the weight loss caused by anorexia(Elnfarawy *et al.*,2021).

Table 2. Effect of *E. elegans* extract on AST, ALT serum of white mice causing chronic hepatitis on CCl_4 model.

Experiment groups	Liver enzyme levels in the blood (U/L)	
	AST	ALT
Control	$356.7±143.5^{AB}$	$144.1±97.8^{A}$
SD100	$441.1±259.0^{AB}$	$168±62.1^{A}$
CCl_4	$442±399^{AB}$	$115.6±69.1^{A}$
Silymarin	$290.2±118.8^{B}$	$105.9±40.1^{A}$
CCl4.SD25	$410.3±130.4^{AB}$	$190.6±112.8^{A}$
CCl_4.SD50	$434±323^{AB}$	$107.5±51.3^{A}$

A, B, C – The difference is statistically significant (p<0.05).

AST and ALT are aminotransferases that are commonly used to assess the extent of liver cell damage. When liver cells are damaged, AST and ALT are released into the blood, causing their activities in the serum to increase (Gowifel *et al.*, 2020; Mi *et al.*, 2019; Tsai *et al.*,2021). Therefore, quantifying serum AST and ALT helps to assess the extent of liver damage. After 6 weeks, the serum AST and ALT enzyme levels in CCl_4-induced chronic hepatitis mice are shown in Table 2.

Table 3. Effect of *E. elegans* extract on white mouse serum bilirubin concentration causing chronic hepatitis on CCl_4model.

Experiment	Serum bilirubin content (mg/dL)		
	Bilirubin T	Bilirubin D	Bilirubin I
Control	$0.2529±0.0741^{BCDE}$	$0.0835±0.0585^{C}$	$0.1694±0.0838^{BCD}$
SD100	$0.2927±0.1083^{ABCD}$	$0.1651±0.1467^{AB}$	$0.1276±0.0693^{CD}$
CCl_4	$0.1678±0.0915^{DE}$	$0.0574±0.0241^{C}$	$0.1642±0.1548^{BCD}$
Silymarin	$0.255±0.0611^{CDE}$	$0.1032±0.01252^{ABC}$	$0.193±0.1106^{ABCD}$
CCl_4.SD25	$0.4473±0.2107^{A}$	$0.1885±0.0962^{A}$	$0.2588±0.1392^{ABC}$
CCl_4.SD50	$0.0782±0.0206^{E}$	$0.0424±0.0079^{C}$	$0.0358±0.0188^{D}$

A, B, C, D – The difference is statistically significant (p<0.05); Bilirubin T – Total bilirubin; Bilirubin D – Direct bilirubin; Bilirubin I – Indirect bilirubin.

The results showed that most of the experiments showed no significant difference. In particular, the concentration of AST and ALT of the CCl_4 group has not shown a clear difference with the control group leading to the CCl_4 group not meeting the requirements for use as a control group for the study. The concentration of AST and ALT of groups with *E. elegans* extract did not show a significant difference compared to the control group, suggesting that the *E. elegans* extract did not affect the health status of mice in the experiment. Therefore, the concentration of AST in the silymarin group, although there was a decrease, was not statistically significant.

The AST and ALT indices of the other experiments showed no clear increase or decrease, so it was not enough to prove the effect of *E. elegans* extract on AST and ALT levels in the serum of white mice with chronic hepatitis on the CCl_4 model(Mi *et al.*, 2019; Tsai *et al.*, 2021).

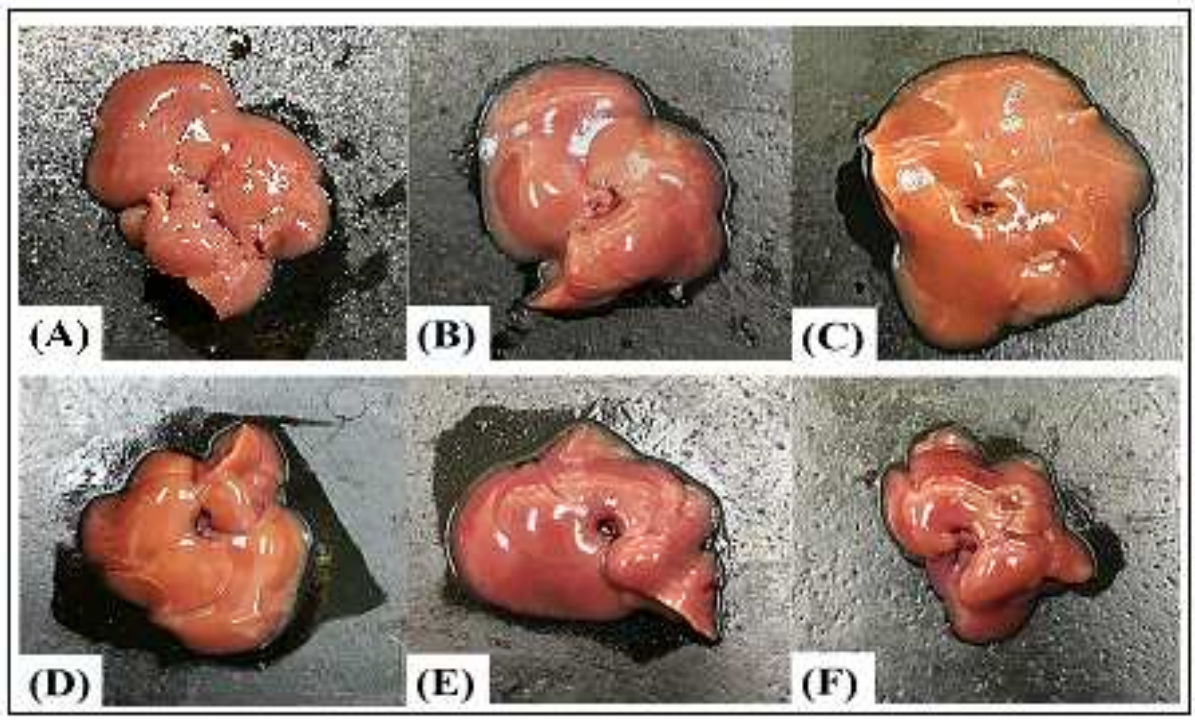

Fig. 1. The general structure of the white mouse liver after 6 weeks of experiments.
(A): Control group; (B): SD100 group; (C): CCl_4 group; (D) Silymarin group; (E): CCl_4.SD25; (F): CCl_4.SD50.

The amount of direct bilirubin (Bilirubin D) or indirect bilirubin (Bilirubin I) increases higher than normal will be able to diagnose diseases of the liver and biliary tract. When the liver is damaged, the bilirubin index will increase abnormally (Nguyen*et al.*, 2012). Therefore, bilirubin quantification helps to assess the extent of liver damage. Table 3 shows the concentration of bilirubin in the serum of white mice with chronic hepatitis on the CCl_4 model after 6 weeks of the experiment.

The results showed that most of the experiments had statistically significant differences. Of which, the concentration of total bilirubin (0.1678±0.0915 mg/dL), Bilirubin D (0.0574±0.0241 mg/dL) and Bilirubin I (0.1642±0.1548 mg/dL) of the CCl_4 group did not show a clear difference compared to the control group, leading to the conclusion that the CCl_4 group was not qualified enough to be used as a control group for the disease under study.

The concentration of Bilirubin T (0.2927±0.1083 mg/dL), Bilirubin D (0.1651±0.1467 mg/dL) and Bilirubin I (0.1276±0.0693 mg/dL) of the *E. elegans* 100% (SD100) group showed no statistically significant difference compared to the control group, indicating that the *E. Elegans* extract did not affect the health status of the mice in the experiment.

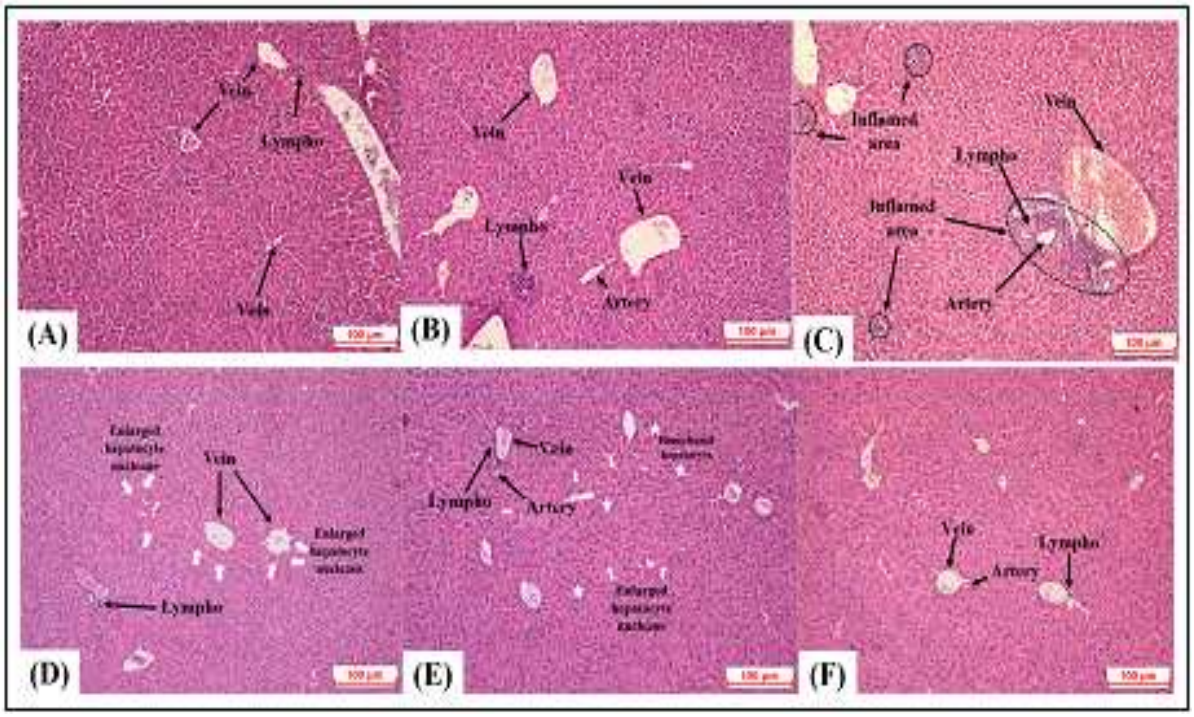

Fig. 2. Themicrostructure of white mouse liver after 6 weeks of experiment.
(A): Control group; (B): SD100 group; (C): CCl_4group; (D) Silymarin group; (E): CCl_4.SD25; (F): CCl_4.SD50.

Compared to the CCl_4 group, the indices of Bilirubin T (0.255±0.0611 mg/dL), Bilirubin D (0.1032±0.0125 mg/dL), and Bilirubin I (0.193±0.1106 mg/dL) of the Silymarin group increased but were not statistically significant. This suggests that the therapeutic effect of Silymarin in this experiment has not been demonstrated. The indices of the *E. elegans* group at concentrations of 25% (CCl_4.SD25) and 50% (CCl_4.SD50) for the treatment of hepatitis in the CCl_4 model showed the ability to reduce bilirubin. In particular, the CCl_4.SD50 group showed a greater reduction in indices when compared to the Silymarin group. However, the indices changed were not statistically significant enough. This demonstrates the long-term therapeutic potential of *E. elegans* extract.

Hepatic histological structure of white mice causing chronic hepatitis on CCl_4model

The general structure of the mouse liver differed markedly between the experiment groups. The mouse liver of the control group (Fig. 1A) and the mouse liver of *E. elegans* extract (SD100)(Fig. 1B) had a smooth, soft liver surface, clearly divided liver lobes, and a crimson color. Compared to the mouse liver of the above two groups, the mouse liver of the CCl_4 group (Fig. 1C) is larger because many liver cells are enlarged, the liver surface is less smooth and soft, and the liver lobes are divided. The lobes of the liver do not have a uniform color, most of them are pale red, and more white spots appear on the surface. Compared to the CCl_4 group, the liver of mice treated with Sylimarin (Fig. 1D) showed significant improvement in general: the surface of the liver was smoother, and white spots were no longer present, indicating a marked reduction in the degree of liver damage. The liver of micetreated with *E. elegans*extractimproved compared to the CCl_4 group and did not differ significantly from the liver of the control group. The liver surface of the micetreated with *E. elegans* extract at concentrations of 25% (Fig. 1E) and 50% (Fig. 1F) was smooth, with a paler crimson liver color than in the mouse liver of the control group. In *E. elegans* extract group at a concentration of 25% (Fig. 1F), there are also some indistinct white spots on the surface of the liver, for 50% concentration (Fig. 1E), white spots do not appear. Both mouse livers of *E. elegans* extract groupswith concentrations of 25% and 50% had a general structure similar to that of the mouse liver of the control group.

The study proceeded to evaluate the liver structure of mice after 6 weeks of the experiment. The results are shown in Fig. 2. The control group (Fig. 2A) and the Silymarin group (Fig. 2B) showed normal liver structure. In the CCl_4 group (Fig. 2C), there was a clear change in structure compared to the control group and the Silymarin group: many hepatocytes were swollen, and fatty and the location of the hepatic sinusoids could not be determined, resulting in the loss of the characteristic structure of the liver.

The macrophages were highly concentrated around the blood vessels. In contrast, the hepatic structure of the mice treated with Silymarin (Fig. 2D) showed significant changes: hepatocytes were not swollen, and lipid droplets were significantly reduced but the structure of the hepatic sinusoids was still not clearly defined.

The number of macrophages was reduced and evenly distributed throughout the liver. This proves that Silymarin is effective in treating liver inflammation, but the liver is still damaged. These results are consistent with studies by a number of authors who have studied the treatment of chronic hepatitis in the CCl_4 model (Duong*et al.*, 2016; Phan *et al.*, 2016, 2018).

In the group of mice treated with 25% *E. elegans* extract (CCl_4.SD25) (Fig. 2E) and 50% *E. elegans* extract (CCl_4.SD50) (Fig. 2F), there were signs of recovery of liver cells. In the CCl_4.SD25 group, hepatocytes were not swollen, did not contain lipid droplets, and showed a clear sinusoid structure. The phagocytic cells were no longer concentrated around the blood vessels. However, there were still some hepatocytes with large nuclei and scattered cells with two nuclei. This proves that *E. elegans* extract at a concentration of 25% can restore liver structure but not completely. In the CCl_4.SD50 group, there were signs of good recovery: the hepatocytes were all normal, not swollen, did not contain lipid droplets, and the sinusoid structure was clear. The phagocytic cells were no longer concentrated around the blood vessels (Shareef *et al.*, 2022).

Conclusion

The restoration of liver histological structure suggests that *E. elegans* extract is effective in treating hepatitis in the CCl_4 model. In particular, *E. elegans* extract at a concentration of 50% has been effective in treating chronic hepatitis. However, this effect has not been clearly demonstrated in liver indices such as AST, ALT, and Bilirubin. To evaluate more comprehensively the ability to treat chronic hepatitis, this study needs to be extended for a longer period of time.

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