EVALUATION OF THE PREVENTIVE EFFECTS OF CIRRHOSIS OF AN XOA PLANT EXTRACT (*Helicteres hirsuta* Lour) IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: To evaluate the prophylactic effects of cirrhosis of Helicteres hirsuta Lour *(HHW) in experimental animals.*

Subjects and methods: White rats were divided into four groups, each with 10 rats. Group 1: the control group drank distilled water at 10 ml/kg body weight per day. Groups 2, 3, and 4 were subcutaneously injected with CCl_4 dissolved in olive oil twice a week for 12 weeks to induce cirrhosis. Group 2: (CCl_4 group) drank distilled water at 10 ml/kg body weight per day. Group 3: (Silymarin group) drank silymarin at a dose of 0.2 g/kg body weight per day. Group 4: (HHW group) drank HHW at a dose of 1.3 g/kg body weight per day.

Results: The activities of AST, ALT, and GGT in the Silymarin and HHW groups were significantly lower than in the CCl_4 group, with $p < 0.001$ and $p < 0.05$, respectively. Histopathological images showed that 7/10 rats in the CCl_4 group developed F4 stage cirrhosis, while in the Silymarin and HHW groups, only 2/10 rats, the majority of rats had liver fibrosis at stages F2 and F3.

Conclusion: HHW at a dose of 1.3 g/kg body weight had the effect of hepatoprotective against liver fibrosis caused by CCl_4 in white rats.

Keywords: Cirrhosis, CCl_4, Helicteres hirsuta Lour*, white rats.*

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Receipt date: January 30, 2024 *Review date: February 27, 2024*
Accepted date: June 30, 2024

1. INTRODUCTION

Cirrhosis is the end stage of chronic liver disease. Prevention and treatment of cirrhosis remain challenging issues in medicine. Many traditional herbal medicines have proven to be effective in preventing and treating cirrhosis. Among them, herbal remedies containing active antioxidant ingredients have been used to protect the liver against fibrosis.

According to folk experience, the *Helicteres hirsuta* L. plant, commonly known as An Xoa, has been used to treat many different diseases such as boils, pain relief, detoxification, dysentery, influenza, smallpox, measles, malaria, poisonous snake bites, as well as liver diseases including cirrhosis and liver cancer. Some studies have also shown that the *Helicteres hirsuta* L. plant has antioxidant, anti-inflammatory, and anti-cancer effects in experiments. From studies on the chemical composition of An Xoa, researchers have identified 28 compounds including 08 terpenoids, 03 steroids, flavonoids, phenolics, lignoids, and 03 other compounds; among which some compounds have antioxidant activities, protect liver cells, and inhibit cancer cell growth such as (±)-pinoresinol, 3b-benzoylbetulinic acid, betulinic acid, lupeol, 4-hydroxybenzoic acid, soscutellarein, protosta-17(20),24-dien-3β-ol, which have toxic effects on various cancer cell lines.

However, currently, there is no comprehensive study on the pharmacological effects of An Xoa in treating liver diseases. This study aims to evaluate the preventive efficacy of An Xoa extract of cirrhosis on animal models. The results of the study serve as a basis for proposing further research on the mechanism of liver protection and other pharmacological effects of this herbal medicine.

2. SUBJECTS AND METHODS

2.1. Subjects

The water extract from the aboveground part of the An Xoa plant (*Helicteres hirsuta* Lour) was collected in March 2019 in A Luoi district, Thua Thien Hue province. The scientific name was determined by Assoc. Prof. Vu Tien Chinh, from the Museum of Nature, Vietnam Academy of Science and Technology (VAST).

2.2. Research Animals

Forty white Wistar rats, without gender distinction, weighing 200±20g each, were provided by the Experimental Animal Department of the Military Medical Academy. The animals were healthy, agile, with smooth fur, raised under standard conditions, at a room temperature of 22°C±2, with a 12/12 day-night cycle, provided with standard food and water for 7 days before the study and throughout the experiment.

2.3. Research Methodology

The aboveground part of the An Xoa plant was dried, crushed to obtain 10.0 kg of dry powder, and boiled three times with water (3 times x 20 liters of water), maintaining a boiling temperature of 55°C for three hours each time. After the third boiling, the entire solution was collected, and concentrated by removing water at 50°C, resulting in 930 g of water extract (HHW). The extraction was conducted at the Center for Training and Research in Toxicology and Radiology, Military Medical Academy.

The experimental model of inducing liver fibrosis followed the method of Gao et al. (2017) with suitable modifications for the laboratory.

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The rats were divided into 4 groups, with 10 rats in each group. Group 1: Control group (physiological control), drank distilled water at 10 ml/kg body weight per day, and injected subcutaneously with olive oil 2 ml/kg body weight. Groups 2, 3, and 4 were induced with liver fibrosis using CCl_4: subcutaneously injected with a CCl_4 solution diluted in olive oil at a ratio of 1/1 (v/v), twice a week on Tuesdays and Fridays, at a dose of 2 ml/kg body weight over 12 weeks. Group 2: (CCl_4 group) rats drank distilled water at 10 ml/kg body weight per day. Group 3: (Silymarin group) rats drank silymarin at a dose of 0.2 g/kg body weight per day. Group 4: (HHW group) rats drank HHW at a dose of 1.3 g/kg body weight per day.

The HHW was dissolved in warm water at 50°C, at a ratio of 1:4 (g/g), and administered to rats orally each morning with a blunt-tipped needle inserted into the stomach continuously for 12 weeks.

Biochemical tests (AST, ALT, GGT) were evaluated before cirrhosis induction and after 3 months of cirrhosis induction and cessation of the study product to assess changes in parameters before and after cirrhosis induction. The rats were euthanized by spinal column dislocation method, the abdominal cavity was opened to observe visceral organs, and the liver was collected for histopathological examination.

2.4. Data Analysis Method

Research data were processed using Excel 16.0 and SPSS 20.0 software. The values of research parameters were presented as mean ± standard deviation

($\overline{X}$ ± SD) with a confidence level of 95%. Pre-post comparisons were made using paired comparison algorithms for non-parametric variables (Wilcoxon test). Independent sample comparisons were made using independent sample comparison algorithms for non-parametric variables (Mann-Whitney test). Compare differences between multiple groups at the same time in the study using the Kruskal-Wallis Test. The difference was statistically significant when $p < 0.05$. Image analysis was performed using ImageJ software version 1.53a.

3. Results

3.1. Biochemical Parameters after Evaluating the Preventive Effect on cirrhosis

Table 3.1. The Activity of Some Liver Enzymes Before the Study

Group of rats	AST before study (u/l)	ALT before study (u/l)	GGT before study (u/l)
Control group	112.59 ± 22.59	46.53 ± 10.75	1.9 ± 2.18
CCl_4	111.58 ± 18.47	54.24 ± 11.52	1.9 ± 1.79
Silymarin	125.26 ± 22.85	52.52 ± 10.28	3.2 ± 1.87
HHW	128.5 ± 23.62	46.06 ± 9.76	2.6 ± 2.01
	p>0.05	p>0.05	p>0.05

Comment: At the pre-study time, the activities of liver enzymes AST, ALT, and GGT between groups of rats had no difference with p>0.05.

Table 3.2. The Activity of Some Liver Enzymes after the Study

Group of rats (n=10)	AST after study (u/l)	ALT after study (u/l)	GGT after study (u/l)
Control group	116.97 ± 19.00	53.55 ± 12.45	1.7 ± 2.11
CCl_4	$2234.7 \pm 325.19^{*}$	$903.37 \pm 183.84^{*}$	$10.1 \pm 3.38^{*}$
Silymarin	$1183.29 \pm 276.57^{*}{}_{\#\#\#}$	$730.55 \pm 157.15^{*}{}_{\#}$	$6.0 \pm 3.74_{\#}$
HHW	$1214 \pm 357.21^{*}{}_{\#\#\#}$	$653.46 \pm 226.01^{*}{}_{\#}$	$7.1 \pm 2.08^{*}{}_{\#}$

**Comparison with the Control group; # Comparison with the CCl4 group.*

**p<0.05, p<0.01, *p<0.001; #p<0.05, ##p<0.01, ###p<0.001 (Mann-Whitney test)*

After the study, the activities of all three enzymes in the control group of rats remained at average levels, while in the remaining groups of rats, all increased sharply.

Notably, the AST activities in the silymarin and HHW groups were significantly lower compared to the CCl_4 group with $p<0.001$. When comparing the liver enzyme activities between the silymarin and HHW groups after inducing fibrosis, no significant difference was observed with $p>0.05$. The ALT and GGT activities in the CCl_4 group were significantly higher compared to the groups supplemented with liver protective agents with $p<0.05$

3.2. Histopathological Images after evaluating the preventive effect on Liver Fibrosis

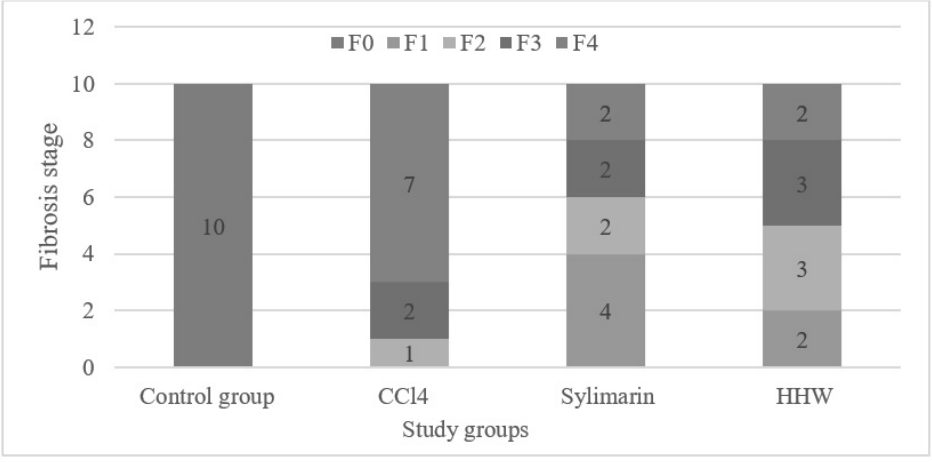
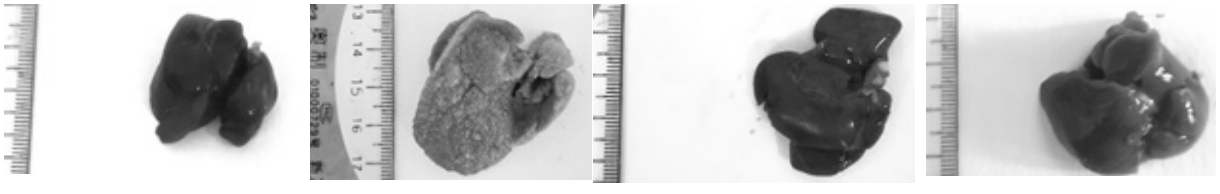


Chart 3.5. Level of liver cirrhosis of rats in each group

After the experiment, all 10 rats in the physiological control group remained at the F0 stage. In the CCl_4 group, the highest number of rats with stage F4 fibrosis was 7/10, with 2 rats with liver fibrosis stage F3. In the silymarin and HHW groups only 2 rats with stage F4 cirrhosis, while the remaining rats in these groups exhibited intermediate stages of fibrosis.

3.2.1. Macroscopic Images



Control group CCl_4 Silymarin HHW

Figure 3.1. Macroscopic Images of rats' liver after the study

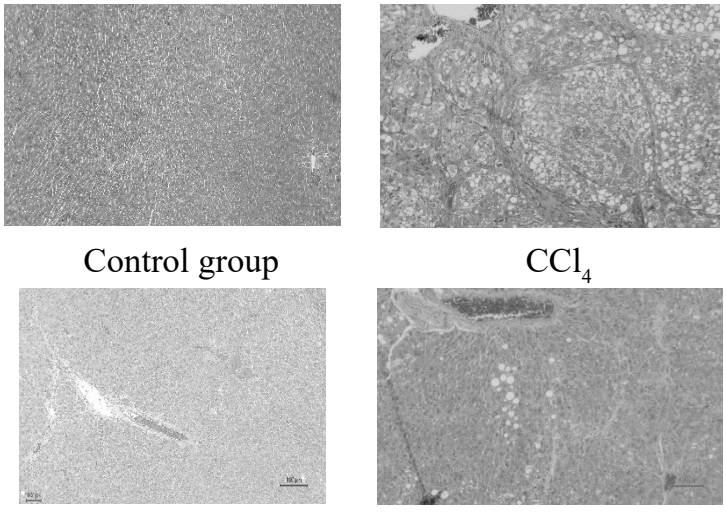
Rats livers of different groups: physiological control, silymarin, and HHW groups, the macroscopic images showed livers with a reddish-brown color, smooth surfaces, no nodules, and no hemorrhage.

For the CCl_4 group: The liver was brown-yellow in color, with a rough surface and numerous unevenly sized nodules ranging from 1-3mm. The liver density was relatively firm, with poor elasticity.

3.2.2. Microscopic Images

Control Group: Microscopic images show normal liver tissue.

CCl_4 Group: Fibrous bands divided the liver tissue longitudinally from the central vein to the portal tract and from the portal tract to the portal tract, with proliferative hepatocytes forming pseudolobules, corresponding to stage F4 fibrosis.



- Brown arrows: Normal liver cells

- Yellow arrows: Portal area

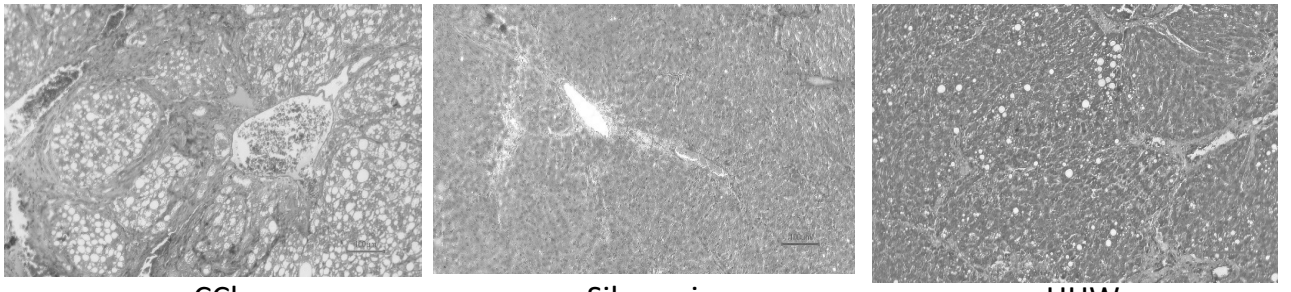
- Red arrows: Hepatocytes with fatty degeneration

- Green arrows: Fibrous bands

Figure 3.2. Histopathological images of liver tissue stained with HE 100x

In the silymarin group, the liver tissue began to exhibit short fibrous bands, extending widely around the portal area and dividing the liver parenchyma. Only some areas of the liver show fibrous bands and images of hepatocyte degeneration. Other areas of the liver still exhibit normal liver tissue. This corresponded to stage F2 fibrosis.

In the HHW group, the liver tissue began to show short fibrous bands, and some bands extended to connect the portal area with the portal area or the portal area with the central lobular region, extending widely and dividing the liver parenchyma. Additionally, there were images of fatty degeneration and granular degeneration of hepatocytes. Image of liver fibrosis stage F2-F3.



CCl_4 Silymarin HHW

- *Blue arrows:Fibrous bands*

Figure 3.3. Histopathological images of rats liver tissue stained with Masson's trichrome (100x)

Microscopic image of rats' liver of the CCl_4 group: hepatocytes exhibit fatty degeneration with large bright cavities in the cytoplasm. There was high-density fibrous connective tissue proliferation (blue color) with dense fibrous bands occupying a relatively large area on the microscopic field. These fibrous bands create numerous bridges between the central vein and the portal area and between portal areas, dividing the liver lobules and forming liver pseudolobules.

Liver microscopic image in the silymarin group, there were sparse, thin fibrous bands with very few bridges between portal areas or between portal areas and central veins. This image corresponded to stages F1-F3 fibrosis. The basic structure of the liver was maintained, without significant disruption of the liver parenchyma structure.

Liver microscopic image in the HHW group, there were a few bridges between portal areas and central veins and between portal areas with portal areas. However, the structure of the liver was still maintained with lobules, portal areas, hepatic trabeculae, and central veins.

4. Discussion

4.1. Biochemical Parameters

The initial parameters included the activities of enzymes AST, ALT, and GGT, which were the most common enzymes in assessing liver cell damage. The results of this study showed that the control group had normal levels of these enzymes, while all other groups showed a significant increase in enzyme activities, indicating liver cell damage in the groups injected with CCl_4. A more detailed comparison within these rat groups showed that the AST activity in the CCl_4 group was higher compared to the groups treated with the hepatoprotective compounds in the study. This showed that both silymarin and HHW could reduce liver enzyme activities. Only the silymarin group had a significantly lower ALT level compared to the control group. In

models of rats with liver cirrhosis by CCl_4, Yuan Y. Z., and colleagues studied the hepatoprotective effects of pomegranate peel and seed extract, and Xiang L. W. studied melon seed extracts showed the effectiveness of these preparations in reducing liver enzymes compared to the control group. Another commonly used liver enzyme parameter to evaluate chronic liver damage and hepatocellular toxicity was GGT. The results of this study showed that GGT in the CCl_4 group was higher compared to the silymarin and HHW groups. This could be explained by the hepatoprotective effects of these extracts, stabilizing the cell membrane and inhibiting the penetration of toxins into the cells, similar to the effects of silymarin. However, further studies providing more specific evidence are needed. To evaluate the efficacy of preventing liver fibrosis by HHW and silymarin, the research team compared biochemical parameters between these two groups. The results showed that after liver fibrosis induction, there was no difference in AST, ALT, and GGT levels between these two groups of rats. This showed that the efficacy of reducing liver enzymes by HHW was similar to that of silymarin at a dose of 200mg/kg body weight.

4.2. Histopathological images

Studies have shown that using scoring systems to assess liver biopsy has many advantages, including standardizing biopsy reports with high consistency among pathologists, uniformity among testing centers, and comparison of subsequent biopsies to monitor disease progression. There were several scoring systems used to assess the degree of fibrosis in liver biopsy samples, such as the Metavir, Knodell Ishak, and Desmet/ Scheuer systems. Among them, the Metavir system evaluated liver fibrosis on a scale of F0-F4 and was the most widely used system in clinical practice.

The results of this study indicated that: In the physiological control group, all livers had normal parenchyma, while in the CCl_4 group, which was only injected with CCl_4 without hepatoprotective products, 7/10 rats exhibited liver fibrosis at stage F4. In the groups of rats injected with CCl_4 and administered silymarin or HHW, pathological examination showed that only hepatocellular degeneration, with most liver fibrosis corresponding to stages F1-F2 and F2-F3, respectively.

This showed that liver damage in the groups that administered silymarin and the research extract occurred more slowly than in the group that only injected CCl_4. When studying the hepatoprotective mechanism of silymarin, studies showed that silymarin had the effect of reducing free radicals that damage cell membranes. Additionally, silymarin stimulated hepatocyte metabolism and activated ribosomal RNA synthesis to promote

protein synthesis, thereby enhancing hepatocyte regeneration. HHW extract also exhibited antioxidant and hepatocyte-protective activities *in vitro*. In vivo studies using HHW on rats help slow down the process of cirrhosis, which may be due to the extract reducing the extent of CCl_4-induced damage or accelerating liver recovery, helping to immediately repair small damage of the liver in the early stages, the results inhibited liver fibrosis in white rats.

To observe the development of fibrotic bands more clearly, liver histological specimens were stained with Masson's trichrome stain. The microscopic images showed that in the CCl_4 group, there were thick fibrotic bands occupying a relatively large area on the slide, along with numerous bridges, indicative of pseudo-lobules. These images were consistent with stage F4 fibrosis and some rats with F3 fibrosis. In contrast, the groups administered silymarin and HHW showed similar microscopic images, with thinner and fragmented fibrotic bands and very few bridges, without disrupting the normal liver structure. This demonstrates that collagen deposition in the liver of the control group was significantly higher compared to the groups administered silymarin and HHW; indicating that these extracts inhibited liver fibrosis. Similar findings were reported in a study on the hepatoprotective effects of pomegranate peel and seed extract by Xiang-lan et al., where Masson's trichrome staining showed higher collagen deposition in the CCl_4-induced toxicity group compared to the group administered the hepatoprotective products. This once again confirmed that HHW extract was effective in protecting the liver, reducing the impact of CCl_4 on the liver, slowing down liver fibrosis progression in white rats, and had a similar effect to silymarin at a dose of 200mg/kg body weight.

5. Conclusion

The use of HHW extract (1.3g/kg body weight) had a hepatoprotective effect against liver fibrosis induced by carbon tetrachloride in white rats. Its effect was similar to silymarin at a dose of 200mg/kg body weight.

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