EVALUATING ACUTE TOXICITY AND HEPATOPROTECTIVE EFFECT OF ICH CAN THANH ORAL SOLUTION IN A PARACETAMOL-INDUCED ACUTE HEPATITIS MOUSE MODEL

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This study investigated the acute toxicity and hepatoprotective effect of Ich can thanh oral solution in a mouse model of paracetamol-induced acute hepatitis. The acute toxicity was assessed through oral administration, and the median lethal dose (LD_{50}) was estimated using the Litchfield-Wilcoxon method. In the paracetamol-induced acute hepatitis model, mice were randomly divided into experimental groups and pretreated orally with distilled water, silymarin (140 mg/kg), or Ich can thanh (4.8 or 9.6 mL/kg/day) for eight consecutive days. Acute liver injury was induced by oral administration of paracetamol (400 mg/kg). Forty-eight hours after induction, serum biochemical parameters, liver weight, hepatic malondialdehyde (MDA) and glutathione (GSH) levels and histopathological changes were assessed. No mortality or toxic signs at doses up to 120 mL/kg (approximately 25 times higher than the intended human dose), indicating that LD_{50} exceeds the highest tested dose. Additionally, Ich can thanh 4.8 mL/kg/day showed a trend towards hepatoprotection, whereas 9.6 mL/kg/day significantly reduced liver weight, decreased AST and ALT activities, increased serum albumin levels, attenuated lipid peroxidation, and improved liver histopathological compared with the model group. In conclusion, Ich can thanh, particularly at 9.6 mL/kg/day, exerts significant hepatoprotective effects in a mouse model of paracetamol-induced acute liver injury.

Keywords: Ich can thanh oral solution, hepatoprotection, acute toxicity, paracetamol-induced acute hepatitis, Swiss mice.

I. INTRODUCTION

Acute hepatitis is characterized by sudden liver inflammation and is most commonly caused by viral infections (hepatitis A-E). Other important etiologies include drug-induced liver injury (e.g., acetaminophen and certain antibiotics), toxic exposures (such as poisonous mushrooms and alcohol), autoimmune disorders and metabolic diseases.[1] Among drug-related causes, paracetamol (acetaminophen) overdose represents one of the leading causes of acute liver injury worldwide. Following toxic exposure, hepatic injury is primarily mediated by the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes intracellular glutathione, induces oxidative stress, disrupts mitochondrial function, and triggers inflammatory cascades that ultimately lead to hepatocellular necrosis. Serum transaminases typically rise within 24-48 hours, and severe cases may progress to acute liver failure.[2]

Despite advances in supportive management, therapeutic options for acute liver injury remain largely limited to removal of the causative agent and symptomatic treatment. Effective agents capable of directly protecting hepatocytes and promoting liver regeneration are still needed. Therefore, considerable attention has been directed toward agents with hepatoprotective

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properties that may attenuate oxidative stress, suppress inflammatory responses, stabilize hepatocyte membranes, and promote hepatic regeneration. In this context, herbal formulations containing bioactive compounds with antioxidant and anti-inflammatory activities have attracted growing interest as adjunctive therapies for liver disorders.[3]

Vietnam possesses abundant medicinal plant resources and a long-standing tradition of using herbal remedies in healthcare. This provides a favourable foundation for the development and investigation of natural products to support liver function. Ich can thanh is formulated as an oral solution containing *Cynara scolymus*, *Adenosma glutinosum*, *Lactuca indica*, *Pueraria thomsonii*, *Silybum marianum*, and *Curcuma longa*. Several individual components of this formulation have been reported to exhibit hepatoprotective effects in experimental animal models.[4-7] However, limited evidence is available regarding the hepatoprotective efficacy of these ingredients when used in combination. Furthermore, data regarding its safety profile remains limited. Therefore, this study aimed to evaluate the acute toxicity and the hepatoprotective effect of Ich can thanh oral solution in a mouse model of paracetamol-induced acute hepatitis.

II. MATERIALS AND METHODS

1. Investigational product

The investigational product was Ich Can Thanh oral solution, manufactured by Hoa Linh Pharmaceutical Co., Ltd. The product was supplied in boxes of 12 vials × 20 mL (Product registration No.: 10593/2021/ĐKSP) and met in-house quality standards. Each 20-mL vial contained 6.2 g of extract prepared from a mixture of dried medicinal herbs equivalent to: 2 g *Cynara scolymus* (artichoke), 2 g *Adenosma glutinosum*, 1.2 g *Lactuca indica*, 1 g *Pueraria thomsonii*, 105 mg *Silybum marianum* extract, and 2.5 mg *Curcuma longa* extract. Batch number: 0522.

Preparation of the test sample for the acute toxicity study: 240 mL of the original Ich can thanh oral solution was concentrated to a final volume of 150 mL to obtain a concentrated preparation. This concentrated solution was used for acute toxicity evaluation and for determining the median lethal dose (LD_{50}) of Ich can thanh. It represents the highest concentration that can be administered to mice via oral gavage.

The proposed human dosage is oral administration, 1-2 times daily, with 1 vial of 20 mL per dose, after meals. In mice, using a conversion factor of 12, the equivalent dose was 4.8 mL/kg/day (*corresponding to a human dose of one 20 mL vial/day for a 50 kg adult*) and 9.6 mL/kg/day (*corresponding to a human dose of two 20 mL vials/day for a 50 kg adult*). Silymarin (Legalon® 70 Protect, MADAUS GmbH, Germany) was used as the positive control. The test product was administered directly as the original oral solution, whereas the positive control was completely dissolved in distilled water before oral gavage.

Animals

Healthy *Swiss albino* mice of both sexes, weighing 25 ± 2 g, were supplied by the National Institute of Hygiene and Epidemiology. Animals were housed in the laboratory of the Department of Pharmacology, Hanoi Medical University, under controlled environmental conditions (temperature 25 ± 1°C, appropriate humidity, and a standard light-dark cycle). They were fed a standard laboratory rodent diet and had free access to water *ad libitum*. Prior to the experiment, the animals were acclimatized to the housing conditions for 7 days and

maintained under these conditions throughout the study.

Chemicals and Equipment

Legalon® 70 Protect MADAUS (MADAUS GmbH, Germany). Efferalgan® 500 mg effervescent tablets (UPSA SAS, France).

Commercial kits for quantification of blood enzymes and metabolites, including ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT, total bilirubin, and albumin, were obtained from Erba and analysed using a semi-automated Erba biochemical analyser (India).

Chemicals used for MDA and GSH assays included 5,5′-dithiobis(2-nitrobenzoic acid) and thiobarbituric acid (Sigma-Aldrich, Germany). Reagents used for histopathological specimen preparation met standard laboratory quality requirements.

2. Methods

Acute toxicity of Ich can thanh oral solution

An acute toxicity study of the investigational product was conducted in mice by oral administration, and the median lethal dose (LD_{50}) was determined using the Litchfield-Wilcoxon method.[8]

Before the experiment, *Swiss albino* mice were fasted overnight and then divided into four groups. These groups were administered Ich can thanh orally in gradually increasing doses to determine the minimal dose that caused 100% mortality and the maximum dose that resulted in no fatality (0% death). The condition of the experimental animals was monitored for 72 hours after Ich can thanh administration. Finally, we cared for the mice until the 14th day of the experiment.

Assess the hepatoprotective effects of Ich can thanh oral solution on paracetamol-induced acute hepatitis in mice

Swiss albino mice were randomly divided into five groups (n = 10 per group):

- Group 1: received distilled water at a volume of 0.2 mL per 10 g of body weight.

- Group 2: received distilled water at 0.2 mL per 10 g of body weight.

- Group 3: received silymarin at 140 mg/kg (administered at 0.2 mL per 10 g of body weight).

- Group 4: received Ich can thanh at 4.8 mL per kg per day.

- Group 5: received Ich can thanh at 9.6 mL per kg per day.

All treatments were administered orally once daily in the morning for eight consecutive days. On day 8, two hours after the final administration (following a 16-18 h fasting period), acute liver injury was induced in Groups 2-5 by oral administration of paracetamol at a dose of 400 mg/kg.[9, 10]

Forty-eight hours after paracetamol administration, blood samples were collected from the carotid artery to measure serum AST, ALT activities, albumin and total bilirubin levels. The livers were excised to determine weight, conduct gross pathological examinations, and assess hepatic malondialdehyde (MDA) and glutathione (GSH) levels across all study groups. Histopathological examination was performed on liver specimens from 30% of mice in each group. Microscopic analyses were carried out at the Department of Laboratory Medicine at the University of Public Health.

Statistical analysis

Statistical analysis was performed using SigmaPlot 12.0 (SYSTAT Software Inc., Richmond, CA, USA). Data distribution was assessed using the Shapiro-Wilk test, and all variables were found to be normally distributed. Data were expressed as mean ± standard

deviation (SD). Differences among groups were evaluated using one-way analysis of variance (one-way ANOVA), followed by the Student-Newman-Keuls post hoc test for pairwise comparisons.

Study setting

The study was conducted at the Department of Pharmacology, Hanoi Medical University.

III. RESULTS

1. Acute toxicity of Ich can thanh oral solution

Mice administered concentrated Ich can thanh at doses ranging from 30 mL/kg to 75 mL/kg showed no sign of acute toxicity. The maximum tolerated dose of Ich can thanh, corresponding to 120 mL/kg of the original solution (approximately 25 times the intended human dose), did not produce any acute toxic effect in mice.

Table 1. Acute toxicity study of Ich can thanh oral solution

Group	n	Dose (mL concentrated solution/kg)	Dose (mL original solution/kg)	Number of deaths	Other abnormal signs
Group 1	10	30 mL/kg	48 mL/kg	0	None
Group 2	10	45 mL/kg	72 mL/kg	0	None
Group 3	10	60 mL/kg	96 mL/kg	0	None
Group 4	10	75 mL/kg	120 mL/kg	0	None

2. Assess the hepatoprotective effects of Ich can thanh oral solution on paracetamol-induced acute hepatitis in mice

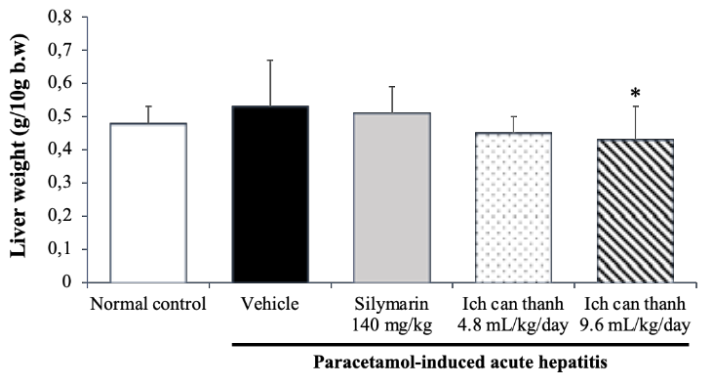

Figure 1. Effects of Ich can thanh on the liver weight of mice.

**p < 0.05, ***p < 0.001: compared to the model group.*

In the paracetamol-induced acute hepatitis mouse model, liver weight in the model group tended to be higher than in the normal control group, although the difference was not statistically significant (p > 0.05). Treatment with silymarin (140 mg/kg) and Ich can thanh at 4.8 mL/kg showed a decreasing trend in liver weight compared with the model group, but this difference was not statistically significant (p > 0.05). However, Ich can thanh at 9.6 mL/kg significantly reduced liver weight compared with the model group (p < 0.05) (shown in Figure 1).

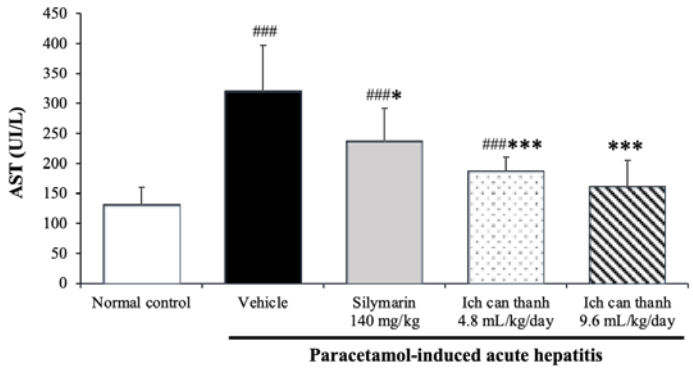

Figure 2. Effects of Ich can thanh on serum AST activity

*###p < 0.001: compared to the normal control group. *p < 0.05, ***p < 0.001: compared to the model group.*

The results shown in Figure 2 indicate that AST activity in the paracetamol-induced acute hepatitis group increased markedly compared with the normal control group (p < 0.001). Treatment with silymarin (140 mg/kg) significantly reduced AST activity compared with the paracetamol-induced acute hepatitis group (p < 0.05). Notably, Ich can thanh at both 4.8 mL/kg and 9.6 mL/kg markedly decreased AST activity compared with the paracetamol-induced acute hepatitis group (p < 0.001).

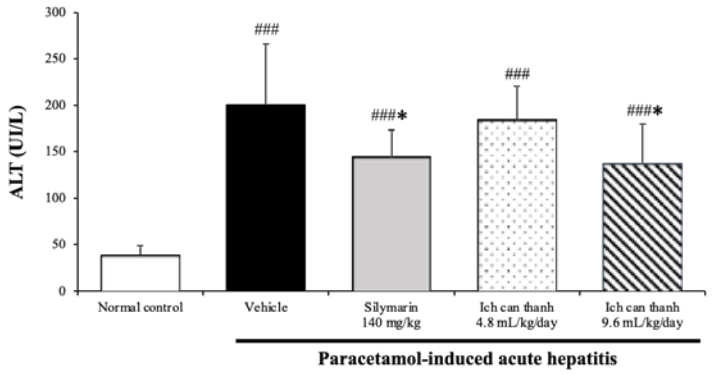

Figure 3. Effects of Ich can thanh on serum ALT activity

*###p < 0.001: compared to the normal control group. *p < 0.05: compared to the model group.*

The results shown in Figure 3 indicate that ALT activity in the paracetamol-induced acute hepatitis group increased markedly compared with the normal control group (p < 0.001). Treatment with silymarin (140 mg/kg) significantly reduced ALT activity compared with the paracetamol-induced acute hepatitis group (p < 0.05). Ich can thanh at 4.8 mL/kg tended to reduce ALT activity, but the difference was not statistically significant (p > 0.05), whereas Ich can thanh at 9.6 mL/kg significantly decreased ALT activity compared with the paracetamol-induced acute hepatitis group (p < 0.05).

Table 2. Effects of Ich can thanh on albumin and bilirubin concentrations

Groups	Albumin (g/dL)	Bilirubin (mg/dL)
Normal control	3.15 ± 0.21	0.80 ± 0.05
Model	3.06 ± 0.22	0.87 ± 0.08#
Silymarin 140 mg/kg	3.10 ± 0.24	0.81 ± 0.05*
Ich can thanh 4.8 mL/kg/day	3.27 ± 0.22*	0.90 ± 0.07
Ich can thanh 9.6 mL/kg/day	3.40 ± 0.19#**	0.87 ± 0.07

*#p < 0.05: compared to the normal control group. *p < 0.05, **p < 0.01: compared to the model group.*

The results shown in Table 2 indicated that albumin levels in the paracetamol-induced acute hepatitis group tended to decrease compared with the normal control group, although the difference was not statistically significant ($p > 0.05$). Silymarin (140 mg/kg) tended to increase albumin levels, but the difference was not statistically significant ($p > 0.05$). In contrast, Ich can thanh at 4.8 mL/kg and 9.6 mL/kg significantly increased albumin levels compared with the paracetamol-induced acute hepatitis group ($p < 0.05$ and $p < 0.01$, respectively). Bilirubin levels in the paracetamol-induced acute hepatitis group were significantly higher than in the normal control group ($p < 0.05$). Treatment with silymarin (140 mg/kg) significantly reduced bilirubin levels compared with the paracetamol-induced acute hepatitis group ($p < 0.05$), whereas Ich can thanh at both 4.8 mL/kg and 9.6 mL/kg did not significantly change bilirubin levels ($p > 0.05$).

Table 3. Effects of Ich can thanh on the MDA and GSH concentrations

Groups	MDA concentration (nmol/100 mg liver)	GSH concentration (μM)
Normal control	13.25 ± 3.28	272.93 ± 69.98
Model	17.32 ± 3.83#	249.05 ± 71.28
Silymarin 140 mg/kg	12.95 ± 3.38#*	271.54 ± 81.95
Ich can thanh 4.8 mL/kg/day	16.57 ± 5.92	312.85 ± 92.11
Ich can thanh 9.6 mL/kg/day	16.82 ± 3.51#	309.37 ± 54.56

*#p < 0.05: compared to the normal control group. *p < 0.05: compared to the model group.*

The results shown in Table 3 indicated that hepatic MDA levels in the paracetamol-induced acute hepatitis group were significantly higher than in the normal control group ($p < 0.05$). Treatment with silymarin (140 mg/kg) significantly reduced hepatic MDA levels compared with the paracetamol-induced acute hepatitis group ($p < 0.05$). Ich can thanh at both 4.8 mL/kg and 9.6 mL/kg tended to decrease hepatic MDA levels; however, the

differences were not statistically significant ($p > 0.05$). Hepatic GSH levels in the paracetamol-induced acute hepatitis group tend to decrease compared with the normal control group ($p > 0.05$). Silymarin and Ich can tend to increase hepatic GSH levels, but the difference was not statistically significant ($p > 0.05$).

Effects of Ich can thanh oral solution on macroscopy and histopathological changes in the mouse liver

Gross examination revealed that livers from the normal control group were dark red in colour, smooth-surfaced, soft, and showed no sign of oedema or congestion. Conversely, livers from the paracetamol-induced acute hepatitis group appeared pale and congested, with multiple necrotic regions, an irregular surface, petechial haemorrhages, and a markedly loose texture. In the silymarin-treated group, the livers were red with mild congestion, lacked clearly visible focal lesions, and had a relatively soft texture. In the Ich can thanh-treated groups, some livers appeared slightly pale with mild congestion and a relatively loose consistency.

Liver tissue from the normal control group exhibited normal hepatic architecture, with no evidence of inflammatory infiltration or fatty degeneration (Figure 4.A). In the paracetamol-induced acute hepatitis group, moderate to severe hepatocellular necrosis was observed (Figure 4.B). In contrast, liver sections from mice treated with silymarin and Ich can thanh showed only mild hepatocellular necrosis (Figures 4.D-4.E).

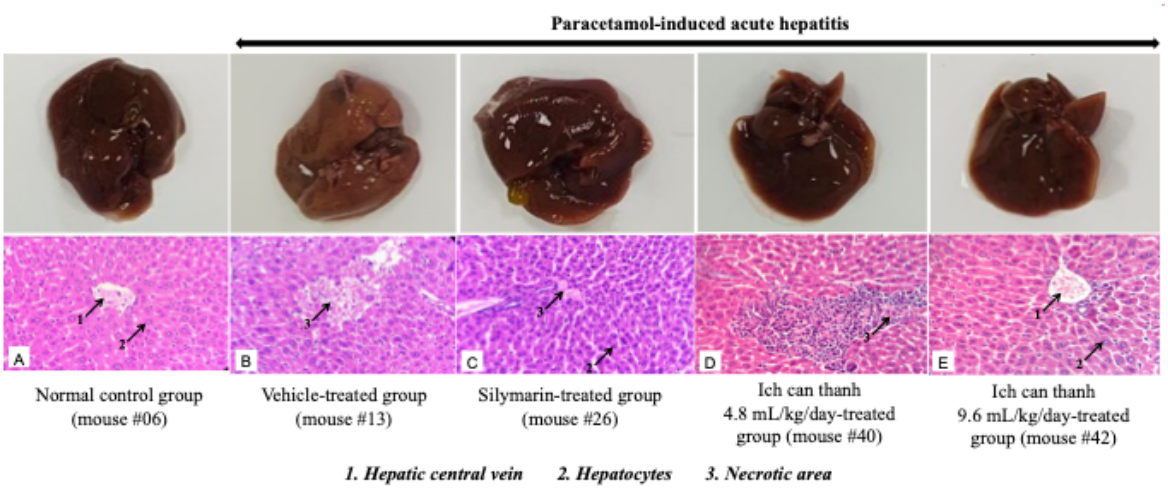

Figure 4. Microscopic images of the mouse liver (H&E staining, ×400)

IV. DISCUSSION

Hepatitis remains a significant global health concern. To evaluate hepatoprotective agents, a variety of experimental liver injury models have been developed, including chemically induced models (e.g., CCl_4, thioacetamide, dimethylnitrosamine, D-galactosamine, ethanol), surgical approaches (e.g., bile duct ligation), and genetically modified animal models. Each model reflects distinct pathogenic mechanisms and is selected based on the specific clinical context and research objective. Models such as CCl_4 and thioacetamide are more commonly used to study chronic liver injury and fibrosis, whereas ethanol-induced

models are relevant to chronic metabolic and inflammatory liver damage. In contrast, paracetamol-induced liver injury is widely used to mimic acute hepatocellular injury with a rapid onset and high reproducibility.[11,12] Therefore, for studies focusing on acute liver injury and hepatoprotective interventions in an acute setting, the paracetamol model is considered appropriate.

Paracetamol overdose results in hepatocellular injury through a well-characterized toxic metabolic pathway. Following cytochrome P450-mediated bioactivation, the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) is formed. Under physiological conditions, NAPQI is rapidly detoxified by conjugation with glutathione. However, excessive exposure depletes intracellular glutathione stores, leading to the accumulation of NAPQI and its covalent binding to cellular macromolecules. This adduct formation disrupts mitochondrial integrity, impairs energy metabolism, and enhances reactive oxygen species generation, ultimately triggering hepatocellular necrosis. In parallel, necrotic cell death promotes the release of damage-associated molecular patterns, which activate inflammatory signalling pathways and further exacerbate hepatic injury. When extensive, these events may culminate in acute liver failure.[13] A dose of 400 mg/kg of paracetamol has been widely used in previous studies to induce acute liver injury in mice; therefore, this dose was selected to establish the experimental model in the present study.[14] In the present study, serum ALT and AST activities were measured, as these enzymes are widely used biomarkers of hepatocellular injury in paracetamol-induced acute liver injury models. In addition, hepatic MDA and GSH levels were determined to assess oxidative stress and antioxidant capacity. MDA is a well-established marker of lipid peroxidation and reflects oxidative damage to cell membranes, whereas GSH serves as a major intracellular antioxidant that protects cells against reactive oxygen species.[15] The findings confirmed the successful establishment of the acute liver injury model, as evidenced by increased liver weight, elevated serum AST and ALT activities, and marked macroscopic and histopathological alterations.

In our study, although AST and ALT levels were significantly lower than in the model group, but remained significantly higher than those in the normal control group, indicating incomplete hepatic recovery. This partial improvement may be related to the relatively short treatment duration in this study. Furthermore, this is an initial study evaluating the hepatoprotective effect of Ich can thanh. Therefore, while the findings suggest a beneficial effect, further studies are required to elucidate the underlying mechanisms, particularly to clarify whether the observed effects are primarily mediated by membrane stabilization, anti-inflammatory activity, or partial antioxidant action. Additionally, paracetamol-induced acute liver injury is primarily characterized by hepatocellular damage, as reflected by elevations in AST and ALT levels.[12] In this study, assessment was performed 48 hours after model induction, a time point at which hepatocellular injury is evident but may not yet significantly impair the liver's synthetic and excretory functions. Consequently, serum albumin and bilirubin levels did not show statistically significant changes and remained within normal ranges.[16] Similar findings have been reported in previous studies using acute paracetamol-induced liver injury models.[17, 18]

Our results indicated that at 9.6 mL/kg/

day, Ich can thanh demonstrated significant hepatoprotective effects, as evidenced by reduced liver weight, markedly decreased serum AST and ALT activities, increased serum albumin levels, a tendency toward reduced hepatic MDA levels, and a tendency toward increased hepatic GSH levels. The observed hepatoprotective effects may, at least in part, be associated with the pharmacological properties of the individual constituents of Ich Can Thanh oral solution.

The herbal components of the formulation have been documented to exert hepatoprotective effects through multiple mechanisms, primarily involving antioxidant, anti-inflammatory, and membrane-stabilizing activities. *Silybum marianum* (milk thistle) possesses the most well-established evidence for hepatoprotection. Its principal active component is silymarin, a lipophilic extract from the seeds composed of three flavonolignans (silybin, silydianin, and silychristin), of which silybin is the most biologically active and accounts for approximately 50-70% of the extract. Silymarin reduces oxidative stress by limiting free radical formation and lipid peroxidation, exhibits antifibrotic properties, and may prevent toxin entry into hepatocytes by inhibiting toxin binding to membrane receptors. In experimental models, silymarin attenuates liver injury induced by acetaminophen, carbon tetrachloride, radiation, iron overload, phenylhydrazine, alcohol, cold ischemia, and *Amanita phalloides* toxins. Clinically, it has been used in the management of alcoholic liver disease, acute and chronic viral hepatitis, and toxin-induced hepatic disorders.[5] Additionally, artichoke (*Cynara scolymus*) contains various bioactive compounds, including polyphenols (such as cynarin, caffeoylquinic acids, and flavonoids) and sesquiterpene lactones. These constituents exhibit potent antioxidant properties by scavenging free radicals and protecting proteins, lipids, and DNA from oxidative damage, thereby mitigating oxidative stress-induced hepatocellular injury. In addition, artichoke extracts have been shown to suppress inflammatory responses by inhibiting NF-κB activation and downregulating pro-inflammatory cytokines, while simultaneously enhancing endogenous antioxidant defences. Experimental studies have demonstrated improvements in liver function, attenuation of histopathological damage, and reduction of hepatic injury markers in toxic models, including alcohol-induced liver injury. Furthermore, artichoke promotes choleresis, enhances cholesterol excretion, and modulates lipid metabolism, thereby reducing hepatic lipid accumulation and improving liver function.[4]

Adenosma glutinosum, rich in flavonoids and coumarins, has also been reported to exhibit hepatoprotective activity in herbal formulations, including reductions in serum transaminases and lipid peroxidation in experimental models of liver injury.[19] Phenylpropanoid compounds isolated from *Lactuca indica* have demonstrated protective effects against CCl_4-induced hepatotoxicity, evidenced by decreased AST and ALT activities, reduced lipid peroxidation, and improved histological architecture. These effects are mainly attributed to antioxidant capacity and stabilization of hepatocyte membranes, thereby limiting necrosis and inflammatory responses within the hepatic parenchyma.[6] Puerarin, an isoflavone derived from *Pueraria thomsonii*, has shown beneficial effects in alcohol-related liver disease and fatty liver conditions. In chronic alcohol-induced liver injury models, puerarin reduced AST, ALT, GGT, and triglyceride levels, while ameliorating steatosis and inflammatory infiltration. Mechanistically, these effects are

associated with modulation of the gut-liver axis, including restoration of intestinal barrier integrity, reduction of circulating endotoxin levels, suppression of Kupffer cell activation, and downregulation of hepatic LPS receptor expression, ultimately leading to decreased TNF-α production and inflammatory signalling. In addition, puerarin exerts antioxidant effects, regulates lipid metabolism, and reduces hepatocyte apoptosis, thereby improving hepatic histological structure. These findings support its potential therapeutic role in liver disorders.[20, 21] Taken together, the hepatoprotective activity of Ich can thanh may result from the synergistic actions of its herbal constituents, mediated by multiple complementary mechanisms, including antioxidant, anti-inflammatory, and metabolic regulatory effects.

In a previous study evaluating the hepatoprotective effects of Ich can thanh in an ethanol-induced liver injury model, the treatment was administered for four weeks and demonstrated significant antioxidant activity, as reflected by improvements in oxidative stress markers. This difference may be explained by the distinct pathophysiological characteristics of the two models. In ethanol-induced liver injury, oxidative stress develops gradually and is closely associated with chronic inflammation and metabolic disturbances, allowing antioxidant interventions to more effectively restore redox balance. In contrast, paracetamol-induced liver injury is characterized by rapid and severe hepatocellular damage, which may limit the recovery of oxidative stress markers such as MDA and GSH within a short experimental timeframe, even when hepatocellular injury is partially attenuated. Additionally, this study was conducted in mice, in which blood sampling is typically limited to a single time point, precluding time-course analysis of biochemical parameters. As an initial investigation into the hepatoprotective effects of Ich can thanh, the present study provides preliminary evidence of both hepatoprotective and antioxidant activities. However, further studies are required to elucidate the underlying molecular mechanisms and to evaluate the effects on drug-metabolizing enzymes and other relevant pathways.

In addition to evaluating the hepatoprotective effects of Ich can thanh oral solution, the present study also conducted a preliminary assessment of its acute toxicity. The absence of mortality or observable clinical signs at doses up to 120 mL/kg of the original solution suggests a wide safety margin for Ich can thanh under acute exposure conditions. Notably, this dose is approximately 25-fold higher than the intended human dose, further supporting its preliminary safety profile. These findings indicate that the oral LD_{50} of the formulation is likely higher than the highest dose tested.

Among the components of Ich can thanh, *Silybum marianum* has the most extensive data on both efficacy and toxicity. Silymarin has shown no major toxicity in animal studies and is considered safe in humans at therapeutic doses; it is well tolerated even at high doses of up to 700 mg three times daily for 24 weeks.[22] In addition, acute toxicity of *Curcuma longa* has also been reported, with no mortality or abnormal clinical signs observed in female rats at a single oral dose of 5000 mg/kg body weight, according to OECD guideline 425.[23] For the remaining components, individual acute toxicity data are limited. However, some studies have evaluated the safety of combined herbal formulations containing ingredients present in Ich can thanh, such as *Cynara scolymus* and *Silybum marianum*. According to OECD guideline 423, an herbal formulation containing *Anethum graveolens*, *Cynara scolymus*, *Citrus aurantium*, *Portulaca oleracea*, and *Silybum marianum* showed no mortality over a 14-day

observation period following a single oral dose of 2000 mg/kg.[24]

However, acute toxicity studies primarily assess short-term adverse effects following single or repeated administration within a limited timeframe and may not fully reflect potential subacute or chronic toxicities. Given that herbal formulations contain multiple bioactive compounds that may exert cumulative or long-term biological effects, additional evaluations are warranted to comprehensively characterize the safety profile of Ich can thanh. Future investigations should include subacute and subchronic toxicity studies, repeated-dose toxicity assessments, and detailed analyses of haematological, biochemical, and histopathological parameters in major organs. Furthermore, evaluating potential hepatotoxicity, nephrotoxicity, and interactions with drug-metabolizing enzymes would provide a more complete understanding of its safety profile.

V. CONCLUSION

The maximum tolerated dose of Ich can thanh was 120 mL/kg of the original solution (approximately 25-fold higher than the intended human dose) and did not produce any sign of acute toxicity. The oral LD_{50} was therefore estimated to be greater than 120 mL/kg in *Swiss albino* mice. Ich can thanh at 4.8 mL/kg/day showed a trend toward hepatoprotection against paracetamol-induced acute liver injury in mice. At 9.6 mL/kg/day, Ich can thanh demonstrated significant hepatoprotective effects, as evidenced by reduced liver weight, markedly decreased serum AST and ALT activities, increased serum albumin levels, a tendency toward reduced hepatic MDA levels, and a tendency toward increased GSH levels in liver homogenates.

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