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Original Article

Oral Acute Toxicity Study of Aqueous Extract of *Chaihu Shugan* Tang Modified with *Adenosma Bracteosum*

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ABSTRACT

Background: The aqueous extract of *Chaihu Shugan Tang* combined with *Adenosma bracteosum* (CAE) has been suggested to exhibit low hepatotoxic potential with enzyme modulation and may support the treatment of hepatobiliary diseases. However, data on its acute toxicity remain limited. **Objectives:** To determine the acute toxicity of the CAE aqueous extract in mice. **Materials and methods:** Mice were divided into seven groups: one control group and six experimental groups receiving increasing doses of the extract (8–64 g/kg body weight). Acute toxicity was assessed using the Litchfield–Wilcoxon method by monitoring mortality and adverse reactions. Hematological and biochemical parameters, as well as histopathological examinations of the liver, kidneys, and spleen, were compared between the experimental and control groups. **Results:** At the highest tested dose (64 g/kg body weight), no mortality was observed. Body weight, hematological parameters, and renal biochemical indices showed no significant abnormalities ($p > 0.05$). However, liver biochemical indices in groups receiving 30.4–64 g/kg differed significantly from the control group ($p < 0.05$). Histopathological examination revealed no signs of damage or pathological changes in the liver, kidneys, or spleen. **Conclusions:** The CAE aqueous extract did not cause acute toxicity in mice at doses up to 64 g/kg body weight. Further subchronic and chronic toxicity studies are required to comprehensively assess its long-term safety.

Keywords: Acute toxicity, *Adenosma bracteosum*, Chaihu Shugan Tang

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INTRODUCTION

Vietnam possesses abundant and diverse medicinal plant resources and inherits a long-standing system of traditional medical knowledge in the use of herbal medicines for disease prevention, treatment, and the promotion of public health[1]. In recent years, traditional herbal formulations have increasingly been prioritized in clinical practice due to their demonstrated therapeutic effectiveness in clinical practice, in some conditions approaching that of conventional pharmaceuticals[2,3], while demonstrating a lower incidence of adverse effects[4]. When adverse events do occur, they are generally mild and predominantly associated with gastrointestinal disturbances[5].

Among the commonly used traditional formulas, *Chaihu Shugan Tang* has been consistently reported to exhibit a favorable safety profile[6-8] and therapeutic effectiveness across a wide range of clinical conditions. Clinical studies have demonstrated that this formula significantly improves symptoms of functional gastrointestinal disorders[6,9], has been associated with reductions in liver enzyme levels and improvements in dyslipidemia in patients with non-alcoholic fatty liver disease[10-12], and provides substantial supportive benefits in the management of emotional disorders such as anxiety and depression[7,13]. Collectively, these findings indicate that *Chaihu Shugan Tang* represents one of the classical traditional formulations with well-established efficacy and a favorable safety profile in contemporary clinical practice.

Adenosma bracteosum Bonati is a medicinal herb that has been traditionally used for the treatment of liver diseases and metabolic disorders. Previous experimental studies have demonstrated a favorable safety profile of this herb, with good tolerability at doses up to 3000 mg/kg body weight, without inducing mortality or overt signs of toxicity[8]. In addition, both the ethanol and aqueous extracts of *Adenosma bracteosum* have exhibited potent inhibitory activity against the enzyme α-glucosidase, with greater efficacy than acarbose[8]. Notably, the compound isoscutellarein-8-O-β-D-glucopyranoside (IG) has been reported to exert approximately tenfold stronger α-glucosidase inhibitory activity compared with acarbose[14], along with several other flavonoids demonstrating similar bioactivity[15]. Furthermore, *Adenosma bracteosum* has shown anticancer potential by inhibiting the proliferation of human cancer cell lines, including NCI-H460 and HepG2 cells[16].

From both theoretical and practical perspectives, the combination of *Chaihu Shugan Tang* with *Adenosma bracteosum* is expected to exert synergistic effects in hepatocyte protection, regulation of hepatobiliary function, and improvement of metabolic disturbances, thereby enhancing supportive therapeutic efficacy in liver-related diseases. However, despite the well-documented efficacy and favorable safety profiles of each component when used individually, the safety of combining a classical herbal formula with a single medicinal herb has not been fully established. Therefore, prior to conducting clinical interventional studies, it is necessary to evaluate the acute toxicity of *CAE* using experimental models, in order to determine its preliminary safety profile and to provide a scientific basis for subsequent investigations.

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MATERIALS AND METHODS

Extract preparation

The composition and dosage of the CAE used in this study are summarized in Table 1.

The daily dose used in this study was 88 g of dried herbal materials, corresponding to one prescription unit for humans. Based on a reference body weight of 50 kg, this dose was equivalent to 1.76 g/kg/day in humans. The human dose was converted to the corresponding dose in mice using a conversion factor of 11.76[17-19], resulting in an equivalent dose of approximately 20.7 g/kg/day. The prepared aqueous extract (semi-solid form of CAE) was administered orally to mice via gastric gavage using a blunt-ended needle. The preparation procedure of the aqueous extract of CAE is presented in Figure 1.

Table 1. Composition of *Chaihu Shugan Tang* modified with *Adenosma bracteosum* (per daily dose)

No.	Herbal material	Scientific name	Weight (g)
1	Bupleurum	*Bupleurum scorzonerifolium* Willd.	12
2	White peony root	*Paeoniae lactiflora* Pall.	16
3	Immature bitter orange	*Citrus aurantium* L.	12
4	Nutgrass rhizome	*Cyperus rotundus* L.	12
5	Szechuan lovage rhizome	*Ligusticum chuanxiong* Hort.	8
6	Dried tangerine peel	*Citrus reticulata* Blanco	6
7	Licorice root	*Glycyrrhiza uralensis* Fisch.	6
8	Adenosma herb	*Adenosma bracteosum* Bonati	16
	Total		88

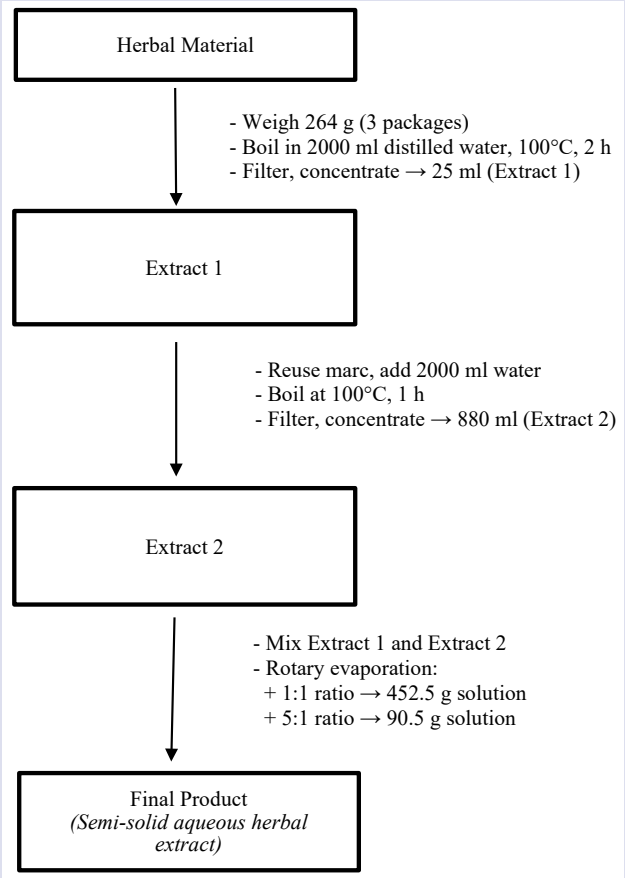

Figure 1. Flow chart of the aqueous extract preparation process

Animals

White mice (*Mus musculus*), of either sex, aged 5–6 weeks (weighing 20 ± 2 g), meeting experimental standards, were supplied by the Institute of Vaccines and Medical Biologicals, Nha Trang City. The animals were acclimatized in the laboratory animal facility under conditions appropriate for the experiment for at least one week prior to the study. Daily observations were conducted to monitor and record experimental outcomes.

White mice were randomly allocated to seven groups (n = 10/group): one control group and six treatment groups receiving the aqueous extract by oral gavage at escalating doses ranging from 8 to 64 g/kg body weight. The total sample size was 70 animals.

Acute toxicity experimental design

The experiment was conducted using a combination of two models: the limit test and the Litchfield–Wilcoxon test. First, the limit test was performed to determine the maximum dose that mice could be administered orally via a gastric gavage needle without causing death. Following this, the Litchfield–Wilcoxon method[20] was applied with one control group and six experimental groups, with doses calculated according to the formula: $D_i = D_1 + (i - 1) \times d$, where D_1 represents the initial physiological dose, d is the common ratio, and i is the group number ranging from 1 to n.

The common ratio was calculated as: $d = (D_{max} - D_{min})/(n - 1)$, where D_{max} is the maximum dose, D_{min} is the minimum dose, and n is the total number of dose groups. Each group of mice was administered the test substance at doses ranging from the highest non-lethal dose to the lowest dose that caused 100% mortality, if any. The animals were monitored for mortality within the first 24 hours, as well as for general clinical signs over 14 days, including abnormalities in feeding behavior, neurological activity, locomotion, climbing, and excretion. Signs of intoxication such as vomiting, convulsions, or agitation were also recorded. Blood samples were collected to assess hematological and biochemical parameters, while histopathological examinations of the liver, kidneys, and spleen were performed in both control and experimental groups. In the case of mortality, necropsy was carried out to evaluate gross organ lesions. The median lethal dose (LD50) was determined based on mortality rates observed within the first 72 hours.

The monitored parameters included (1) hematological indices (e.g., red blood cell count, white blood cell count, platelet count), and liver and kidney function tests (AST, ALT, urea, and creatinine); (2) macroscopic examination of the liver, kidneys, and spleen; and (3) microscopic evaluation of liver and kidney tissue sections stained with hematoxylin and eosin (H&E).

All experimental procedures in this study were conducted in accordance with the recommendations of the OECD. The study complied with these guidelines and received approval from the Institutional Animal Ethics Committee of Can Tho University of Medicine and Pharmacy (25.002.HV.CTUMP/PCT).

Data analysis

The results were expressed as mean ± standard deviation ($\bar{X} \pm SD$). Differences between groups were analyzed using One-way ANOVA followed by Tukey's post hoc test with SPSS version 20.0. A p-value of less than 0.05 was considered statistically significant.

RESULTS

General health status of mice and changes in body weight

After administration of the CAE at doses ranging from the lowest dose of 8 g/kg body weight/24 h (equivalent to 88 g of dried herbal

materials per day in humans, with a conversion factor of 11.76) to the highest dose of 64 g/kg body weight (equivalent to 704 g of dried herbal materials per day in humans), with an oral administration volume of 0.2 mL/10 g per time, twice daily within 24 h. Mice administered up to 64 g/kg body weight, which is the maximum feasible oral dose for LD50 evaluation, showed no abnormal clinical symptoms during the first 24 h and throughout the following 14 days. No mortality occurred in any group. The animals maintained good appetite, normal locomotor activity, no neurological or digestive disorders, smooth fur, clear eyes, clean perianal region, and well-formed stools (Table 2).

The body weight of mice was measured before and after 14 days of administration of the aqueous extract of CAE. Results in table 3 showed that the body weight of mice increased significantly in each group when comparing before and after treatment ($p < 0.05$). However, there was no statistically significant difference ($p > 0.05$) in the degree of weight gain among the groups receiving the extract compared to the control group. This indicates that the aqueous extract of CAE did not significantly affect the body weight development of mice during the study period.

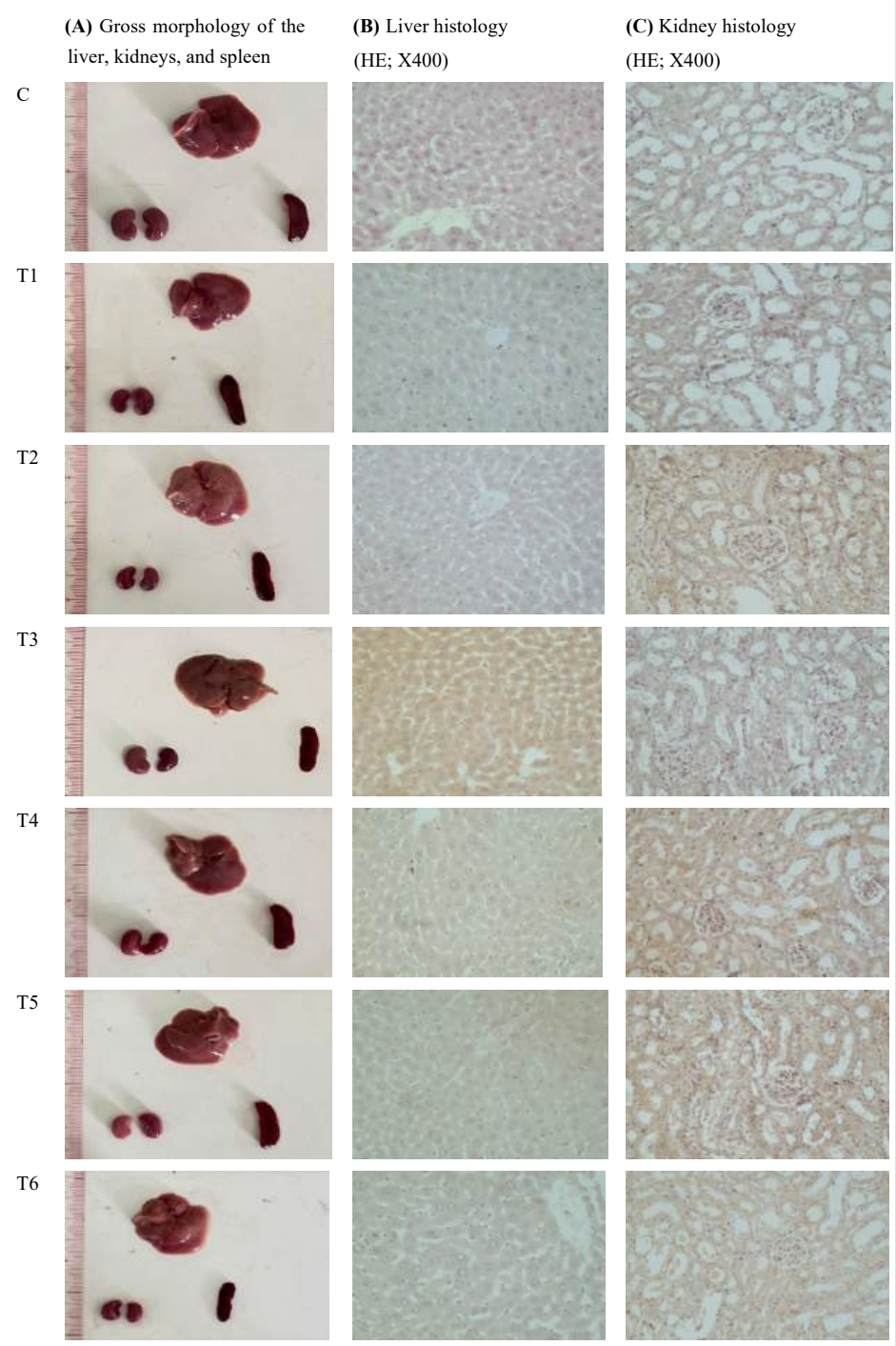

Figure 2. (A) Gross morphology of the liver, kidneys, and spleen. (B) Liver histology (hematoxylin and eosin stain, HE; ×400). (C) Kidney histology (hematoxylin and eosin stain, HE; ×400).

Table 2. Effects of the aqueous extract of *Chaihu Shugan Tang* modified with *Adenosma bracteosum* on the general health status of mice

Group	Number of mice (n)	Dose (g/kg body weight/24 h)	Mortality rate (%)	Other abnormal signs
Group 1	10	8	0	None
Group 2	10	19.2	0	None
Group 3	10	30.4	0	None
Group 4	10	41.6	0	None
Group 5	10	52.8	0	None
Group 6	10	64	0	None

Table 3. Effects of the aqueous extract of *Chaihu Shugan Tang* modified with *Adenosma bracteosum* on body weight of mice

Group	Observation index	Time point (Day 0)	Time point (Day 14)
C (0)	Body weight % body weight change	19.86 ± 0.52 ↑10.22%	21.89 ± 0.48
T1 (1)	Body weight % body weight change	19.62 ± 0.34 ↑12.6%	22.09 ± 0.35
T2 (2)	Body weight % body weight change	19.43 ± 0.54 ↑11.52%	21.67 ± 0.51
T3 (3)	Body weight % body weight change	19.42 ± 0.51 ↑13.07%	21.96 ± 0.46
T4 (4)	Body weight % body weight change	19.66 ± 0.33 ↑11.29%	21.88 ± 0.36
T5 (5)	Body weight % body weight change	19.75 ± 0.42 ↑10.58%	21.84 ± 0.37
T6 (6)	Body weight % body weight change	19.76 ± 0.47 ↑8.2%	21.4 ± 0.36

Hematological indices

After 14 days of treatment, no significant differences were observed among groups in terms of red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, white blood cell count, and platelet count in mice (Table 4).

Kidney and liver function

After 14 days of administration of the aqueous extract at the tested doses, no statistically significant differences were observed among groups in blood creatinine and urea indices. This indicates that under the tested doses and duration, the aqueous extract did not cause significant effects on kidney function in mice (Table 5).

At day 14, ALT and AST indices showed significant changes. Specifically, for ALT, groups 3, 4, 5, and 6 were significantly different ($p < 0.05$) from the control group (group 0) when analyzed using the Tukey HSD test. For AST, group 0 also differed significantly from groups 1, 3, 4, 5, and 6 ($p < 0.05$), while group 2 differed significantly from groups 3, 4, 5, and 6. Overall, ALT and AST levels tended to decrease in the higher-dose groups, particularly in groups 3, 4, 5, and 6 (Table 6).

Histopathological evaluation

After 14 days of the study, macroscopic examination of the liver, kidneys, and spleen in the treated groups showed that these organs exhibited a uniform dark brown-red color, smooth surfaces, no nodules or signs of hemorrhage, and retained elasticity when pressed. No differences were observed compared with the control group (Figure 2-A). In both control and treated groups, the hepatic lobules were clearly observed, and hepatocytes were arranged in regular cords. No hepatocyte necrosis, degeneration, or inflammatory infiltration was observed. The central vein was neither dilated nor congested (Figure 2-B). In both control and treated groups, the renal tissue structure appeared normal. Glomerular capillaries and renal tubules were intact. No necrosis of tubular epithelial cells or inflammatory cell infiltration was detected (Figure 2-C).

DISCUSSION

Traditional medicine and herbal preparations have been used for thousands of years in Vietnam as well as in many Asian countries and worldwide. With their important role in disease prevention, treatment, and community health care, herbal medicines have become an indispensable component of traditional health systems. In the current context, the demand for traditional medicine and herbal products continues to rise, accompanied by increasing attention from many countries in developing national standards for herbal medicines and establishing production guidelines to ensure the quality, efficacy, and safety of herbal and traditional medicines[20]. Vietnam possesses abundant medicinal resources and inherits a rich legacy of traditional knowledge in the use of medicinal plants and classical formulas for disease treatment and health promotion[1,37]. The country has been promoting the modernization of traditional formulations and herbal medicines to improve preservation, transportation, and usage[21]. However, the safety and therapeutic efficacy of traditional medicines and herbal products have not been fully investigated. Acute toxicity testing provides essential information for classifying the toxicological profile of a drug, predicting symptoms, anticipating treatment for acute poisoning, and establishing dose ranges for subsequent toxicological and pharmacological studies, as well as defining the safety margin[20].

The present study demonstrated that oral administration of CAE at doses ranging from 8 g/kg body weight/24 h to 64 g/kg body weight, with a dosing volume of 0.2 mL/10 g twice daily, caused no mortality within 24 hours of administration and throughout the 14-day observation period. The highest administered dose was 64 g/kg/24 h. Throughout the study period, observations of mice in all experimental groups indicated normal feeding behavior and locomotor activity, with no signs of neurological or gastrointestinal disturbances. The animals exhibited smooth fur, clear eyes, dry perianal regions, well-formed stools, and no abnormal clinical manifestations. As no mortality was observed at the maximum tolerated dose, the median lethal dose (LD_{50}) of the oral liquid extract could not be determined. The favorable

Table 4. Effects of the aqueous extract of *Chaihu Shugan Tang* modified with *Adenosma bracteosum* on some hematological indices

Indicator	Group	Day 14 (Mean ± SD)
Red blood cell count (10^{12}/L)	C (0)	9.13 ± 0.63
	T1 (1)	7.79 ± 1.86
	T2 (2)	7.67 ± 1.33
	T3 (3)	8.42 ± 0.47
	T4 (4)	8.66 ± 0.88
	T5 (5)	8.93 ± 0.8
	T6 (6)	8.5 ± 1.13
Hemoglobin concentration (g/L)	C (0)	142.6 ± 9.76
	T1 (1)	134.8 ± 24.31
	T2 (2)	150.2 ± 19.07
	T3 (3)	133.4 ± 7.72
	T4 (4)	136.6 ± 11.08
	T5 (5)	142.2 ± 5.31
	T6 (6)	140.0 ± 9.26
Hematocrit (%)	C (0)	0.47 ± 0.04
	T1 (1)	0.45 ± 0.08
	T2 (2)	0.48 ± 0.09
	T3 (3)	0.43 ± 0.02
	T4 (4)	0.43 ± 0.04
	T5 (5)	0.44 ± 0.04
	T6 (6)	0.44 ± 0.05
Mean corpuscular volume (fL)	C (0)	50.43 ± 1.48
	T1 (1)	50.91 ± 2.17
	T2 (2)	55.06 ± 8.9
	T3 (3)	50.61 ± 2.03
	T4 (4)	50.37 ± 1.25
	T5 (5)	49.33 ± 2.94
	T6 (6)	49.7 ± 4.09
White blood cell count (G/L)	C (0)	11.87 ± 1.56
	T1 (1)	8.93 ± 2.23
	T2 (2)	11.27 ± 1.43
	T3 (3)	10.86 ± 2.41
	T4 (4)	10.42 ± 2.63
	T5 (5)	10.14 ± 2.24
	T6 (6)	9.68 ± 1.83
Platelet count (G/L)	C (0)	689.0 ± 130.32
	T1 (1)	586.1 ± 215.37
	T2 (2)	557.3 ± 101.55
	T3 (3)	616.4 ± 150.52
	T4 (4)	648.0 ± 295.22
	T5 (5)	632.8 ± 208.68
	T6 (6)	588.6 ± 199.58

Table 5. Effects of the aqueous extract of *Chaihu Shugan Tang* modified with *Adenosma bracteosum* on blood urea and creatinine concentrations in mice

Indicator	Group	Day 14 (Mean ± SD)
Urea (mmol/L)	C (0)	6.41 ± 1.32
	T1 (1)	5.83 ± 1.04
	T2 (2)	7.22 ± 1.2
	T3 (3)	6.17 ± 1.52
	T4 (4)	6.1 ± 1.04
	T5 (5)	5.89 ± 1.07
	T6 (6)	4.87 ± 1.54
Creatinine µmol/L	C (0)	71.7 ± 13.89
	T1 (1)	71.2 ± 15.4
	T2 (2)	96.0 ± 11.93
	T3 (3)	79.2 ± 32.88
	T4 (4)	93.5 ± 10.13
	T5 (5)	88.6 ± 16.9
	T6 (6)	76.0 ± 17.0

Table 6. Effects of the aqueous extract of *Chaihu Shugan Tang* modified with *Adenosma bracteosum* on liver function

Indicator	Group	Day 14 (Mean ± SD)
AST (UI/L)	C (0)	310.5 ± 107.07
	T1 (1)	187.1 ± 63.22
	T2 (2)	348.8 ± 73.23
	T3 (3)	120.6 ± 47.33
	T4 (4)	205.0 ± 66.06
	T5 (5)	194.5 ± 38.71
	T6 (6)	158.0 ± 57.99
ALT (UI/L)	C (0)	159.5 ± 71.41
	T1 (1)	173.6 ± 74.67
	T2 (2)	135.8 ±42.37
	T3 (3)	52.8 ± 19.4
	T4 (4)	58.0 ± 13.36
	T5 (5)	75.9 ± 22.38
	T6 (6)	67.7 ± 53.3

tolerability observed across all tested dose levels allows the conclusion that the oral LD_{50} of the extract exceeds 64 g/kg body weight[22,23]. These findings indicate that CAE is well tolerated even at very high doses and possesses a wide safety margin. This observation is consistent with findings reported in previous acute toxicity studies of multi-herbal formulations and combined herbal extracts. Similar results have been documented in studies evaluating the acute toxicity of various herbal preparations, in which the LD_{50} commonly exceeds 5000 mg/kg. Notably, Ahmad et al. (2014) reported that *Euphorbia hirta* did not induce mortality at a dose of 5000 mg/kg and was classified as "practically non-toxic" according to the OECD toxicity classification criteria[24,25]. Similarly, Samat et al. (2020) demonstrated that their standardized herbal formulation did not induce mortality and was well tolerated across a wide dose range[26]. Likewise, Sharma et al. (2018) reported that the tested herbal preparations did not produce acute toxicity even when administered at high doses[27]. Consistent with these findings, Olorunnisola et al. (2012) showed that *Vernonia amygdalina* extract was well tolerated at very high doses (5000 mg/kg), with no observed mortality or abnormal changes in behavior, biochemical parameters, or histopathological features, thereby reinforcing the notion that multi-herbal preparations generally exhibit a high safety profile when administered orally[28]. In addition, Alafiatayo et al. (2019) reported no signs of acute toxicity following the administration of *Caesalpinia bonduc*, even at doses exceeding 2000 mg/kg[29]. These findings are consistent with those of Le Minh Hoang (2024), who reported that administration of a high-dose extract of Gui Zhi Tang (Cinnamon Twig Decoction) modified with G. asiatica to mice—at 10 times the therapeutic dose—did not result in LD_{50} determination and showed no abnormalities in treated animals[30]. Accordingly, the median lethal dose (LD_{50}) of CAE was determined to be greater than 64 g/kg body weight. This value is substantially higher than the threshold of 5000 mg/kg, which is classified by the OECD as "practically non-toxic," indicating that the liquid extract exhibits very low acute toxicity and is well tolerated even when administered at high doses.

Regarding the general condition, after 14 days of treatment, mice in both control and experimental groups remained active, with smooth fur, normal skin and mucosa, normal feeding behavior, and well-formed stools. These results suggest that the extract did not affect the general condition of mice during the study period.

Body weight is considered a sensitive index for evaluating toxic symptoms and is generally regarded as an indicator of toxicity when fluctuations exceed 10%[18]. Statistical analyses revealed no significant changes in body weight gain ($p > 0.05$). The body weight variation did not exceed 10% (increase or decrease). This indicates that administration of CAE at doses ranging from 8 g/kg to 64 g/kg body weight did not affect weight gain in mice. This parameter is widely regarded as an important indicator in safety evaluation. The observed trend is consistent with findings from other acute toxicity studies of herbal preparations. Ahmad et al. (2014) and Samat et al. (2020) likewise reported stable body weight gain over a 14-day observation period, even when the test substances were administered at high doses[26,31].

Hematological parameters, including red blood cell count, white blood cell count, platelet count, hematocrit, and hemoglobin levels, showed no statistically significant differences among the groups ($p > 0.05$), indicating that the liquid extract did not adversely affect the hematopoietic system and did not induce acute hematological toxicity. Concurrently, serum urea and creatinine levels exhibited no statistically significant changes ($p > 0.05$), demonstrating that renal function was maintained and not adversely affected throughout the study period. These observations are consistent with the safety profile reported in previous studies. Alafiatayo et al. (2019) demonstrated that *Caesalpinia bonduc* extract did not alter hematological or biochemical parameters even at very high doses (≥2000 mg/kg), reflecting good tolerability and the absence of acute toxicity[29]. Similar findings were reported by Albrakati (2021), in which stable urea–creatinine and hematological indices were observed in control and treated groups, with the author emphasizing that the lack of change in these parameters constitutes important evidence of preserved renal function and the absence of hematological disorders[32]. Furthermore, a meta-analysis conducted by Razmpoosh et al. (2020) confirmed that herbal preparations administered orally generally do not cause significant fluctuations in urea and creatinine levels[33], reinforcing the notion that herbal-derived products typically possess a wide safety margin with respect to renal and protein metabolic function. Collectively, the stability of both hematological parameters and renal function indices in the present study indicates that CAE does not induce acute hematological or nephrotoxic effects, even when administered at high doses.

Regarding liver function, both ALT and AST levels tended to decrease in the high-dose groups, particularly in groups 3, 4, 5, and 6, with statistically significant differences compared with controls ($p < 0.05$). The observed reduction in liver enzyme levels was not accompanied by any adverse outcomes, such as mortality, clinical signs of toxicity, other biochemical abnormalities, or histopathological damage on either macroscopic or microscopic examination. According to OECD guidelines for acute toxicity assessment, elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are considered typical indicators of acute hepatic injury; in contrast, decreases in these enzymes are generally not regarded as manifestations of toxicity and may instead reflect physiological variation or hepatoprotective activity of the test substance. This

interpretation is consistent with the known pharmacological properties of the constituent herbs in *Chaihu Shugan Tang*. Multiple studies have demonstrated that *Bupleurum falcatum* exerts hepatoprotective, anti-inflammatory, and antioxidant effects, leading to reductions in liver enzyme levels in experimental models of hepatic injury induced by carbon tetrachloride (CCl_4) or paracetamol (Li et al., 2017; Kim et al., 2015)[34,35]. Similarly, *Adenosma bracteosum* contains thymol, linalool, and various flavonoids with potent antioxidant activity, which have been shown to reduce ALT and AST levels and to improve hepatic histoarchitecture in animal models of toxic liver injury (Nguyen et al., 2020; Tran et al., 2018)[8,36]. Furthermore, the findings of the present study are consistent with toxicity reports on other herbal preparations, such as those by Alafiatayo et al. (2019) for *Caesalpinia bonduc* and by Olorunnisola et al. (2012) for *Vernonia amygdalina*, both of which demonstrated no evidence of hepatotoxicity and stable liver enzyme levels even at high doses[29,28]. Taken together, the reduction in ALT and AST observed in the present study is more likely indicative of the hepatoprotective potential of the liquid extract rather than a sign of toxicity, and is fully consistent with the established pharmacological properties of the herbal components involved.

Macroscopic examination of the liver, kidneys, and spleen in both the experimental and control groups revealed normal morphology, with no alterations in color, structure, or tissue elasticity. Histopathological findings were fully consistent with the gross observations, as no evidence of tissue damage was detected, including cellular degeneration, necrosis, inflammatory infiltration, congestion, or interstitial edema. As histopathological evaluation is considered the gold standard in acute toxicity assessment, the absence of structural alterations indicates that the liquid extract did not induce toxicity in target organs, particularly the liver and kidneys, which are highly susceptible to toxic insults. These findings are consistent with previously published reports on the toxicity profiles of herbal preparations. Samat et al. (2020), in their evaluation of the acute and subacute toxicity of a standardized herbal formulation, reported completely normal hepatic and renal histology, with no evidence of necrosis or interstitial inflammation[26]. Similarly, Alafiatayo et al. (2019) demonstrated that *Caesalpinia bonduc* seed extract did not cause histopathological alterations in the liver or kidneys of experimental animals[29]. Collectively, these observations further reinforce the evidence that herbal formulations generally possess a wide safety margin and rarely induce target-organ toxicity when administered orally.

CONCLUSION

The results of this study demonstrate that CAE did not induce acute toxicity at any of the tested dose levels, including the maximum dose of 64 g/kg body weight. No mortality, clinical signs of toxicity, or behavioral abnormalities were observed, and hematological parameters, biochemical indices, and renal function remained within normal ranges. Histopathological examination of the liver, kidneys, and spleen revealed no structural damage, thereby confirming the absence of target-organ toxicity. Although extrapolation of dosage from animal models to humans remains limited, the findings of this study indicate that the liquid extract possesses a relatively wide safety margin and provides preliminary safety data to support further experimental and clinical investigations.

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REFERENCES

1. Nam NH, Huong LTT, et al. (2023) A review on ethnomedicinal plants in Vietnam and their bioactive compounds. Natural Product Communications 18(7).
2. Lai X, Wang K, Zhai X, Li Y, Hu Z, et al. Efficacy and safety of Chinese herbal medicine compared with losartan for mild essential hypertension: a randomized, multicenter, double-blind, noninferiority trial. Circ Cardiovasc Qual Outcomes. 2022;15:e007923.
3. Yu X, Li L, Ma X, et al. The efficacy and safety of the Chinese herbal formula JTTZ for the treatment of type 2 diabetes with obesity and hyperlipidemia: a multicenter randomized, positive-controlled, open-label clinical trial. Int J Endocrinol. 2018;2018:9519231.
4. Tian JX, Lian FM, Tong XL, et al. Efficacy of Chinese herbal medicine compared with metformin for glucose regulation and cardiovascular risk factors in type 2 diabetes with dyslipidaemia: a multicenter randomized clinical trial. Lancet Diabetes Endocrinol. 2021
5. Jafari Z, Hadipour M, Dehghani F, et al. The effect of Glycyrrhiza glabra L. on primary dysmenorrhea compared with ibuprofen: a randomized, triple-blind controlled trial. Iran J Pharm Res. 2019;18(4):2202–2210.
6. Yang N, Liu Y, Xu H, et al. Modified Chaihu Shugan powder for functional dyspepsia: a meta-analysis of randomized controlled trials. Evid Based Complement Alternat Med. 2013;2013:791724.
7. Wang W. Clinical Efficacy of Modified Chaihu Shugan Tang in the Treatment of Hypertension with Comorbid Anxiety (柴胡疏肝汤加减治疗高血压病伴焦虑的临床疗效研究). Int J Clin Res. 2025;9(9):53–57.
8. Nguyen NH, Nguyen HT, Nguyen TH, et al. Potential antidiabetic activity of extracts and isolated compound from Adenosma bracteosum (Bonati). Int J Pharm Sci Rev Res. 2020;64(1):123–130.
9. Wang YJ, Zhou J, Li YN, et al. Efficacy of Chaihu Shugan San in the Treatment of Functional Dyspepsia with Liver–Stomach Disharmony (柴胡疏肝散治疗功能性消化不良肝胃不和证的疗效研究). J Integr Tradit Chin West Med Dig. 2023;31(10):751–755.
10. Zhang YY, Zhang Y, Xu H, et al. Therapeutic effect of Chaihu-Shugan-San in fatty liver disease: a meta-analysis of randomized controlled trials. Int J Clin Exp Med. 2018;11(12):12880–12888.
11. Qu X, Jiang Y, Wang W, et al. Chaihu-Shugan-San for patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Medicine (Baltimore). 2025;104(20).
12. He SY, Chen YF, Liu Y, et al. Mechanisms of Chaihu Shugan San in the Prevention and Treatment of Non-Alcoholic Fatty Liver Disease: A Systems Pharmacology Study (柴胡疏肝散防治非酒精性脂肪肝作用机制研究. 系统药理学研究). 2024.
13. Sun Y, Zhao H, Wang H, et al. Treatment of depression with Chai Hu Shu Gan San: a systematic review and meta-analysis of 42 randomized controlled trials. BMC Complement Med Ther. 2018;18:21.
14. Nguyen NH, Pham QT, Luong TNH, Le HK, Vo VG. Potential antidiabetic activity of extracts and isolated compound from Adenosma bracteosum. Biomolecules. 2020;10(2):201.
15. Nguyen NH, Duong TH, Nguyen HT, et al. New halogenated flavonoids from Adenosma bracteosum and Vitex negundo and their α-glucosidase inhibition. Chem Biodivers. 2023;20(7):e202300390.
16. Nguyen NH, Ta QTH, Pham QT, et al. Anticancer activity of novel plant extracts and compounds from Adenosma bracteosum in human lung and liver cancer cells. Molecules. 2020;25:2912.
17. Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human studies revisited. FASEB Journal, 22(3), 659–661.
18. U.S. Food and Drug Administration. (2005). Guidance for industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers.
19. Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. Journal of basic and clinical pharmacy, 7(2), 27–31.

20. Ministry of Health (2015), Guidance on preclinical and clinical trials of traditional oriental medicines and herbal medicines issued with Decision No. 141/QD-K2DT dated October 27, 2015, 2015.
21. Government (2019), Decision No. 1893/QD-TTg dated December 25, 2019, of the Prime Minister approving the Program for the development of traditional medicine combined with modern medicine until 2030, 2019.
22. OECD. Test No. 420: Acute Oral Toxicity – Fixed Dose Procedure. OECD Guidelines for the Testing of Chemicals; 2001.
23. OECD. Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals; 2001.
24. OECD. Test No. 425: Acute Oral Toxicity – Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals; 2008.
25. OECD. Guidance Document on Considerations for Acute Oral Toxicity Testing. Series on Testing and Assessment No. 240; 2016
26. Samat NA, Zaidan S, Shamsuddin AF, et al. Acute and subchronic oral toxicity of a standardized herbal formulation. BMC Complement Med Ther. 2020;20:32.
27. Sharma V, Sharma A, Kansal L. Toxicity assessment of herbal preparation. Toxicol Rep. 2018;5:580–586.
28. Olorunnisola SK, Bradley G, Afolayan AJ. Acute and sub-acute toxicity studies of Vernonia amygdalina leaf extract in rats. J Med Plants Res. 2012;6(18):3517–3521
29. Alafiatayo AA, Lai KS, Syahid A, et al. Evaluation of acute toxicity of Caesalpinia bonduc seed extract. Sci Rep. 2019;9:16951.
30. Le Minh Hoang, Nguyen Duy Khuong, Tran Viet Anh, Nguyen Thi Thuy Linh, Nguyen Thi Huyen Trang, Nguyen Thi Thu Thuy (2024) Evaluation acute and subchronic oral toxicity of "quyen ty thang increase cinnamomum, spina gleditsciae" in mice. Cantho Journal of Medicine and Pharmacy 71: 174–180.
31. Ahmad M, Mohammed S, Zaini A, et al. Acute and subacute toxicity studies of Euphorbia hirta extract in rodents. J Ethnopharmacol. 2014;152(3):548–556.
32. Albrakati A. Aged garlic extract rescues ethephon-induced kidney damage by modulating oxidative stress, apoptosis, inflammation and histopathology. Environ Sci Pollut Res. 2021;28(6):6818–6829
33. Razmpoosh E, Safi S, Abdollahi N, et al. The effect of Nigella sativa on liver and kidney parameters: a systematic review and meta-analysis of RCTs. Pharmacol Res. 2020;156:104767.
34. Li X, Wang H, Wen X, et al. Protective effect of Bupleurum falcatum against CCl_4-induced liver injury. J Ethnopharmacol. 2017;196:232–240.
35. Kim HY, Kim JK, Choi JH, et al. Hepatoprotective effect of Bupleurum falcatum on paracetamol-induced liver damage. Molecules. 2015;20(4):5804–5815.
36. Tran KQ, Pham QT, Le HK, et al. Antioxidant and hepatoprotective activities of flavonoids isolated from Adenosma bracteosum. J Pharm Pharmacogn Res. 2018;6(5):346–356
37. Nguyen, P. H., Tran, V. D., Pham, D. T., Dao, T. N. P., & Dewey, R. S. (2021). Use of and attitudes towards herbal medicine during the COVID-19 pandemic: A cross-sectional study in Vietnam. European Journal of Integrative Medicine, 54, 101328.

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