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# ACUTE TOXICITY STUDY AND EFFECTS OF DRY EXTRACT OF "THANG THANH GIANG TROC" ON GENERAL CONDITION AND RENAL FUNCTION IN EXPERIMENTAL ANIMALS

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## ABSTRACT

**Objective:** To study the acute toxicity and effects of the dried extract of "clearing and purifying" on the general health and kidney function of experimental animals .

**Research subjects and methods:**

Acute toxicity of the dried extract was assessed in white mice using the Litchfield–Wilcoxon method.

The effects of the extract on general health and kidney function were evaluated in 50 white rats with chronic kidney disease induced by 5/6 nephrectomy. The rats were divided into 5 groups: TTGT-1 (administered the dried extract at a dose of 0.735 g/kg/day), TTGT-2 (1.47 g/kg/day), the model group (administered distilled water), the reference group (enalapril 10 mg/kg/day), and the surgical control group (no kidney removal, administered distilled water). The treatment lasted 60 days. Evaluation parameters included general condition, serum urea and creatinine levels, kidney weight, and renal histopathology before and after treatment.

**Results:** The maximum dose of 30 g/kg (23.6 times the expected therapeutic dose) caused no mortality or signs of toxicity. After 60 days, serum urea and creatinine levels in the TTGT-1 and TTGT-2 groups significantly decreased compared with baseline and the model group ($p<0.01$).

**Conclude:**

The dried extract "transforms purity into impurity". Achieved safety in acute toxicity testing.

The dried extract "ascends the pure and descends the impure" Doses of 0.735 g/kg/24h and 1.47 g/kg/24h reduced serum urea and creatinine levels in experimentally resected white rats with chronic kidney disease.

***Keywords:*** Thang thanh giang troc, chronic kidney disease, 5/6 nephrectomy.

## 1. INTRODUCTION

Chronic kidney disease (CKD) is a condition of prolonged structural damage or functional decline of the kidneys, seriously affecting the health of patients and increasing the risk of death. The disease is often associated with many chronic diseases such as diabetes, hypertension, or metabolic disorders, and is on the rise globally. According to the International Society of Nephrology, about 10-13% of the adult population worldwide suffers from chronic kidney disease, creating a significant burden on the healthcare system [1]. The progression of the disease often occurs silently over a long period and can lead to end-stage renal failure if not controlled in time. Kidney replacement therapies such as dialysis or kidney transplantation help prolong life but are expensive and require specialized technical conditions [2]. In traditional medicine, chronic kidney disease is considered a consequence of functional disorders of the internal organs, especially related to the kidneys, spleen, and lungs, leading to the indistinguishment of clear and turbid substances and stagnation of fluids and dampness. Many traditional medicine treatments have been studied to improve symptoms, enhance kidney function, and limit disease progression [3]. The "thang thanh giang troc thang" formula has been used at the Department of Nephrology and Urology of Tue Tinh Hospital for many years and has an 80% treatment success rate for patients with chronic kidney disease [4]. However, the use of decoctions has many limitations in terms of preparation time and storage. Therefore, to make it convenient for users, we have initially produced a dry extract called "thang thanh giang troc". To ensure scientific accuracy and comprehensively evaluate the effects of the formula, this study was conducted to evaluate the acute toxicity and effects of the dry extract on the general health and kidney function in experimental animals.

## 2. MATERIALS, SUBJECTS, AND METHODS OF RESEARCH

### 2.1. Research materials

#### *2.1.1. Research Product*

The “Thang Thanh Giang Troc” dried extract was derived from the traditional herbal formula “On Dam Thang”, consisting of the following medicinal materials: Rhizoma Smilacis glabrae, Radix et Rhizoma Salviae miltiorrhizae, Cortex Eucommiae, Flos Styphnolobii japonici immaturi,

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Radix Astragali membranacei, Herba Centellae asiaticae, Radix Polygoni cuspidati, Rhizoma Pinelliae preparatum, Pericarpium Citri reticulatae perenne, Caulis Bambusae in Taeniis, Fructus Aurantii, Faeces Bombycum, Rhizoma Rhei and Radix Achyranthis bidentatae.

All herbal materials were authenticated and met the quality standards of the Vietnamese Pharmacopoeia V and the institutional standards. The dried extract was prepared by aqueous extraction, followed by concentration and drying to obtain a dried extract for experimental use.

***2.1.2. Control drug used in the study:*** Enalapril 10mg.

**2.2. Research Subjects**

Acute toxicity studies were conducted on healthy Swiss white mice of both sexes, weighing 18-22g.

The study on the effects of the "clearing and purifying" dry extract was conducted on healthy, adult male Wistar white rats weighing 200-250g.

The laboratory mice were provided by the Animal Department of the Military Medical Academy.

**2.3. Location and time of study**

The study was conducted from April 2024 to October 2024 at the Department of Pharmacology, Institute of Pharmacy Training – Military Medical Academy, and at the Department of Pathology and Forensic Medicine of the 103 Military Hospital.

**2.4. Research Methodology**

***2.4.1. Methods for studying the acute toxicity of the dried extract of "thang thanh giang troc"***

Determine the LD50 of the dry extract of "clearing and purifying" in white mice by oral administration using the Litchfield - Wilcoxon method and according to the guidelines of the Ministry of Health and the World Health Organization (WHO).

Swiss white mice were randomly divided into 5 groups of 10 each. They ¬were given the "clearing and purifying" dry extract orally at a volume of 0.2 ml/10 g body weight/ dose, but with gradually increasing doses, up to a maximum of 3 times/24 hours, with each dose administered at least 3 hours apart. The mice were fasted for 12 hours before administration but had access to plenty of water . The condition of the mice was assessed at 72 hours and 7 days after administration.

***2.4.2. Research method on the effects of "clearing and purifying" dry extract on a mouse model of chronic kidney disease with 5/6 nephrectomy.***

Rats successfully modeled for CKD were randomly assigned into four experimental groups (n = 10 each), and an additional control group (n = 10) was used for physiological comparison.

*Group 1 (Control):* Rats underwent a sham operation (no kidney removal) and were given distilled water orally.

*Group 2 (Model):* Rats underwent 5/6 nephrectomy and were given distilled water orally.

*Group 3 (Reference):* Rats underwent 5/6 nephrectomy and received Enalapril at a dose of 10 mg/kg/day administered orally.

*Group 4 (TTGT-1):* Rats underwent 5/6 nephrectomy and received the Thang Thanh Giang Troc extract at a dose of 0.735 g/kg/day administered orally.

*Group 5 (TTGT-2):* Rats underwent 5/6 nephrectomy and received the Thang Thanh Giang Troc extract at a dose of 1.47 g/kg/day administered orally.

All treatments were administered once daily by oral gavage for 60 consecutive days.

The rats were given the test sample, reference drug, or distilled water in batches for 60 days to evaluate the effect of the "clearing and purifying" dry extract .

## 3. RESULTS

**3.1. Results of acute toxicity study of "thang thanh giang troc" dried extract**

Results of monitoring the general condition and mortality rate of mice.

After ingestion of the sample, the mice were monitored for 72 hours and until day 7. Mice in all groups were active, ate normally, and showed no signs of neurological, respiratory, or autonomic nervous system disorders. At doses of 6.0–30.0 g/kg body weight, no deaths or signs of toxicity were observed. Even at the maximum dose of 30.0 g/kg (23.6 times the expected effective dose), no toxicity was detected, indicating that the dry extract of "thang thanh giang troc" has high safety in acute toxicity testing.

**3.2. Research results on the effects of the dry extract of "clearing and reducing turbidity" on a mouse model of chronic kidney disease with 5/6 nephrectomy.**

***3.2.1. Results of assessing the general condition and weight of the mice***

The results of the general status assessment showed that mice in the surgical groups with the model exhibited more ruffled fur, darker fur color, reduced activity and appetite compared to the surgical control group, with these symptoms becoming increasingly pronounced over time. The general status of mice in the reference groups (treated with enalapril) and the groups treated with TTGT improved better (less ruffled fur, better activity and appetite) compared to the model group.

The results of the weight assessment of the mice are presented in Table 1.

**Table 1. Effect of "clearing and purifying" dry extract on mouse weight. ($\bar{X}$ ± SD, n = 10 in each batch)**

| Research group | Mouse weight (g ) | | | $p_{b-a}$ | $p_{c-a}$ | $p_{c-b}$ |
|---|---|---|---|---|---|---|
| | Immediately after the second PT (a) | 15 days after the second PT (b) | After taking the medication for 60 days (c) | | | |
| Control (1) | 198.08 | 208.21 | 236.18 | < 0.05 | < 0.01 | < 0.01 |
| | ± 18.92 | ± 20.69 | ± 19.54 | | | |
| Model (2) | 196.36 | 195.62 | 193.95 | > 0.05 | > 0.05 | > 0.05 |
| | ± 19.05 | ± 19.55 | ± 19.28 | | | |
| Reference (3) | 191.82 | 189.82 | 218.32 | > 0.05 | > 0.05 | < 0.05 |
| | ± 18.62 | ± 18.62 | ± 20.14 | | | |

| Research group | Mouse weight (g ) | | | $p_{b-a}$ | $p_{c-a}$ | $p_{c-b}$ |
|---|---|---|---|---|---|---|
| | Immediately after the second PT (a) | 15 days after the second PT (b) | After taking the medication for 60 days (c) | | | |
| TTGT- 1 (4) | 195.16 | 194.95 | 215.91 | > 0.05 | > 0.05 | < 0.05 |
| | ± 17.63 | ± 19.26 | ± 21.06 | | | |
| TTGT-2 (5) | 193.95 | 193.08 | 224.06 | > 0.05 | > 0.05 | < 0.05 |
| | ± 20.06 | ± 18.98 | 21.65 | | | |
| $P_{2-1}$ | > 0.05 | < 0.05 | < 0.01 | - | - | - |
| $P_{3,4,5-1}$ | > 0.05 | < 0.05 | < 0.05 | - | - | - |
| $P_{3,4,5-2}$ | > 0.05 | > 0.05 | < 0.05 | - | - | - |
| $P_{4.5-3}$ | > 0.05 | > 0.05 | > 0.05 | - | - | - |
| $P_{5-4}$ | > 0.05 | > 0.05 | > 0.05 | - | - | - |

Table 1 shows that the weight of mice between the treatment groups at baseline did not differ significantly ($p > 0.05$). After 60 days of treatment, the weight of mice in the treatment groups increased compared to before treatment ($p < 0.05$) and was higher than that of the control group, while the weight of the control group did not increase ($p < 0.05$).

***3.2.2. Results of urea and creatinine assessment in mouse blood***

**Table 2. Effect of "clearing and purifying" dry extract on serum urea concentration in rats. ($\bar{X}$ ± SD, n = 10 in each batch)**

| Research group | Serum urea concentration (mmol/l) | | | $p_{b-a}$ | $p_{c-a}$ | $p_{c-b}$ |
|---|---|---|---|---|---|---|
| | Before surgery | 15 days after the second surgery | After taking the medication for 60 days | | | |
| Control (1) | 5.72 ± 0.71 | 5.88 ± 0.84 | 6.01 ± 0.98 | > 0.05 | > 0.05 | > 0.05 |
| Model (2) | 5.68 ± 0.74 | 8.96 ± 0.98 | 10.56 ± 1.12 | > 0.05 | < 0.01 | < 0.001 |
| Reference (3) | 5.81 ± 0.88 | 8.81 ± 1.01 | 8.24 ± 0.96 | > 0.05 | < 0.01 | < 0.05 |
| TTGT- 1 (4) | 5.48 ± 0.69 | 8.68 ± 0.92 | 8.59 ± 0.91 | > 0.05 | < 0.01 | > 0.05 |
| TTGT-2 (5) | 5.92 ± 0.85 | 8.75 ± 0.93 | 7.90 ± 0.85 | > 0.05 | < 0.01 | < 0.05 |
| $P_{2-1}$ | > 0.05 | < 0.01 | < 0.001 | - | - | - |
| $P_{3,4,5-1}$ | > 0.05 | < 0.01 | < 0.01 | - | - | - |
| $P_{3,4,5-2}$ | > 0.05 | > 0.05 | < 0.01 | - | - | - |
| $P_{4.5-3}$ | > 0.05 | > 0.05 | > 0.05 | - | - | - |
| $P_{5-4}$ | > 0.05 | > 0.05 | < 0.05 | - | - | - |

Table 2 shows that after 60 days of treatment, serum urea levels in the control group remained unchanged ($p > 0.05$), while the model group showed a significant increase compared to previous time points and compared to the control group ($p < 0.001$). Compared to the model group, all drug-treated groups showed a decrease in serum urea ($p < 0.01$), with the TTGT-2 group showing the greatest decrease, which was significantly lower than TTGT-1 ($p < 0.05$).

**Table 3. Effect of "clearing and purifying" dry extract on serum creatinine concentration in rats. ($\bar{X}$ ± SD, n = 10 in each batch)**

| Research group | Serum creatinine concentration (µmol/l) | | | $p_{b-a}$ | $p_{c-a}$ | $p_{c-b}$ |
|---|---|---|---|---|---|---|
| | Before surgery | 15 days after the second surgery | After taking the medication for 60 days | | | |
| PT certificate (1) | 89.20 ± 9.85 | 90.10 ± 9.92 | 91.10 ± 10.21 | > 0.05 | > 0.05 | > 0.05 |
| Model (2) | 88.90 ± 9.54 | 139.60 ± 14.26 | 241.38 ± 26.52 | > 0.05 | < 0.01 | < 0.001 |
| Reference (3) | 86.90 ± 9.65 | 140.80 ± 15.06 | 131.60 ± 14.96 | > 0.05 | < 0.01 | < 0.05 |
| TTGT- 1 (4) | 86.10 ± 9.52 | 138.80 ± 14.16 | 133.99 ± 14.73 | > 0.05 | < 0.01 | > 0.05 |
| TTGT-2 (5) | 85.90 ± 9.46 | 141.50 ± 13.96 | 130.60 ± 13.65 | > 0.05 | < 0.01 | < 0.05 |

| Research group | Serum creatinine concentration (µmol/l) | | | $p_{b-a}$ | $p_{c-a}$ | $p_{c-b}$ |
|---|---|---|---|---|---|---|
| | Before surgery | 15 days after the second surgery | After taking the medication for 60 days | | | |
| $P_{2-1}$ | > 0.05 | < 0.01 | < 0.001 | - | - | - |
| $P_{3,4,5-1}$ | > 0.05 | < 0.01 | < 0.01 | - | - | - |
| $P_{3,4,5-2}$ | > 0.05 | > 0.05 | < 0.01 | - | - | - |
| $P_{4.5-3}$ | > 0.05 | > 0.05 | > 0.05 | - | - | - |
| $P_{5-4}$ | > 0.05 | > 0.05 | < 0.05 | - | - | - |

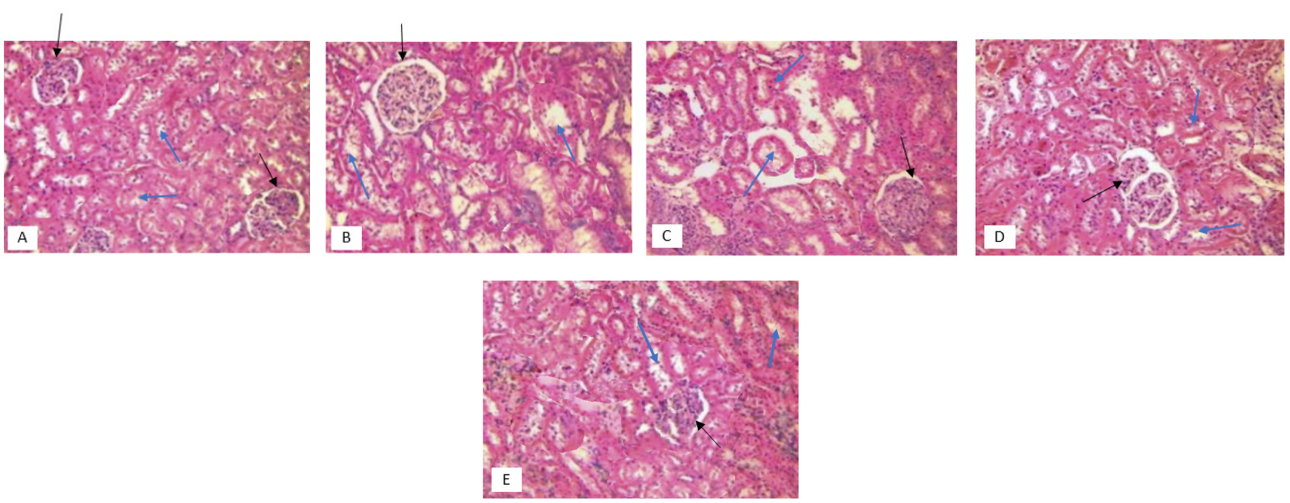
Table 3 shows that, after 60 days of treatment, compared to the control group, the serum creatinine concentration of rats in the drug-treated groups decreased significantly ($p < 0.01$). Serum creatinine concentration of rats in TTGT-2 group. The reduction was the greatest, and the difference was statistically significant compared to group TTGT-1 ($p < 0.05$) .

***3.2.6. Results of weight and microscopic assessment of mouse kidneys***

Compared to the model group, the kidney weight of rats in the reference groups, TTGT-1, and TTGT-2 decreased by 13.89%, 15.15%, and 16.41%, respectively, with statistically significant differences ($p < 0.05$). Comparing the three reference groups, TTGT-1 and TTGT-2, there was no significant difference in kidney weight ($p > 0.05$)(Table 4).

**Table 4. Effect of "clearing and purifying" dry extract on the kidney weight of rats ($\bar{X} \pm SD$, n = 10 in each batch)**

| Research group | Weight of mouse kidneys (g/100g mouse) | % reduction compared to the model batch |
|---|---|---|
| Model (2) | 0.396 ± 0.046 | - |
| Reference (3) | 0.341 ± 0.042 | 13.89% |
| TTGT- 1 (4) | 0.336 ± 0.051 | 15.15% |
| TTGT-2 (5) | 0.331 ± 0.049 | 16.41% |
| $P_{3,4,5-2}$ | < 0.05 | - |
| $P_{4.5-3}$ | > 0.05 | - |
| $P_{5-4}$ | > 0.05 | - |




*A. Control group PT (5 mice); B. Model group (12 mice); C. Reference group (24 mice); D. TTGT-1 group (36 mice); E. TTGT-2 group (45 mice); 1. Glomerulus (black arrow); 2. Renal tubule (blue arrow)*

**Figure 1. Microscopic images of mouse kidneys in the study groups (HE x 200)**

Images of the kidneys of mice with chronic kidney disease in the PT control group (image A) show normal renal parenchyma, renal cortex with glomeruli, renal tubules, and intertubular blood vessels with normal structure. Histological images of the kidneys of mice in the model group (image B) show interstitial dilation, glomerular hypertrophy, and tubular dilation. Histological images of the kidneys of mice in the reference group (image C), TTGT-1 group (image D), and TTGT-2 group (image E) show significantly improved renal lesions compared to the model group (image B).

## 4. DISCUSSION

### 4.1. Discussion on the acute toxicity of the "clearing and purifying" dried extract.

Results showed that the dry extract of "thang thanh giang troc" did not cause acute toxicity when administered orally to white mice. At the maximum dose of 30.0 g/kg body weight (the limit of what could be given orally in 24 hours), no deaths or abnormal symptoms were observed during the first 72 hours and 7 days of observation. This dose was 23.6 times higher than the

expected effective dose; however, the mice maintained normal conditions such as smooth fur, clear eyes, normal eating and activity. Therefore, the $LD_{50}$ of the oral preparation under experimental conditions could not be determined. The results indicate that the dry extract of "Thăng Thanh Giáng Trọc" has a high safety profile, meeting the objective of acute toxicity assessment in the study.

**4.2. Discussion on the effects of the dried extract of "thang thanh giang troc" on a mouse model of chronic kidney disease with 5/6 nephrectomy**

***4.2.1. The effect of the "clearing and clearing" dry extract on the general condition of mice***

The dried extract of "clearing and purifying" herbs should be taken continuously. The study duration did not affect the overall condition of the mice. Mice in both the control group and the groups treated with the "clearing and clearing" dry extract showed positive results. They are all behaving normally. The mice have smooth fur, normal skin and mucous membranes, normal eating habits, and formed stools.

***4.2.2. Effect of "clearing and purifying" dry extract on the weight of mice***

After 60 days of treatment, the weight of mice in the treated groups increased compared to before treatment. The weight of mice in the model group did not increase. Compared to the model group, the weight of mice in the drug-treated groups was statistically significantly higher ($p < 0.05$). Thus, the "raising the clear and lowering the turbid" dry extract did not affect the weight of the mice.

***4.2.3. Effect of "clearing and purifying" dry extract on serum urea and creatinine concentrations in rats***

Increased serum urea is a common manifestation in chronic kidney disease due to impaired glomerular filtration function, leading to the accumulation of nitrogenous metabolic products in the blood [6]. In a 5/6 nephrectomy mouse model, after 60 days of treatment, serum urea levels in the groups treated with the "rising clear, falling turbid" dry extract decreased significantly compared to the control group ($p < 0.01$). This result shows that the preparation has the ability to support the improvement of nitrogenous metabolic disorders in chronic kidney disease.

Furthermore, serum creatinine is an important indicator reflecting the degree of renal function decline. After 60 days of drug use, serum creatinine levels in the treatment groups decreased significantly compared to the control group ($p < 0.01$). This indicates that the dried extract of "thang thanh giang troc" has an effect on improving biochemical indicators related to renal function in a mouse model of chronic kidney disease induced by 5/6 nephrectomy.

***4.2.3. Effects of the "clearing and purifying" dry extract on the weight and kidney microscopy of rats***

The study results showed that kidney weight in the reference groups, TTGT-1 and TTGT-2 decreased by 13.89%, 15.15%, and 16.41%, respectively, compared to the model group ($p < 0.05$). This indicates that the treatment measures were effective in limiting renal hypertrophy, a common condition in chronic kidney disease.

Histological results of the kidney showed that the surgical control group had normal kidney structure, while the model group exhibited characteristic lesions such as interstitial dilation, glomerular hypertrophy, and tubular dilation. In the reference groups, TTGT-1 and TTGT-2, histological lesions were significantly reduced, and glomerular and tubular structures improved compared to the model group. These results indicate that the dried extract of "thăng thanh giáng trọc" has a protective and improving effect on kidney tissue damage in the mouse model of chronic kidney disease.

## 5. CONCLUSION

**5.1. Conclusion on the acute toxicity of the dried extract of "clearing and purifying"**

LD50 of the "thang thanh giang troc" dry extract was found after oral administration in white mice. At the highest dose administered to mice, 30.0 g/kg body weight - 23.6 times the expected effective dose-no mice died or showed signs of toxicity. Therefore, it can be confirmed that the "thang thanh giang troc" dry extract is effective. Achieved safety in acute toxicity testing.

**5.2. Conclusion on the effects of the "clearing and purifying" dry extract on a model of chronic kidney disease with 5/6 nephrectomy in white rats.**

The dried extract "transforms purity into impurity". Doses of 0.735 g/kg/24h and 1.47 g/kg/24h were effective in preventing and inhibiting the progression of chronic kidney disease in a 5/6 nephrectomy model in white rats. Specifically:

- Reduced indicators assessing renal function impairment: blood urea and creatinine compared to the control group.

- Reduced renal hypertrophy and renal histopathological changes through indicators such as: reduced renal weight, improved renal histopathological lesions (such as interstitial dilation, glomerular hypertrophy, and tubular dilation).

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