STUDY ON THE EFFECT OF THE DRY EXTRACT THANG THANH GIANG TROC ON BLOOD PRESSURE AND URINARY INDICES IN A RAT MODEL OF CHRONIC KIDNEY DISEASE INDUCED BY FIVE-SIXTHS NEPHRECTOMIES

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ABSTRACT

Objective: *To investigate the effect of the dry extract of Thang Thanh Giang Troc on blood pressure and urinary indices in a rat model of chronic kidney disease induced by five-sixths kidney resection.*

Subjects and methods*: This study was conducted according to the method of Lu JR et al. (2014). Forty Wistar rats with CKD induced by 5/6 nephrectomy were divided into four groups: TTGT-1 and TTGT-2 (administered Thang Thanh Giang Troc extract at doses of 0.735 g/kg/day and 1.47 g/kg/day, respectively), the model group (given distilled water), and the reference group (treated with Enalapril at 10 mg/kg/day). In addition, a separate control group of 10 rats underwent sham operation without kidney removal and received distilled water. The intervention lasted 60 days, during which blood pressure and urine parameters were evaluated before and after treatment.*

Results: *After 60 days of treatment, systolic, diastolic, and mean blood pressures, 24-hour urine volume, and proteinuria in the TTGT-1 and TTGT-2 groups were significantly reduced compared with pre-treatment levels and with those in the model group ($p<0.01$).*

Conclusion: *The dry extract of Thang Thanh Giang Troc, administered at doses of 0.735 g/kg/day and 1.47 g/kg/day, produced statistically significant antihypertensive and renoprotective effects in the 5/6 nephrectomy rat model of chronic kidney disease ($p<0.05$). This experimental model offers a reliable foundation for future pharmacological and therapeutic research.*

Keywords*: Thang Thanh Giang Troc, chronic kidney disease, 5/6 nephrectomy, rats.*

INTRODUCTION

Chronic kidney disease (CKD) is a common and serious condition, often secondary to systemic disorders such as diabetes, hypertension, and gout [1]. It represents a global public health burden with a rising incidence and high treatment costs [2]. According to the Global Burden of Disease Study (2010), CKD ranked 27th among causes of death worldwide in 1990 and increased to 18th by 2010 [3]. CKD develops insidiously with complex pathophysiology and multi-organ effects. Without timely management, it can progress to end-stage renal failure, threatening both health and life. Current renal replacement therapies such as hemodialysis, peritoneal dialysis, and kidney transplantation can prolong survival but are costly and unsuitable for early or mid-stage disease. Traditional medicine, with its advantages of fewer side effects, accessibility, and potential to alleviate symptoms and prevent complications, plays an important role in CKD management. The prescription Thang Thanh Giang Troc Thang has been clinically used for many years at the Nephrology and Urology Department of Tue Tinh Hospital, achieving an efficacy rate of approximately 80% in CKD patients [4]. However, the decoction form is inconvenient for modern use due to preparation time and preservation difficulties. To improve patient compliance, a dry extract form (Thang Thanh Giang Troc) was developed. To scientifically evaluate its pharmacological effects, this study aimed to assess the influence of the Thang Thanh Giang Troc dry extract on blood pressure and renal function indices in a rat model of CKD induced by 5/6 nephrectomy.

SUBJECTS AND METHODS OF THE STUDY

Research product

The dry extract Thang Thanh Giang Troc was produced by the Bach Thao Duoc Institute of Medicine and Pharmacy, based on the traditional prescription Thang Thanh Giang Troc Thang. The original formula derives from the classical On Dom Thang prescription, supplemented with Radix *Achyranthis bidentatae*, Radix et Rhizoma *Salviae miltiorrhizae*, Radix *Polygoni cuspidati*, Faeces *Bombycis*, Herba *Centellae asiaticae*, Rhizoma *Rhei*, Cortex *Eucommiae*, Flos *Styphnolobii japonici* imaturi, and Radix *Astragali membranacei*. All herbal ingredients

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complied with the standards of the Vietnamese Pharmacopoeia V and institutional quality control regulations.

The reference drug Enalapril (10 mg tablets, batch no. ELP0424, expiry date 06/2026, manufactured by STADA-VN JSC, Vietnam) was used for comparison.

Experimental animals

Adult male Wistar rats weighing 200–250 g, healthy and clinically normal, were obtained from the Animal Department of the Military Medical Academy. The rats were acclimatized for one week under standard laboratory conditions (temperature 22 ± 2°C, humidity 55 ± 10%, 12-hour light/dark cycle) with free access to food and water.

Study period and location

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.

Experimental methods

Establishment of the Chronic Kidney Disease Model:

The rat model of CKD was established following the protocol of Lu JR *et al.* (2014) [5], using a two-step 5/6 nephrectomy procedure:

Step 1: Surgical removal of two-thirds of the left kidney under anesthesia.

Step 2: After 15 days of recovery, the entire right kidney was removed.

Fifteen days after the second surgery, the success of CKD induction was confirmed when the rat survived with a healed wound and showed biochemical signs of renal dysfunction (blood urea, serum creatinine, and 24-hour urinary protein increased ≥1.5 times compared to pre-surgery values).

Experimental Design:

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.

Blood pressure, 24-hour urine output, and 24-hour urinary protein levels were measured at three time points: before surgery, 15 days after the second nephrectomy, and after 60 days of intervention.

Data processing

Data were expressed as Mean ± SD. Statistical analyses were performed using SPSS version 20.0. Given the study design involving multiple groups and repeated measurements on the same experimental subjects, a repeated-measures ANOVA was employed to assess time-dependent changes and between-group differences. When global significance was observed, pairwise comparisons were conducted using Bonferroni-adjusted post-hoc tests to control for multiple comparisons. Statistical significance was set at $p<0.05$.

Ethics in research

The study was approved by the Ethics Committee of the Vietnam University of Traditional Medicine. All experimental procedures were conducted in accordance with the Guidelines for Basic Appraisal of Preclinical Research Results for Drugs, Traditional Medicines, Vaccines, and Biological Products (Ministry of Health, Vietnam). Animal numbers were kept to a minimum necessary to achieve reliable results. Any rats that died during the experiment or at its completion were handled according to biosafety regulations. All efforts were made

RESULTS

Table 1. Effects of dry extract of Thang Thanh Giang Troc on systolic blood pressure of rats ($\bar{X}$ ± SD, n = 10 per group)

Research batch	Systolic blood pressure of rats (mmHg)			p_{b-a}	p_{c-a}	p_{c-b}
	Before surgery (a)	15 days after 2nd PT (b)	After taking the medicine for 60 days (c)			
Control (1)	117.52±15.71	115.84±17.40	116.48±12.82	> 0.05	> 0.05	> 0.05
Model (2)	118.55±14.62	130.21±18.28	156.46±13.09	> 0.05	< 0.001	< 0.01

References (3)	119.89±12.75	131.23±15.71	139.09±13.47	> 0.05	< 0.05	> 0.05
TTGT- 1 (4)	119.80±13.62	129.16±10.55	142.85±15.56	> 0.05	< 0.01	< 0.05
TTGT-2 (5)	120.76 ± 9.46	130.13±16.30	137.06±12.29	> 0.05	< 0.05	> 0.05
p_{2-1}	> 0.05	> 0.05	< 0.001	-	-	-
$p_{3,4,5-1}$	> 0.05	> 0.05	< 0.01	-	-	-
$p_{3,5-2}$	> 0.05	> 0.05	< 0.01	-	-	-
p_{4-2}	> 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	-	-	-

After 60 days of taking the drug, compared with the model group, the systolic blood pressure of rats in the reference group and TTGT-2 group decreased significantly with p<0.01; and in TTGT-1 group, it decreased significantly with p<0.05.

Table 2. Effects of dry extract of Thang Thanh Giang Troc on diastolic blood pressure of rats ($\bar{X}$ ± SD, n = 10 per group)

Research batch	Rat diastolic blood pressure (mmHg)			p_{b-a}	p_{c-a}	p_{c-b}
	Before surgery (a)	15 days after 2nd PT (b)	After taking the medicine for 60 days (c)			
Control (1)	97.76±13.25	95.63±15.32	109.56±16.28	> 0.05	> 0.05	> 0.05
Model (2)	100.13±11.12	110.33±16.47	141.74±22.61	> 0.05	< 0.001	< 0.01
References (3)	99.41 ± 9.75	109.50±14.71	118.81±22.50	> 0.05	< 0.05	> 0.05
TTGT- 1 (4)	99.89 ± 13.80	109.63±16.92	122.45±17.69	> 0.05	< 0.01	< 0.05
TTGT-2 (5)	98.96 ± 11.84	109.32±15.79	113.86±16.32	> 0.05	< 0.05	> 0.05
p_{2-1}	> 0.05	> 0.05	< 0.001	-	-	-
$p_{3,4,5-1}$	> 0.05	> 0.05	> 0.05	-	-	-
$p_{3,4-2}$	> 0.05	> 0.05	< 0.05	-	-	-
p_{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	-	-	-

After 60 days of taking the drug, compared with the model group, the diastolic blood pressure of rats in the reference group and TTGT-1 group decreased statistically significantly with p<0.05 and in TTGT-2 group decreased statistically significantly with p<0.01.

Table 3. Effects of dry extract of Thang Thanh Giang Troc on mean blood pressure of rats ($\bar{X}$ ± SD, n = 10 per group)

Research batch	Mean blood pressure of rats (mmHg)			p_{b-a}	p_{c-a}	p_{c-b}
	Before surgery (a)	15 days after 2nd PT (b)	After taking the medicine for 60 days (c)			
Control (1)	105.90±14.26	103.96±16.07	112.41±11.79	> 0.05	> 0.05	> 0.05
Model (2)	107.72±12.37	118.52±16.62	147.80±17.08	> 0.05	<0.001	<0.01
References (3)	107.85±10.81	118.45±14.90	127.16±17.95	> 0.05	< 0.05	>0.05
TTGT- 1 (4)	108.09±14.89	117.68±13.07	130.85±15.64	> 0.05	< 0.01	< 0.05
TTGT-2 (5)	107.94±12.40	117.89±14.96	123.42±13.39	> 0.05	< 0.05	> 0.05

p_{2-1}	> 0.05	> 0.05	< 0.001	-	-	-
$p_{3,4,5-1}$	> 0.05	> 0.05	> 0.05	-	-	-
$p_{3,4-2}$	> 0.05	> 0.05	< 0.05	-	-	-
p_{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	-	-	-

Compared with the model batch, the average blood pressure values of rats in the reference batch and TTGT-1 batch decreased statistically significantly with p<0.05 and in TTGT-2 batch decreased statistically significantly with p<0.01.

Table 4. Effects of dry extract of Thang Thanh Giang Troc on 24-hour urine output of rats ($\bar{X}$ ± SD, n = 10 per group)

Research batch	24-hour urine output (ml)			p_{b-a}	p_{c-a}	p_{c-b}
	Before surgery	15 days after 2nd PT	After taking the medicine for 60 days			
Control (1)	14,93± 3,12	13,65±2,97	15,01 ±4,17	> 0,05	> 0,05	> 0,05
Model (2)	14,77± 3,63	21,15±2,63	30,28±5,68	< 0,05	< 0,001	< 0,01
References (3)	13,97± 3,01	20,89±4,06	21,13±3,07	< 0,05	< 0,05	> 0,05
TTGT- 1 (4)	15,09± 4,02	21,94±3,97	23,08±3,76	< 0,05	< 0,05	> 0,05
TTGT-2 (5)	15,36± 3,84	21,05±2,85	20,97±3,31	< 0,05	< 0,05	> 0,05
p_{2-1}	> 0,05	< 0,05	< 0,001	-	-	-
$p_{3,4,5-1}$	> 0,05	< 0,05	< 0,01	-	-	-
$p_{3,4,5-2}$	> 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	-	-	-

After 60 days of taking the drug, compared to the model batch, the 24-hour urine output of rats in the reference batch and TTGT-1 and TTGT-2 batches decreased statistically significantly with p<0.01.

Table 5. Effect of dry extract Thang Thanh Giang Troc on the content the 24-hour proteinuria content in rats with chronic kidney disease and 5/6 nephrectomy ($\bar{X}$ ± SD, n = 10 per group)

Research batch	24-hour urinary protein of rats (mg/24h)			p_{b-a}	p_{c-a}	p_{c-b}
	Before surgery (a)	15 days after 2nd PT (b)	After taking the medicine for 60 days (c)			
Control (1)	235.68±42.32	243.27±37.42	252.03±41.27	> 0.05	> 0.05	> 0.05
Model (2)	219.85±39.75	358.31±52.45	482.19±58.66	< 0.05	<0.001	<0.01
References (3)	251.12±40.58	364.62±55.12	376.82±51.84	< 0.05	< 0.05	> 0.05
TTGT- 1 (4)	208.96±36.83	349.84±43.96	361.96±45.76	< 0.05	< 0.05	> 0.05
TTGT-2 (5)	261.09±32.64	351.86±54.16	358.13±53.26	< 0.05	< 0.05	> 0.05

p_{2-1}	> 0.05	< 0.05	< 0.001	-	-	-
$p_{3,4,5-1}$	> 0.05	< 0.05	< 0.01	-	-	-
$p_{3,4,5-2}$	> 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	-	-	-

After 60 days of taking the drug, compared with the model batch, the 24-hour proteinuria of rats in the reference batch and the TTGT-1, TTGT-2 batches decreased statistically significantly with p <0.01.

DISCUSSION

Chronic kidney disease is the most common cause of secondary hypertension and is an independent risk factor for cardiovascular morbidity and mortality [6]. When the renal arteries are narrowed, renal blood flow is reduced, leading to activation of the renin-angiotensin-aldosterone system (RAAS). Increased renal renin secretion stimulates the production of angiotensin II and aldosterone, causing vasoconstriction and sodium retention, thereby increasing extracellular fluid volume and blood pressure. This mechanism is one of the main causes of secondary hypertension from chronic kidney disease, and causes severe complications, especially chronic kidney failure. The kidney plays a central role in blood pressure regulation through the control of sodium excretion and extracellular fluid volume. According to Guyton, this regulation is impaired in patients with chronic kidney disease, leading to persistent hypertension. Animal experiments have shown that when blood flow to the kidneys is isolated and pressure increases, the kidneys respond by increasing sodium excretion, which helps to reduce blood pressure to normal levels. However, when the kidneys are damaged or have limited function, this ability is impaired, leading to sodium overload and hypertension. In particular, the use of substances such as angiotensin or aldosterone, or the removal of large parts of the kidney, will weaken the ability to excrete sodium, causing a more severe increase in blood pressure. In the early stages, hypertension often appears due to increased extracellular fluid volume, leading to increased cardiac output, leading to increased systolic blood pressure. However, when cardiac output and extracellular fluid volume are normalized, increased peripheral resistance becomes the main factor causing increased diastolic blood pressure. From these mechanisms, CKD is not only the cause of hypertension but also creates a pathological spiral, in which hypertension contributes to kidney damage, further impairs kidney function, leading to rapid progression of chronic kidney failure and worsening hypertension [7]. The dry extract with the function of "raising the clear and lowering the turbid" has been shown to reduce systolic, diastolic, and mean arterial blood pressure in rats with chronic kidney disease.

Proteinuria is not only a manifestation of glomerular damage but also a prognostic factor, related to inflammation, renal fibrosis and impaired filtration function [8]. After 60 days of taking the drug, compared to the model group and before taking the drug, the 24-hour urinary protein concentrations of the rat in the drug-taking groups decreased significantly ($p<0.01$). This shows that the dry extract of Thang Thanh Giang Troc had the effect of reducing the 24-hour urinary protein content of rat with chronic kidney disease and 5/6 kidney resection, and indirectly shows that the dry extract of Thang Thanh Giang Troc had the effect of improving kidney function and protecting kidney cells.

In the study, after 60 days of taking the drug, compared with the model group, the 24-hour urine output of rat in the reference group and TTGT-1 and TTGT-2 groups decreased statistically significantly with $p<0.01$. This is explained by the fact that normally the kidneys have the ability to concentrate and dilute urine. When drinking a lot of water, the kidneys increase the excretion of excess water, reducing the specific gravity of urine to the maximum level. On the contrary, in conditions of water deficiency, the kidneys increase water retention, concentrate urine, increase osmolarity, and increase the specific gravity of urine. When suffering from chronic

kidney disease, the kidneys will lose the ability to concentrate and dilute urine. In chronic kidney disease, progressive and irreversible nephron loss impairs the kidneys ability to concentrate and dilute urine. Even when the remaining nephrons function at their maximum capacity, they cannot fully compensate, resulting in impaired water excretion [8].

CONCLUSION

The Thang Thanh Giang Troc dry extract at doses of 0.735 g/kg/day and 1.47 g/kg/day significantly reduced blood pressure and improved renal function in rats with CKD induced by 5/6 nephrectomy ($p<0.05$). The established model demonstrated stable pathophysiological characteristics and maintained a 100% survival rate, indicating its suitability for further pharmacological and therapeutic investigations. These findings provide scientific evidence supporting the potential clinical application of Thang Thanh Giang Troc as a complementary therapy in the management of chronic kidney disease.

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