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Original Research Article

Antihypertensive Effects of TCT: "Thanh Can Thang", a Vietnamese Tradition Medicine Remedy, in a Cortisone Acetate Rat Model

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ABSTRACT

"Thanh Can Thang" (TCT) has been used in Vietnamese traditional medicine to treat clinical hypertension. The remedy contains 10 medicinal herbs, including *Ramulus cum Unco Uncariae*, *Radix Scutellariae*, *Flos Chrysanthemi indici*, *Herba Loranthi Gracifilolii*, *Achyranthes bidentata* Blume, *Dipsacus japonicas*, *Alisma orientalis*, *Ligusticum wallichii*, *Fossilia Ossis Mastodi*, and *Zizyphus jujube*. This study aimed to investigate the *in vivo* anti-hypertensive effects of TCT in Wistar rats, using a cortisone acetate model. Hypertension was induced with daily subcutaneous administration of 2.5 mg/kg/day cortisone acetate and 1% sodium chloride in drinking water for 28 days. Two different doses of TCT (14.56 and 43.68 g/kg/day) were used. TCT treatment at both doses resulted in significant decrease in systolic, diastolic, and average blood pressure values. Interestingly, 24 h after the last administration of cortisone acetate, both tested TCT doses were able to restore the levels of systolic, diastolic, and average blood pressure values back to the levels observed in the control group, hence ameliorating hypertension induced by cortisone acetate. This study verifies the usefulness of the traditional medicine TCT in hypertension treatment.

Keywords: Vietnamese traditional medicines, Thanh Can Thang, Anti-hypertension, Cortisone acetate.

Introduction

Hypertension represents a common health-related challenge in Vietnam and the world at large. Globally, high blood pressure affects millions of people in developing countries.[1,2] Each year, approximately 17.5 million people die from cardiovascular diseases, with 35–40% of these deaths associated with increased blood pressure.[3-5] In Vietnam, the rate of hypertension has also increased rapidly. A 2015 Vietnam Heart Association survey found that 5.454 adults over the age of 25 suffered from hypertension out of a total population of 44 million people in 8 provinces nationwide. The current hypertensive population of Vietnam is estimated to be 6.85 million people and if no effective treatments are developed, approximately 10 million cases are estimated to develop by 2025.[6] Hypertension is a silent disease, with few warning signs, most of which are nonspecific, and patients often are unaware of any changes in their health status until a stroke occurs. The currently available drugs for hypertension treatment can be prescribed in a diverse combination and dosages. However, anti- hypertensives can be costly and associated with many side effects such as headaches, dizziness, hot flashes, fatigue, and weakness.

In Vietnam, the "Thanh Can Thang" (TCT) remedy has been used clinically to treat hypertension, associated with conditions such as headache, and insomnia.[7] The TCT formulation contains 10 medicinal herbs, including Câu đằng (*Ramulus cum Unco Uncariae*, *Uncaria* sp.), Hoàng cầm (*Radix Scutellariae*, Lamiaceae), Cúc hoa (Flos *Chrysanthemi indici*), Tang ký sinh (*Herba Loranthi Gracifilolii*, Loranthaceae), Ngưu tất (*Achyranthes bidentata* Blume., Amaranthaceae), Tục đoạn (*Dipsacus japonicus* Miq., Dipsacaceae). Others are Trạch tả (*Alisma orientalis* Sam. Juzep., Alismataceae), Xuyên khung (*Ligusticum wallichii* Franch., Apiaceae), Long cốt (*Fossilia Ossis Mastodi*) and Đại táo [*Zizyphus jujube* Mill. var. inermis (Bge.) Rehd., Rhamnaceae]. These ingredients were chosen based on the theory of traditional medicine, which aims to treat the manifestations of disease symptoms in a bid to treating an underlying disease, such as hypertension. Based on the pharmacological effects associated with each ingredient, the ingredients are classified as being: the sovereign (*Ramulus cum Unco Uncariae*), minister (Radix Scutellariae and Flos *Chrysanthemi indici*), assistant (Herba Loranthi Gracifilolii, *Achyranthes bidentata*, *Dipsacus japonicas*, *Alisma orientalis*, *Ligusticum wallichii*, and Fossilia Ossis Mastodi), or courier (*Zizyphus jujube*) components[7]. To develop a scientific basis for the wide application of TCT in the treatment of hypertension and to prepare products for use by the wider community, we conducted a study examining the antihypertensive effects of TCT in experimental animals with cortisone acetate-induced hypertension.

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Materials and Methods

"Thanh can thang" ingredients

The ingredients of the polyherbal remedy are show in Table 1. The medicinal materials in the remedy are used in the form of dried herbs and meet the Pharmacopoeia Fifth standards established by Vietnam. TCT was extracted by an automatic decoction system at Tue Tinh Oriental Hospital, Vietnam Traditional Medicine and Pharmacy Institute. The aqueous extract of the remedy was prepared in different concentrations, depending on the required dose for oral administration. The dosage was calculated according to the dry medicinal package/kg/day or drug package/kg/day.[8] According to established guidelines, the expected dose for human use is 104 g/person/day. According to the conversion coefficient from humans to experimental animals, the equivalent dose in rats, using a conversion coefficient of 0.7, the expected effective dose for rats is 14.56 g/kg/day.[7]

Table 1: Thanh Can Thang **TCT** ingredients

No	Local names	Scientific names	Amount (g)
1	Câu đằng	*Uncaria rhynchophylla* (Miq) Jach	12
2	Hoàng cầm	*Scutellaria baicalensis* Georgi	12
3	Cúc hoa	*Chrysanthemum indicum* L.	06
4	Tang ký sinh	*Loranthus parasiticus* (L.) Merr	12
5	Ngưu tất	*Achyranthes bidentata* Blume	10
6	Tục đoạn	*Dipsacus japonicus* Miq.	12
7	Trạch tả	*Alisma orientale* (Sam.) Juzep	10
8	Xuyên khung	*Ligusticum chuanxiong* Hort.	08
9	Long cốt	*Os Draconis*	10
10	Đại táo	*Ziziphi jujube*	12

Animals

Wistar rats (both sexes, 200-220 g) were provided by the Department of Animals Study, Vietnam Military Medical Academy. The rats were allowed to acclimatize in the experimental animal laboratory for at least one week before conducting the experiment. The animals were provided with standard animal feed and had free access to clean water. This animal experimental protocol has been approved by Ethical Committee of Vietnam Military Medical University on October 18, 2019 with registration number IACUC-092/19.

Drugs/Chemicals/Equipment

Hydrochlorothiazide, cortisone acetate (Pharmacompass, USA), olive oil, 0.5% sodium carboxymethyl cellulose, and 1% NaCl were provided by the Vietnam National Institute of Drug Quality Control. The non-invasive blood pressure (NIBP) system for rats, including a NIBP Controller and a pulse transducer/pressure cuff for the NIBP system, was purchased from AD Instruments (New Zealand). A rodent warmer, for the body temperature control of laboratory rodents, and a metabolic chamber for rats, with waste collection, were purchased from Ugo-Basile (Italy).

Animal experiments

Increased blood pressure was induced in the rats by the subcutaneous administration of 2.5 mg/kg/day cortisone acetate and oral administration of 1% sodium chloride (NaCl) in water continuously for 28 days according to the method described by Knowlton, with slight modifications[9]. The rats were divided into 5 groups, containing 10 animals per group: group 1 (control group, given distilled water); group 2 (model, hypertension induced by cortisone acetate); group 3 (reference, cortisone acetate-induced hypertension and oral hydrochlorothiazide at 25 mg/kg/day); group 4 (treatment 1, cortisone acetate-induced hypertension and 14.56 g/kg/day TCT); and group 5 (treatment 2, cortisone acetate-induced hypertension and 43.68 g/kg/day TCT).

Rats were given distilled water, hydrochlorothiazide or TCT (oral with a specialized curved needle) continuously for 7 days, starting from day 21 of cortisone acetate injection. During oral administration of drug and TCT; rats in groups 2 to 5 continued to be injected with cortisone acetate at a dose of 2.5 mg/kg/day and to drink 1% sodium chloride solution. Systolic and diastolic blood pressure and heart rate were measured using a non-invasive blood pressure measuring system on the tail, before the first injection of cortisone acetate, before drug administration (day 21 of cortisone acetate injection), and 24 hours after the last injection of cortisone acetate (day 28). The average rat blood pressure was calculated using the following formula:

Mean blood pressure = 2/3 diastolic pressure + 1/3 systolic pressure.

Statistical analyses

The collected data were analyzed using SPSS 20.0 software. Average values and percentages were calculated. Differences in mean values were compared using analysis of variance (ANOVA) for normally distributed data, and the Mann-Whitney U test for non-normally distributed data. Differences were considered significant at $p < 0.05$.

Results and Discussion

Measurement of systolic blood pressure in rats

The systolic blood pressure was measured before the first cortisone acetate injection and 21 days after the start of cortisone acetate injections. The results in Table 2 showed that the systolic blood pressure of the rats in the control (121.52 ± 11.96 mmHg), positive control (121.39 ± 10.23 mmHg), TCT treatment 1 (121.81 ± 10.20 mmHg), and TCT treatment 2 groups (121.56 ± 14.27 mmHg) showed no significant differences in blood pressure prior to cortisone acetate injections ($p > 0.05$). However after 21 days of daily cortisone acetate administration, the mean systolic blood pressure increased to 140.15 ± 12.94 mmHg in the hypertensive model. With the administration of hydrochlorothiazide and the two different doses of TCT, the systolic blood pressure remained approximately 139 mmHg. However, on the day after the last cortisone acetate injection (day 28), the systolic blood pressure levels of rats treated with hydrochlorothiazide, TCT at 14.56 g/kg/day and TCT at 43.68 g/kg/day, were reduced to 127.22 ± 13.32, 129.02 ± 11.71, and 122.74 ± 12.02 mmHg, respectively. These results were significantly lower than those for the untreated model at the same time and represented significant reductions compared with the respective values after 21 days of cortisone acetate injections ($p < 0.05$ and $p < 0.01$). The values for the treatment groups the day after the last injection were equivalent to the value for the control group and the respective initial values for each group ($p > 0.05$, Table 2). The TCT treatment 2 group achieved a higher systolic blood pressure decrease than either the reference group or the TCT treatment 1 group although no significant difference was observed between the reference and TCT treatment 1 groups ($p > 0.05$).

Measurement of diastolic blood pressure

As shown in Table 3, the diastolic blood pressures were measured before the first cortisone acetate administration (a), 21 days after the start of cortisone acetate injections (b), and 24 hours after the final cortisone acetate injection (c). No significant differences were observed in the mean diastolic blood pressure of the rats in the control group, cortisone acetate administration, 25 mg/kg/day hydrochlorothiazide, 14.56 g/kg/day TCT and 43.68 g/kg/day TCT groups before cortisone acetate injections ($p > 0.05$). When examined 24 hours after the last cortisone acetate injection, the diastolic blood pressure in the hydrochlorothiazide, TCT 1 and TCT 2 groups significantly decreased to 110.15 ± 16.68, 111.23 ± 15.48, and 103.29 ± 16.25 mmHg, respectively. These results were significantly lower than that for the untreated model and lower than the values after 21 days of cortisone acetate injections ($p < 0.05$ and $p < 0.01$). TCT treatment 2 showed a better decrease in diastolic blood pressure than either the reference group or TCT treatment 1, however, no significant

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difference was identified among these groups ($p > 0.05$). At the higher TCT dose, the diastolic blood pressure level was similar to that in the control group and the value before hypertension induction ($p > 0.05$, Table 3).

The mean blood pressure

The average blood pressure of rats were calculated from diastolic blood pressure and systolic blood pressure which measured before the first cortisone acetate injection (a), 21 days after the start of cortisone acetate injections (b), and 24 h after the last administration of cortisone acetate (c). The average blood pressure of the rats in the hypertension model (group 2), and those treated with the reference compound (group 3) and TCT treatments 1 (group 4) and 2 (group 5) showed no significant differences in average blood pressure the day before administration of cortisone acetate ($p > 0.05$, Table 4). After 21 days of cortisone acetate injections, the average blood pressure of all hypertension-induced groups increased significantly compared to that of the control group ($p > 0.05$). When measured 24 hours after the last cortisone acetate injection, the average blood pressures of rats treated with hydrochlorothiazide (group 3), TCT treatment 1 (group 4), and TCT treatment 2 (group 5) were lower than that in the untreated model group (group 2, $p < 0.01$ and $p < 0.001$). The average blood pressure in the TCT treatment 2 group (group 5) was somewhat similar to that observed for baseline (116.26 ± 7.23 vs 115.18 ± 12.09 mmHg, $p > 0.05$).

Heart rates

Heart rate refers to the number of contractions performed by the heart each minute. In our experiment, the heart rates were observed for all groups, as shown in Table 5. After 21 days of cortisone acetate treatment, the heart rates of all the groups increased significantly compared to the control ($p > 0.05$). When measured 24 hours after the last cortisone acetate injection, the heart rates of the rats in the hydrochlorothiazide, TCT treatment 1 and TCT treatment 2 groups were significantly lower than that in the untreated cortisone acetate group (group 2, $p > 0.05$). Interestingly, the heart rate of the TCT treatment 2 group was similar to the baseline level (116.32 ± 7.97 vs 115.56 ± 10.87, time/min $p > 0.05$).

Hypertension is one of the most common diseases that affects the populations of developed countries, the incidence of which has continued to increase over time, representing a major public health problem. To study the effects of drugs used in the management of hypertension, many hypertensive models have been used. These include renal and blood vessel-related hypertension, dietary hypertension, endocrine hypertension, nervous hypertension, psychological hypertension, and genetic hypertension. Corticosteroids are commonly used to induce hypertension in experimental rats, such as cortisone acetate and deoxycorticosterone acetate (DOCA).[9] The daily administration of cortisone acetate, at 2.5 mg/kg daily, in rats and mice given a NaCl supplemented drink causes blood pressure to increase rapidly over 3 weeks. In addition, research has also shown that cortisone acetate does not damage kidney tissue or alter the concentrations of electrolytes in the serum.[11,12] This method is simple, stable and suitable for the *in vivo* evaluation of the antihypertensive effects of potential agents. In the evaluation of antihypertensive effect, the ability to monitor blood pressure is imperative. The measurement technique used in the present study was a convenient method with high accuracy involving a non-invasive blood pressure measurement from the tail.

Two different doses of TCT were administered after 21 days of hypertension induction with cortisone acetate at 2.5 mg/kg and both doses significantly decreased the systolic, diastolic, and average blood pressure levels in rats compared to the untreated hypertensive group. Interestingly, 24 h after the last administration of cortisone acetate, both tested TCT doses were able to restore systolic, diastolic and average blood pressure to levels similar to those observed in the control group ($p < 0.01$). The blood pressure levels for both tested TCT doses returned to the levels they were prior to hypertension induction ($p > 0.05$). The high TCT dose (43.68 g/kg/day) produced a higher decrease than the lower dose (14.56 g/kg/day), but this difference was not statistically significant ($p > 0.05$). TCT remedy has been used to treat hypertensive patients with symptoms including phlegm, headaches, dizziness, and asthenia. The antihypertensive effect of this remedy is most likely as a result of the combination of the herbs. Previously published studies have examined the effects of the individual ingredient found in TCT for lowering blood pressure. David *et al.*[13] have reported the antihypertensive effects of *Ramulus cum Uncis Uncariae* on a hypertensive hamster model. The antihypertensive effects of *Radix Scutellariae* in albino rats using the theophylline model of hypertension have also been reported.[8] Dai *et al.*[14] reported the antihypertensive effects of *Flos Chrysanthemi* and *Jujube* extract which were shown to have antihypertensive effects in albino rats when hypertension was induced by L-NAME.[15] These findings indicates that the ingredients in this remedy may have an important synergism against high blood pressure.

Table 2: Systolic blood pressure of rats treated with TCT and hydrochlorothiazide

Groups	Systolic blood pressure (mmHg)			
	Before administration of cortisone acetate (a)	**Day 21 of cortisone acetate injection (b)**	**24 h after the last administration of cortisone acetate (c)**	**P-values**
Control (1)	121.52 ± 11.96	120.98 ± 9.65	121.99 ± 10.91	-
Cortisone acetate (2)	121.15 ± 14.71	140.15 ± 12.94	144.24 ± 15.43	-
Hydrochlorothiazide 25 mg/kg/day (3)	121.39 ± 10.23	139.54 ± 14.74	127.22 ± 13.32	$p_{c\text{-}b} < 0.001$ $p_{c\text{-}a} > 0.05$
Treatment 1, TCT 14.56 g/kg/day (4)	121.81 ± 10.20	139.05 ± 12.44	129.02 ± 11.71	$p_{c\text{-}b} < 0.001$ $p_{c\text{-}a} > 0.05$
Treatment 2, TCT 43.68 g/kg/day (5)	121.56 ± 14.27	138.93 ± 14.16	122.74 ± 12.02	$p_{c\text{-}b} < 0.001$ $p_{c\text{-}a} > 0.05$
P-values	> 0.05	$p_{2,3,4,5\text{-}1} < 0.01$ $p_{3,4,5\text{-}2} > 0.05$ $p_{3\text{-}4} > 0.05$ $p_{3,4\text{-}5} > 0.05$	$p_{3,4,5\text{-}1} > 0.05$ $p_{1,5\text{-}2} < 0.01$ $p_{3,4\text{-}2} < 0.05$ $p_{3\text{-}4} > 0.05$ $p_{3,4\text{-}5} > 0.05$	-

Each value represents the mean ± standard deviation for 10 animals in each group (One-way ANOVA and LSD tests).

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Table 3: Diastolic blood pressure of rats treated with TCT and hydrochlorothiazide

Group	Diastolic blood pressure (mmHg)			
	Before administration of cortisone acetate (a)	**Day 21 of cortisone acetate injection (b)**	**24 h after the last administration of cortisone acetate (c)**	**P-values**
Control (1)	102.51 ± 12.63	101.56 ± 6.95	102.69 ± 10.99	-
Cortisone acetate (2)	102.12 ± 13.13	125.00 ± 16.40	129.81 ± 13.98	-
Hydrochlorothiazide 25 mg/kg/day (3)	102.04 ± 18.94	125.05 ± 20.88	110.15 ± 16.68	$p_{c-b} < 0.001$ $p_{c-a} > 0,05$
Treatment 1, TCT 14.56 g/kg/day (4)	102.33 ± 13.20	125.11 ± 17.79	111.23 ± 15.48	$p_{c-b} < 0.001$ $p_{c-a} > 0,05$
Treatment 2, TCT 43.68 g/kg/day (5)	102.92 ± 15.62	124.96 ± 17.91	103.29 ± 16.25	$p_{c-b} < 0.001$ $p_{c-a} > 0.05$
P-values	> 0.05	$p_{2,3,4,5-1} < 0.01$ $p_{3,4,5-2} > 0,05$ $p_{3-4} > 0.05$ $p_{3,4-5} > 0.05$	$p_{3,4,5-1} > 0.05$ $p_{1,5-2} < 0.01$ $p_{3,4-2} < 0.05$ $p_{3-4} > 0.05$ $p_{3,4-5} > 0.05$	-

Each value represents the mean ± standard deviation for 10 animals per group (One-way ANOVA and LSD tests).

Table 4: Measurement of average blood pressure in rats

Group	Average blood pressure of rats (mmHg)			
	Before administration of cortisone acetate (a)	**Day 21 of cortisone acetate injection (b)**	**24 h after the last administration of cortisone acetate (c)**	**P-values**
Control (1)	115.18 ± 12.09	114.45 ± 6.80	115.56 ± 10.87	-
Cortisone acetate (2)	114.87 ± 13.96	135.10 ± 11.74	139.43 ± 13.73	-
Hydrochlorothiazide 25 mg/kg/day (3)	114.94 ± 8.42	134.71 ± 10.59	121.53 ± 10.88	$p_{c-b} < 0.001$ $p_{c-a} < 0.05$
Treatment 1, TCT 14.56 g/kg/day (4)	115.32 ± 6.61	134.40 ± 8.56	123.09 ± 7.66	$p_{c-b} < 0.001$ $p_{c-a} < 0.05$
Treatment 2, TCT 43.68 g/kg/day (5)	115.35 ± 8.28	134.27 ± 9.00	116.26 ± 7.23	$p_{c-b} < 0.001$ $p_{c-a} > 0.05$
P-values	> 0.05	$p_{2,3,4,5-1} < 0.001$ $p_{3,4,5-2} > 0.05$ $p_{3-4} > 0.05$ $p_{3,4-5} > 0.05$	$p_{3,4,5-1} > 0.05$ $p_{1,5-2} < 0.001$ $p_{3,4-2} < 0.01$ $p_{3-4} > 0.05$ $p_{3,4-5} > 0.05$	

Each value represents the mean ± standard deviation for 10 animals (One-way ANOVA and LSD tests).

Table 5: Heart rates of the rats

Group	Heart rate (beats/min)			
	Before administration of cortisone acetate (a)	**Day 21 of cortisone acetate injection (b)**	**24 h after the last administration of cortisone acetate (c)**	**P-values**
Control	115.18 ± 12.09	114.45 ± 6.80	115.56 ± 10.87	-
Cortisone acetate	114.87 ± 13.96	129.45 ± 12.29	139.43 ± 13.73	-
Hydrochlorothiazide 25 mg/kg/day	114.94 ± 8.42	129.03 ± 10.59	120.49 ± 9.41	$p_{c-b} < 0.01$ $p_{c-a} > 0.05$
Treatment 1, TCT 14.56 g/kg/day	115.23 ± 6.84	128.74 ± 8.17	119.98 ± 8.38	$p_{c-b} < 0.01$ $p_{c-a} > 0.05$
Treatment 2, TCT 43.68 g/kg/day	115.16 ± 7.15	128.58 ± 9.17	116.32 ± 7.97	$p_{c-b} < 0.01$ $p_{c-a} > 0.05$
P values	> 0.05	$p_{2,3,4,5-1} < 0.01$ $p_{3,4,5-2} > 0.05$ $p_{3-4} > 0.05$ $p_{3,4-5} > 0.05$	$p_{3,4,5-1} > 0.05$ $p_{1,3,4,5-2} < 0.01$ $p_{3-4} > 0.05$ $p_{3,4-5} > 0.05$	

Each value represents the mean ± standard deviation for 10 replicates (One-way ANOVA and LSD tests). (n = 10)

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Conclusion

The remedy TCT was able to reduce systolic, diastolic and the average blood pressure values in cortisone acetate-induced hypertensive rats. The effects of TCT were similar to that of hydrochlorothiazide, a known antihypertensive agent. This study gives credence to the usefulness of TCT in the management of hypertension in traditional medicine. However, further studies are recommended to ascertain the mechanisms of action associated with its antihypertensive effect.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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