Uttar Pradesh Journal of Zoology

Volume 44, Issue 21, Page 350-355, 2023; Article no.UPJOZ.2883
ISSN: 0256-971X (P)

A Study on Pharmacological Evaluation of Diuretic Activity of *Tinospora cordifolia* Stem Ethanolic Extract

Syed Safiullah Ghori [a*], Syeda Seema Zaidi [a], Mohammed Sufian Aamer [b], Daraksha Fatima [a] and Abdul Hannan [b]

[a] Department of Pharmacology, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad - 500 001, India.
[b] Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad - 500 001, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i213708

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Vishnu A. Adole, Science and Commerce College, India.
(2) R. Vijayaraghavan, University of Madras, India.
(3) Jayaprada Chunduri, University of Mumbai, India.

Original Research Article

Received: 01/08/2023
Accepted: 09/10/2023
Published: 19/10/2023

ABSTRACT

This study aimed to assess the diuretic effects of *Tinospora cordifolia* stem ethanolic extract in Swiss Albino Rats. The administration of the higher dose (200 mg/kg) of the ethanolic extract derived from the stem part of *Tinospora cordifolia* resulted in a significant increase in diuresis during the 6th hour, with a measured output of 1.93 + 1.21ml, compared to the control group (0.38+ 0.12 ml). This effect was also compared to the reference standard, furosemide, which produced an output of 2.45 + 1.12ml ($p < 0.05$). Similarly, the higher dose (400 mg/kg) of *Tinospora cordifolia*

**Corresponding author: Email: safiullahghori@gmail.com;*

stem extract demonstrated significant diuretic activity (2.70 + 1.15 ml) compared to the control group (1.14 + 0.09 ml) ($p < 0.05$). The increase in urine output was statistically significant ($p < 0.01$). The study also analysed the impact of furosemide (10 mg/kg) and *Tinospora cordifolia* extracts at doses of 200 mg/kg and 400 mg/kg on the excretion of electrolytes (Na+ and K+) in the 6-hour urine samples. The pH of the urine samples following treatment with *Tinospora cordifolia* stem extract at doses of 200 mg/kg and 400 mg/kg was measured to be 5.48 and 6.79, respectively. The results obtained in this study provide a quantitative basis for explaining the diuretic activity of *Tinospora cordifolia*. The extract displayed notable diuretic activity, thus supporting its traditional use as a diuretic in ethno-pharmacology. Further investigations, including phytochemical and pharmacodynamic studies, are necessary to identify the active constituents responsible for this activity and to understand the exact mechanism of the diuretic effect exhibited by the aqueous extract of *Tinospora cordifolia*.

Keywords: Tinospora cordifolia; diuretic; swiss albino; extracts; urine.

1. INTRODUCTION

"Diuresis is the excretion of urine, especially when excessive (polyuria). The term collectively denotes the physiologic processes underpinning increased urine production by the kidneys during the maintenance of fluid balance" [1]. "Diuresis may be caused by certain medical conditions or by taking medications that increase urine output. Lifestyle factors can also lead to these conditions. Diuretics are medications that induce the elimination of Na+ & water in urine, leading to a net loss. However, it is important to note that despite the ongoing diuretic effect, the body's compensatory homeostatic mechanisms work to restore Na+ balance. As a result, a certain degree of persistent Na+ deficit and reduction in extracellular fluid volume is observed" [2]. "Diuretics help to alleviate pulmonary congestion and peripheral edema, leading to a reduction in cardiac workload, oxygen demand, and plasma volume. Consequently, this reduction contributes to lowering blood pressure"[3]. "Potassium-sparing diuretics are either aldosterone antagonists or directly inhibit Na+ channels in the DT and CD cells. Osmotic diuretics inhibit the reabsorption of sodium and water, increasing the osmolarity of the blood and the renal filtrate. Loop diuretics act by inhibiting the Na+/K+/2Cl- transporter protein, present in the walls of the ascending loop of Henle. These agents cause a reduction in the reabsorption of NaCl or salt, which significantly increases diuresis. Thiazide diuretics are medium efficacy diuretics with primary site of action in the cortical diluting segment or early DT. They inhibit Na+Cl- symport at the luminal membrane. Carbonic anhydrase inhibitors inhibit the enzyme carbonic anhydrase, which has the effect of decreasing the reabsorption of bicarbonate in the proximal tubule. This leads to retention of potassium in the urine and decreased sodium absorption. Decreased sodium absorption leads to a decrease in the reabsorption of water" [4]. *Tinospora cordifolia* , known as "Guduchi" in Sanskrit, is a member of the Menispermaceae family which contains a variety of chemical constituents that belong to diverse classes, including alkaloids, glycosides, steroids, phenolics, aliphatic compounds, and polysaccharides. The leaves of *T. cordifolia* is notably abundant in protein (11.2%), as well as calcium and phosphorus [5,6].

"In the stem of *Tinospora cordifolia* , clerodane Furano diterpene glycosides such as Amritoside A, B, C, and D have been identified, and their structures have been determined through various spectroscopic investigations" [7-9]. "*Tinospora cordifolia* is widely utilised in traditional Ayurvedic medicine and is recognized for its diverse therapeutic properties" [10,11]. "These properties include the treatment of ailments such as jaundice, rheumatism, urinary disorders, skin diseases, diabetes, anaemia, inflammation, allergic conditions, anti-periodic effects, as well as radioprotective properties"[12,13]. It is additionally referred as Rasayan Dravya in ayurveda for these properties Despite an extensive literature survey, diuretic activity of *Tinospora cordifolia* was not found. Hence, in the present investigation an attempt was made to investigate the diuretic potential of the plant.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant

Stem of the plant was procured from an authenticated plant supplier and identified by a botanist.

2.2 Preparation of Extract

The freshly collected plant material was dried at room temperature. Exposure to sunlight was avoided to prevent loss of active constituents. After drying, plant material was cut into pieces. Dried stem was powdered using a ball mill and sieved through sieve no. 44 to produce uniformity. The coarse powder obtained was extracted with petroleum ether to remove fatty substances. The marc was extracted with chloroform and ethanol separately in soxhlet apparatus for 7 days and filtered. Solvent was evaporated to dryness on a water bath in a tared flat-bottomed Petri dish. Residue was weighed (16%).

2.3 Experimental Animals

Approval from the Institute of Animal Ethics Committee (IAEC) was obtained to acquire healthy adult albino Wistar rats of either sex, weighing between 180-220 g. These rats were sourced from a registered animal house. Before the initiation of the experiment and grouping, a period of 7 days was dedicated to acclimatising the rats. The rats were housed in clean and dry plastic cages, maintaining a temperature of 25±2 °C and a relative humidity of 45-60%, with a 12-hour light and 12-hour dark cycle. They were provided with standard feed and water ad *libitum*. To prepare for the experiments, the animals underwent overnight fasting and were deprived of food and water. The experimental procedures strictly adhered to the guidelines set by the institutional animal ethics committee, with reference number IAEC/EXP/29/409/2017/EXP/89/2018.

2.4 Evaluation of Diuretic Activity

The rats were randomly selected and divided into five groups, labelled Groups I, II, III, and IV, each consisting of six animals. Individual numbering was assigned to each rat within their respective groups. To begin the experiment, all rats were administered a dose of 0.9% sodium chloride solution at a rate of 10 ml/kg body weight. The group breakdown and treatments were as follows:

Group I (Control): Received normal saline solution (10 ml/kg, intraperitoneal administration).
Group II (Standard): Administered the standard drug furosemide (10 mg/kg body weight) in a 0.9% sodium chloride solution.
Group III (Test-I): Given the plant aqueous extract (200 mg/kg body weight) suspended in a 0.9% sodium chloride solution.
Group IV (Test-II): Administered the plant aqueous extract (400 mg/kg body weight) suspended in a 0.9% sodium chloride solution.

Following the respective treatments, the rats were placed in specially designed metabolic cages, with three animals per cage. These metabolic cages allowed for the separation of urine and faeces. Urine was collected in graduated vials, and the total volume was measured after intervals of 6 and 10 hours, with regular hourly monitoring. The mean urine volume was calculated as ml/100 g of body weight. The room temperature was maintained at approximately 25±0.5 °C throughout the experiment. During this period, no food or water was provided to the animals. Subsequently, the volume of urine, as well as the levels of Na+, K+, and Cl− ions in the urine, were determined to assess the diuretic activity.

2.5 Measurement of Urine Output and Electrolyte Analysis

At the end of the experimental period, the urine samples collected from each rat were used to calculate urine output, urine yield, electrolyte (Na+ and K+) concentrations, and pH. These measurements were expressed as mequiv/100 g body weight. The concentrations of Na+ and K+ were determined using a Sherwood Flame Photometer 410, which was calibrated using standard solutions with varying concentrations of Na+ and K+. The pH of the urine samples was measured using a pH meter.

2.6 Statistical Analysis

All the values were expressed as mean^{+} - SEM. The results were analysed for statistical significance by using one-way ANOVA followed by Dunnett's test. $P<0.05$ was considered significant.

3. RESULTS

Based on the mortality result of the sighting study, the starting dose in the main study is decided and carried out with six animals per dose level (1000 mg/kg). Based on the mortality result on 14^{h} day of observation, the doses for *in vivo* study were selected.

Table 1. Result of phytochemical screening of *Tinospora cordifolia* stem extract

Chemical Constituents	Ethanolic Extract
Carbohydrates	-
Glycosides	+
Alkaloids	+
Flavonoids	+
Phenols	+
Fixed oils	-
Steroids	+
Saponins	-
Gums & mucilage	-
Proteins & free amino acids	-

Phytochemical Screening of Tinospora cordifolia Stem Ethanolic Extract-
(+) → Positive (-) → Negative

Table 2. Result of acute oral toxicity for *Tinospora cordifolia* stem extract

Treatment	Dose mg/kg	Mortality	Urination	Body colour changes	Locomotion	Body weight
TCSE	5 mg/kg	-	-	-	-	-
TCSE	5 mg/kg	-	-	-	-	-
TCSE	200 mg/kg	-	-	-	-	-
TCSE	1000 mg/kg	+	- mild	-	- mild	-

The higher dose of the aqueous extract of the stem part of *Tinospora cordifolia* (200 mg/kg) showed marked diuresis (1.93 + 1.21 ml) during the 6th h versus control (0.38+ 0.12 ml), compared with a reference standard (furosemide 2.45 + 1.12 ml) ($p < 0.05$).

The higher dose (400 mg/kg) of *Tinospora cordifolia* stem extract also showed significant ($p < 0.05$) diuretic activity (2.70 + 1.15ml) versus control (1.14 + 0.09 ml). The urine output was significant ($p < 0.01$).

The effect of Furosemide (10 mg/kg) and the extract of *Tinospora cordifolia* (200 mg/kg and 400 mg/kg) on Electrolyte (Na+ and K+) excretion in the 6 hrs urine is presented in Table 4. The plant extract significantly enhanced the excretion of electrolytes ($p<0.05$) which was comparable to that of Furosemide. Na+ concentration of test extract was 490±2.71 & 533 ± 3.27 µmole/kg against the control which was 410 ± 4.15 µmole/kg.

The effect of Furosemide (10 mg/kg) and the extract of *Tinospora cordifolia* (200 mg/kg and 400 mg/kg) on Electrolyte (Na+ and K+) excretion in the 6 hrs urine are depicted graphically in Figs. 1 & 2.

Table 3. Effect of *Tinospora cordifolia* stem extract on urine volume (ml/100 mg) of albino rats (n = 6)

Group	Dose	1 Hr	4 Hrs	6Hrs
Control group	Saline 10 ml/ Kg	0.2 ± 0	0.38± 0.12	1.14 ± 0.09
Standard Group	Furosemide 10 mg/kg	1.72 ±0.10	1.92 ± 1.15	2.45 ± 1.12
Test Group –I	TCSE extract 200 mg/kg	0.38 ±0.11	0.82 ± 1.12	1.93 ± 1.21
Test Group –II	TCSE extract 400 mg/kg	0.5 ± 0.29	1.45 ± 1.01	2.70 ± 1.15

Table 4. Effect of *Tinospora cordifolia* stem extract on urine volume (ml/100 mg) of albino rats (n = 6)

Group	Dose	1 Hr	4 Hrs	6 Hrs
Control group	Saline 10 ml/ Kg	0.2 ± 0	0.38± 0.12	1.14 ± 0.09
Standard Group	Furosemide 10 mg/kg	1.72 ±0.10	1.92 ± 1.15	2.45 ± 1.12
Test Group –I	TCSE extract 200 mg/kg	0.38 ±0.11	0.82 ± 1.12	1.93 ± 1.21
Test Group –II	TCSE extract 400 mg/kg	0.5 ± 0.29	1.45 ± 1.01	2.70 ± 1.15

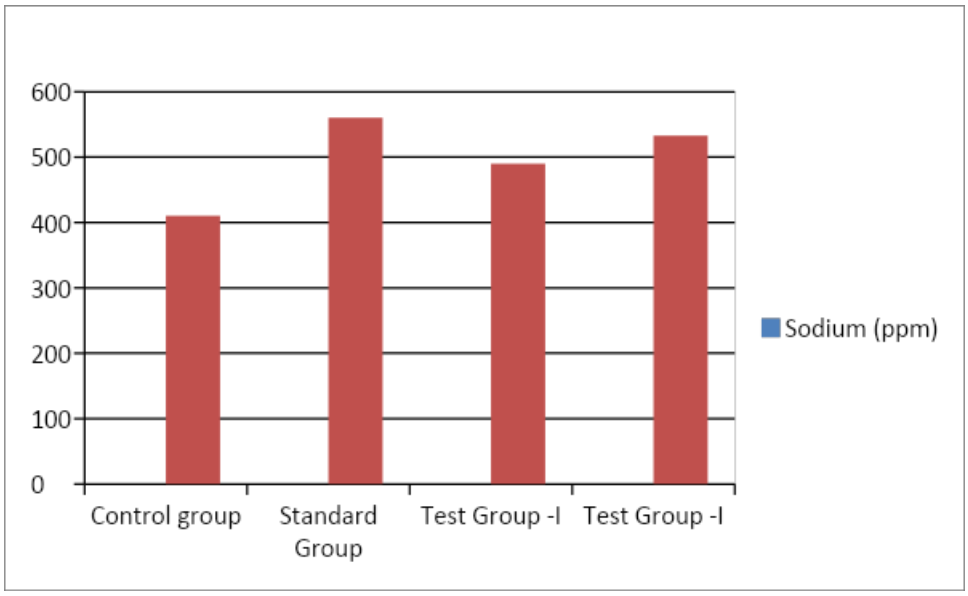

Fig. 1. Effect of Ethanolic Extract of *Tinospora cordifolia* on urine pH [Sodium (ppm)]

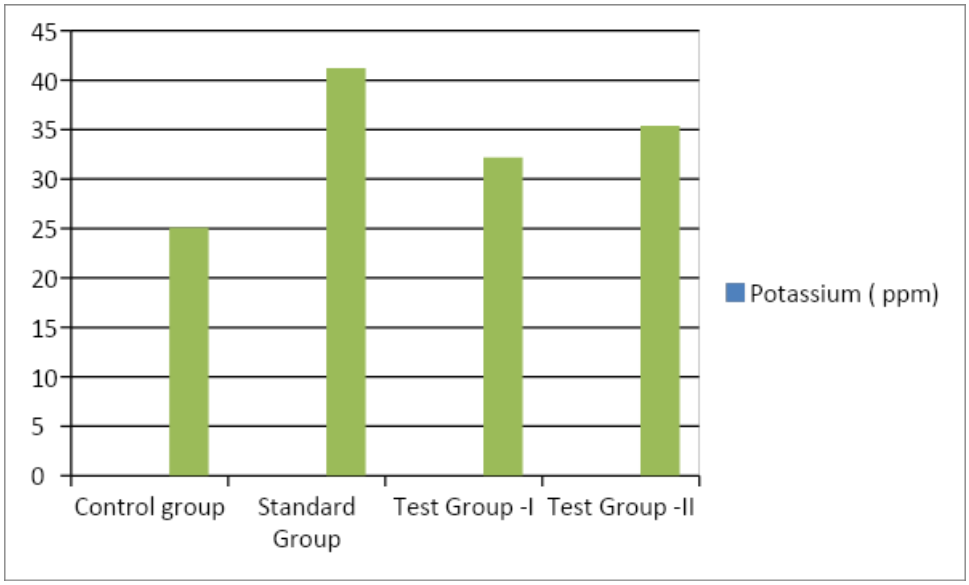

Fig. 2. Effect of Ethanolic Extract of *Tinospora cordifolia* on urine pH [Potassium(ppm)

4. DISCUSSIONS AND CONCLUSION

This study aimed to provide scientific evidence for the diuretic activity of *Tinospora cordifolia*, a plant traditionally used for its diuretic properties. Diuretic therapy carries the risk of severe electrolyte disorders and toxicities, highlighting the need for safety profile studies using different extract doses. This research contributes to the diverse range of diuretic treatments available for the safe and effective management of edema and cardiovascular diseases. Recent reports have shown "the compounds and their biological roles in *Tinospora cordifolia* extract. Such properties may be exploited for production of new formulations, which may be better and promising than conventional ones" [13].

Although the observed potassium levels in the urine samples were low, it is important to note that many diuretics cause potassium loss, which can lead to hypokalemia [14,15]. "The increased sodium and water excretion activity, as demonstrated by the natriuretic effects, strongly supports the potential use of the plant for antihypertensive purposes. The use of herbal

medicine as a treatment modality has significantly increased over the last decade" [16]. "This is due to several factors, the principal of which is that herbal medicine is a cheaper alternative with fewer undesired side effects" [17]. The regulation of sodium and potassium is closely associated with the renal control of acid-base balance.

Statistical analysis of the data showed its significance when compared with the normal control group. This finding indicates that the component of *Tinospora cordifolia* responsible for diuresis exhibits a quick onset of action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shils, Maurice Edward, Shike, Moshe. Modern Nutrition in Health and Disease. Lippincott Williams & Wilkins; 2006. ISBN 9780781741330. Retrieved 2015-05-11.
2. Agrawal SS, Paridhavi M. Herbal drugs technology. 1st ed. Hyderabad: Universities Press (India) Private Limited. 2007;545-46.
3. Jain DL, Baheti AM, Parakh SR, Ingale SP, Ingale PL. Study of antacid and diuretic activity of ash and extracts of *Musa sapientum* L. fruit peel. Pharmacog Magazine. 2007;3:116-9.
4. Tripathi KD. Essentials of Medical pharmacology, 6th Edition, pg: 561-563,
5. Rana V, Thakur K, Sood R, Sharma V, Sharma TR. Genetic diversity analysis of *Tinospora cordifolia* germplasm collected from northwestern Himalayan region of India. J Genet. 2012;91:99–103.
6. Chaudhary N., Siddiqui M.B., Khatoon S. Pharmacognostical evaluation of *Tinospora cordifolia* (Willd) Meirs and identification of biomarkers. J. Res. Indian Med. 2014;13:543–550.
7. Maurya R., Manhas L.R., Gupta P., Mishra P.K., Singh G., Yadav P.P. Amritosides A, B, C and D: clerodane furano diterpene glucosides from *Tinospora cordifolia*. Phytochemistry. 2004;5:2051–2055.
8. Abhijeet R, Mokat D. On vegetative propagation through stem cuttings in medicinally lucrative *Tinospora species*. J. Pharmacogn. Phytochem. 2018;2:2313–2318.
9. Sumran G, Aggarwal A. Prospect of Indian herbs as sources of antioxidants in 14 combating oxidative stress. Chem. Biol. Interface. 2019;9:1–20.
10. Meena AK, Singh A, Panda P, Mishra S, Rao MM. *Tinospora cordifolia*: Its bioactivities & evaluation of physicochemical properties. IJPPR. 2010; 2:50–55.
11. Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S. Immunomodulatory active compounds from *Tinospora cordifolia*. J. Ethnopharmacol. 2012;141:918–926.
12. Goel HC, Prasad J, Singh S, Sagar RK, Agrawala PK, Bala M, Sinha AK, Dogra R. Radioprotective potential of an herbal extract of *Tinospora cordifolia*. J. Radiat. Res. 2004;45:61–68.
13. Tambekar DH, Khante BS, Chandak BR, Titare AS, Boralkar SS, Aghadte SN. Screening of antibacterial potentials of some medicinal plants from Melghat forest in India. Afr J Tradit Complement Altern Med. 2009;6:228–32.
14. Khitan ZJ, Shweihat YR, Tzamaloukas AH, Shapiro JI. Dietary potassium and cardiovascular profile. Results from the modification of diet in renal disease dataset. Journal of Clinical Hypertension. 2018;611–612. DOI: 10.1111/jch.13207
15. Gumz ML, Lynch IJ, Greenlee MM, Cain BD, Wingo CS. The renal H+-K+-ATPases: physiology, regulation, and structure. American Journal of Physiology: Renal Physiology. 2010;298:F12–F21. DOI: 10.1152/ajprenal.90723.2008
16. Frishman WH, Beravol P, Carosella C. Alternative and complementary medicine for preventing and treating cardiovascular disease. Dis. Mon. 2009;55:121–192. DOI: 10.1016/j.disamonth.2008.12.002.
17. Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T, et al. Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: comparison with Captopril. Phytomedicine. 2011;18:251–258. DOI: 10.1016/j.phymed.2010.08.016