

Cardioactive and Antihypertensive Properties of *Tinospora crispa*: An *In vivo* Approach

Juhaer Anjum¹, Fahima Aktar¹, Jakir Ahmed Chowdhury², Abu Asad Chowdhury¹, Shaila Kabir¹, Md. Al Amin Sikder¹ and Md. Shah Amran¹

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka
Dhaka -1000, Bangladesh

²Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka
Dhaka- 1000, Bangladesh

(Received: May 16, 2023; Accepted: September 8, 2023; Published (web): October 25, 2023)

ABSTRACT: *Tinospora crispa*, a potent medicinal plant with multiple clinical indications, has a recorded history of ethnopharmaceutical use as an antihypertensive and has also been reported for its cardioactive properties. The antihypertensive and cardioactive properties of the plant were investigated in two different animal models of hypertension (ethanol-induced and digoxin-induced). In both models, the plant's ethanolic extract was observed to exert a dose-dependent effect. Furthermore, treatment with the ethanolic extract resulted in no undesirable changes in liver and kidney function parameters such as serum SGOT, SGPT and creatinine levels. The ethanolic extract of the plant may, therefore, be considered safe and effective for the treatment of hypertension, although further extensive research into this is warranted.

Key words: *Tinospora crispa*, antihypertensive, cardioactive, ethanol, digoxin, SGOT, SGPT and creatinine.

INTRODUCTION

Wherever and whenever any civilization had arisen, we would find that man grasped from nature whatever might protect them from diseases, and this is to fulfill one of man's basic needs of medical treatment, *i.e.*, health. Fossils from plants with medicinal properties have been found, indicating that early men used these plants as drugs against various illnesses.¹ We, therefore, see that the use of plant preparations in the treatment of various diseases is an age-old practice. In the present day, the World Health Organization (WHO) is also giving emphasis on the concomitant use of plant-based formulations or herbal drugs, which are largely based on plant materials to ensure total health coverage. A large number of herbal drugs are known to be used in the treatment of cardiovascular disorders in different

corners of the world. Plant-based medicines are also used in Bangladesh in the form of Ayurveda, Unani and herbal formulations.

Tinospora crispa is a potent medicinal plant with multiple clinical indications.² The plant has a recorded history of ethnopharmaceutical use as an antihypertensive in several communities. The plant has also been reported for its cardioactive properties. In isolated male Sprague Dawley rat-aorta and atria, different extracts (water, chloroform, petroleum-ether and methanol) and their fractions were reported to exert dose-dependent α and β adrenoceptor antagonistic activity.³ Moreover, the n-butanol fraction prepared from the aqueous extract of the plant exerted positive chronotropic and hypotensive activity in a murine model.⁴ In a previous computational study, several of its constituents were observed to be potentially active against a number of molecular targets for hypertension management.⁵ This study aimed to investigate the cardioactive actions of *T. crispa* by using different *in vivo* methods as well as assess the biosafety profile of the drug.

Correspondence to: Md. Shah Amran
E-mail: amranms@du.ac.bd
Mobile: 01718-617915

Dhaka Univ. J. Pharm. Sci. **23**(1): 1-6, 2024 (June)
DOI: <https://doi.org/10.3329/dujps.v23i1.74085>

MATERIALS AND METHODS

The whole plant of *T. crispa* was collected from local sources, and then the specimen was certified by the experts of Bangladesh National Herbarium at Mirpur and provided the accession number for future reference (DACB-66767). The plant was carefully powdered coarsely after shade drying. Then, the extraction of the powdered plant was done by using 70% ethanol. The extract was filtered after every 3 days. Later on, a rotary evaporator was used to dry the extract using low temperature and pressure, and the obtained extract was preserved.

Ketalar (Ketamine hydrochloride) (Popular Pharmaceuticals Ltd.) and ethanol (99.9%, HPLC grade) (Merk Germany) were used, and biochemical kits and reagents were purchased from a local supplier.

One hundred and five (105) healthy rats (Wistar rats) of either sex weighing between 150-200 g were purchased from Department of Pharmacy, Jahangir Nagar University, Savar, Dhaka and each of them was nourished carefully by keeping them in the animal house of Institute of Food and Nutrition Science of the University of Dhaka in a well-controlled environment (relative humidity 55%, 12 ± 1 h light/dark cycle and temperature 25 ± 3°C) for 2 weeks. All rats were provided with a standard food

supplement and filtered water. To establish the ethanol-induced hypertension model, rats weighing 180 to 220 g initially were randomly allocated to either the ethanol-fed or control group. The method of ethanol feeding was that described by Chan and Sutter in which rats received 5% ethanol (v/v) in the drinking water for the first week, 10% for the next 2 weeks, and 20% from Weeks 3-6.⁶ The control group was fed pure water *ad libitum*. Daily food and water consumption were recorded. On this regimen, average daily ethanol consumption was 10 to 11 g/kg. Blood pressure was determined by the tail-cuff method at weeks 0, 2 and 3-6 in rats. Blood pressure was measured by direct arterial cannulation at week 6. All experiments were conducted between 0730 h and 1130 h to minimize the diurnal variation in ethanol metabolism. Arrhythmia was induced by the administration of 0.5 mg/kg of digoxin through the intra-peritoneal route.⁷

After that, considering equal body mass index, 21 groups were created, where each group was constituted of 5 animals. All of the experimental procedures were carried out according to the Institutional Animals Ethics Committee (IEAC), Biological Sciences Faculty, University of Dhaka (Ref. No.:158/Biol.ScS). The animal grouping is presented in Table 1.

Table 1. Protocol of evaluation of the cardioactive potential of extract of *T. crispa*.

Group number	Group status	Treatment specimen	Volume of treatment specimen (mg/kg)	Group abbreviation
1	Negative Control	Physiological saline	10mL/kg	C
2	Disease Control	Ethanol (Et)	11mg/kg [5-20% (v/v)]	Et
3	Disease Control	Digoxin (dig)	0.5 mg/kg	Dig
4	Dig + Atenolol	Atenolol (At)	300 mg/kg + 0.8 mg/kg	Dig + At 0.8
5	Dig + Atenolol	Atenolol (At)	300 mg/kg + 1.6 mg/kg	Dig + At 1.6
6	Dig + Atenolol	Atenolol (At)	300 mg/kg + 2.4 mg/kg	Dig + At 2.4
7	Et + Atenolol	Atenolol (At)	11 mg/kg + 0.8 mg/kg	Et + At 0.8
8	Et + Atenolol	Atenolol (At)	11 mg/kg + 1.6 mg/kg	Et + At 1.6
9	Et + Atenolol	Atenolol (At)	11 mg/kg + 2.4 mg/kg	Et + At 2.4
10	Dig + <i>T. crispa</i>	<i>T. crispa</i>	300 mg/kg + 200 mg/kg	Dig + Tc200
11	Dig + <i>T. crispa</i>	<i>T. crispa</i>	300 mg/kg + 400 mg/kg	Dig + Tc400
12	Dig + <i>T. crispa</i>	<i>T. crispa</i>	300 mg/kg + 800 mg/kg	Dig + Tc800
13	Et + <i>T. crispa</i>	<i>T. crispa</i>	11 mg/kg + 200 mg/kg	Et + Tc200
14	Et + <i>T. crispa</i>	<i>T. crispa</i>	11mg/kg + 400 mg/kg	Et + Tc400

15	Et + <i>T. crispa</i>	<i>T. crispa</i>	11 mg/kg + 800 mg/kg	Et + Tc800
16	Atenolol	Atenolol (At)	0.8 mg/kg	At 0.8
17	Atenolol	Atenolol (At)	1.6 mg/kg	At 1.6
18	Atenolol	Atenolol (At)	2.4 mg/kg	At 2.4
19	<i>T. crispa</i>	<i>T. crispa</i>	200 mg/kg	Tc200
20	<i>T. crispa</i>	<i>T. crispa</i>	400 mg/kg	Tc400
21	<i>T. crispa</i>	<i>T. crispa</i>	800 mg/kg	Tc800

Various parameters were monitored to evaluate the biological effects of the extract. Heart rate (HR) and blood pressure, both systolic (SBP) and diastolic (DBP), of rats of different groups were determined before and after administration of a specified amount of either ethanol or digoxin, standard drug atenolol or extract of *T. crispa* at different doses by using blood pressure measuring apparatus. Blood samples were collected from the control and experimental animals to analyze different cardiac parameters (total cholesterol, LDL, HDL and triglyceride, etc.) using a Humalyzer (Blood analyzer) machine. The safety profile of the plant extract has also been measured by conducting organ function tests for the liver and the kidney (SGPT, SGOT, creatinine).

RESULTS AND DISCUSSION

Both ethanol and digoxin were observed to have a marked effect on the heart rates, the systolic and diastolic blood pressures of the experimental animals. Conditioning with ethanol resulted in more drastic increases than digoxin in all three parameters. However, in both animal models, the experimental extract was recorded to successfully revert the elevated conditions to normal, and a dose-dependent response was noted. The extract was more successful in bringing down the heart rate than the control drug atenolol in the ethanol-induced model, although this was not the case in the digoxin-induced model. The control drug and the extract had comparable effects on the systolic pressure of the experimental animals in both models. The heart rates and blood pressures (both systolic and diastolic) in different groups of rats after administration of different medicaments are presented in Figure 1.

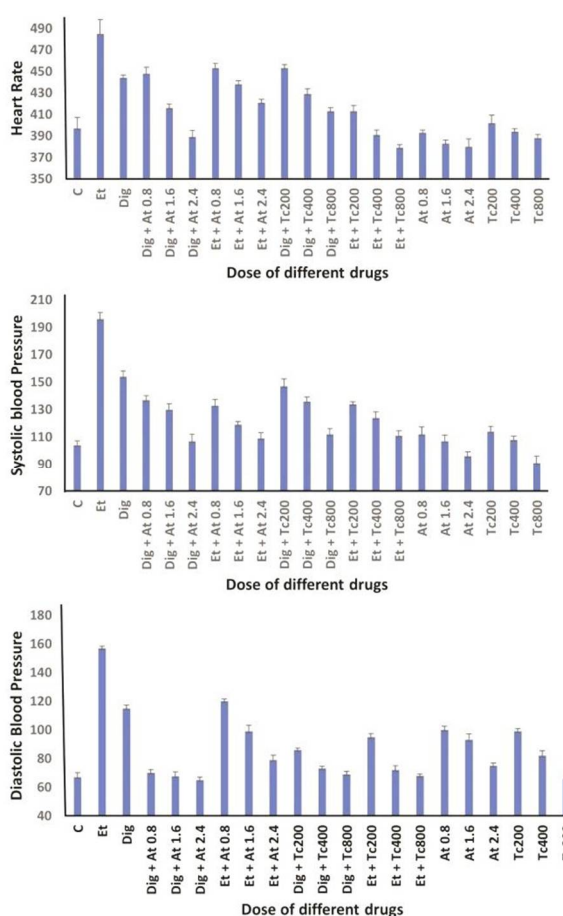


Figure 1. Heart rate, systolic blood pressure and diastolic blood pressure in different groups of rats before and after administration of different medicaments.

The plant has also previously been reported for its cardioactive and antihypertensive properties. Bakhari and Isa, 2010 have previously reported that several extracts (aqueous, petroleum-ether, methanol and chloroform) and fractions of the plant is capable of antagonizing both the α and β adrenoceptor.³ Praman *et al.*, 2011 and 2013 also reported the cardio-modulatory activity of the plant extract, indicating the inhibition of β 1 and β 2 adrenoceptors

along with the involvement of non-cholinergic and non-adrenergic pathways.^{4,8} *T. crisper* constituents cycloeucalenol, N-formylornociferine and cycloeucalenone have also been reported to be cardio-modulatory.^{9,10} In a prior computational assay, five phytoconstituents of *T. crisper* yielded a higher binding affinity to $\alpha 2A$ adrenergic receptor than the respective control, and nine phytoconstituents similarly showed promise with the $\beta 1$ adrenergic receptor.⁵

The SGOT and SGPT tests were blood tests that are part of the liver profile. It measured two liver enzymes, called Serum Glutamic-Oxaloacetic Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT). SGOT is now usually called aspartate aminotransferase (AST) and SGPT is called alanine transferase (ALT). SGOT and SGPT tests (or AST test and ALT test) evaluated how much of both liver enzymes were present in the blood before and after administration of different medicaments. A high level of SGOT and SGPT are released into the blood as a sign of liver damage, cancer, or other diseases. The well-functioning of the liver, a vital organ of the body, depends on different enzyme levels.¹¹ As parameters of liver function, SGOT and SGPT were measured before and after administration of a specified amount of either digoxin, ethanol, standard drug atenolol, or extract of *T. crisper* at different doses according to the protocol shown in Table 1. The data are presented in Figure 2.

Serum creatinine (a blood measurement) is an important indicator of kidney health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate) and adenosine triphosphate (ATP, the body's immediate energy supply source). Healthy kidneys filter creatinine out of the blood. Creatinine is excreted from the body as a waste product through urine. The functioning of the kidney, a vital organ of the body, was assessed by measuring the creatinine level before and after administration of a specified amount of either digoxin, ethanol, standard drug

atenolol, or extract of *T. crisper* at different doses according to the protocol shown in Table 1. The data are presented in Figure 3.

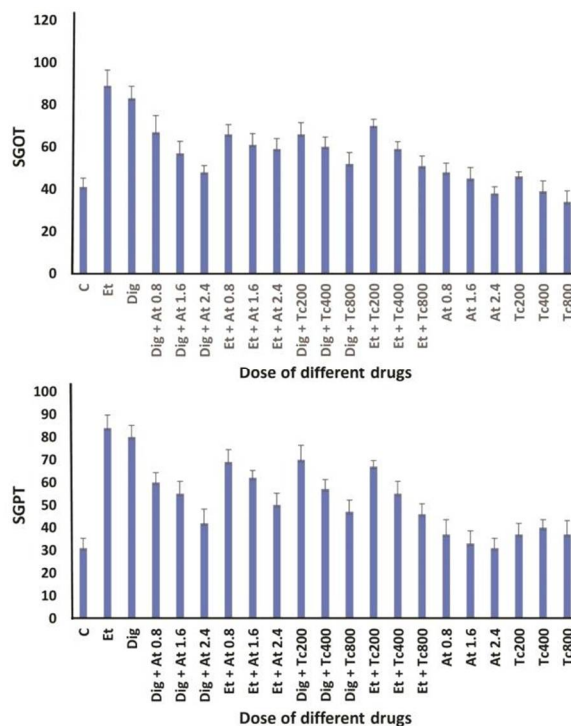


Figure 2. SGOT and SGPT levels in different groups of rats before and after administration of different medicaments.

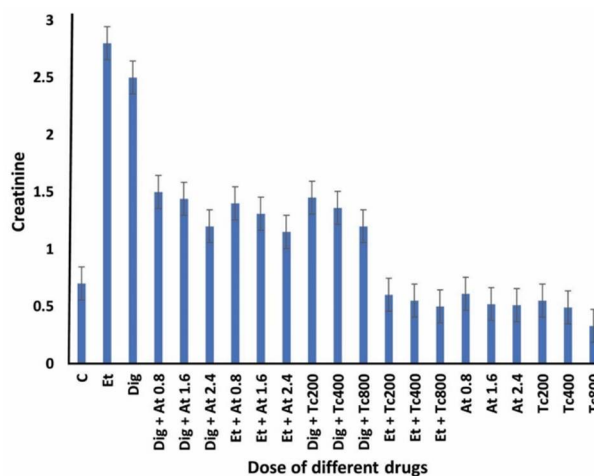


Figure 3. Creatinine levels in different groups of rats before and after administration of different medicaments.

In the normal, non-conditioned rats, the plant extract was not observed to significantly alter the levels of SGOT, SGPT or serum creatinine on its own, indicating that the extract exerts no harmful

effect on normal kidney and liver functions. Moreover, in a dose-dependent fashion similar to the control, the extract reduced the levels of SGOT, SGPT and serum creatinine after the levels spiked following digoxin or ethanol treatment. These findings were found to be both parallel to and conflicting with previous reports, as the safety status of the plant is a matter of debate in relevant circles.¹²⁻¹⁸ The ethanolic and methanolic extracts of the plant and chloroform and n-hexane fractions of the latter were communicated to yield no toxic effects in murine models. In male Balb/C mice, 50-200 mg/kg of the ethanolic extract was safely tolerated, and in Swiss Albino mice, doses up to 2000 mg/kg were not found to be of concern.^{12,13} A few contrasting studies have presented data that establishes the plant as hepatotoxic. Kadir *et al.*, 2011 reported that the ethanolic extract increased levels of serum AST, ALT, bilirubin and G-glutamyl transferase, as well as causing hepatocyte damage.¹⁴ ALT and AST elevation have also been reported in other studies.^{15,16} There have been two clinical instances of *T. crispa*-associated toxicity, a 49-year-old male patient partaking a herbal preparation of the plant and a 57-year-old man who consumed the aqueous extract of the plant.^{17,18} Overall, the safety status of the plant is contested still and requires further intensive investigation.

CONCLUSION

This manuscript explored and investigated the cardioactive and antihypertensive activity of a common medicinal plant of Bangladesh, *T. crispa*, as well as assessed its biosafety profiles in terms of hepato- and nephrotoxicity. The ethanolic extract of the plant was observed to be both cardioactive and antihypertensive; however, the safety profile could not be established beyond any doubt. Experimental findings regarding the biosafety of the plant were found to be encouraging. However, prior contrasting reports warrant further investigation. Overall, further investigations into the plant and its constituents can potentially yield novel lead compounds for further development of new therapeutic agents.

ETHICAL DECLARATION:

Ref. No.:158/Biol.ScS

AUTHOR CONTRIBUTIONS

MSA has conceived the original idea, JA and FA extensively reviewed the literature, and JAC, AAC, SK, and MAAS critically reviewed the overall activities. All the authors read the article meticulously and agreed to submit the article.

FUNDING

This work has been supported by a grant from the Centennial Research Grants of Dhaka University, Grant No.: Regi/Admin-3/47855, Dated:03-06-2021.

ACKNOWLEDGMENTS

We express our gratitude to the authority of the Department of Pharmaceutical Chemistry for using the computers and other facilities of the Molecular Pharmacology and Herbal Drug Research Laboratory established under the HEQEP Project.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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