Original Article

Evaluation of Cardiovascular and Pharmacodynamic Effects of Single and Multiple Doses of Aqueous and Hydro-Alcoholic Extracts of *Tinospora cordifolia* in Healthy Human Male Subjects

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ABSTRACT

Objective: To evaluate the cardiovascular and pharmacodynamic effects of single and multiple doses of *Tinospora cordifolia* (Aqueous and Hydro-alcoholic extracts) in healthy human male subjects.

Method: Randomized, double blind, placebo-controlled dose ranging study. After approval from the institutional ethics committee, written informed consent was taken from subjects. Eligible subjects were allocated to the single and multiple dose groups, with six subjects in each group. The active treatment (Aqueous and Hydro-alcoholic extracts of *Tinospora cordifolia*) and placebo capsules were administered in 2:1 ratio in all the study groups. Vital parameters and pharmacodynamic assessment of cardiac profiling were performed using cold pressor test (CPT), tilt table and platelet aggregation tests. The data was presented as mean ± SD. No statistical tests were applied as the sample size was less (n=6 in each group).

Result: During CPT, *Tinospora cordifolia* attenuated the rise in SBP, DBP and pulse rate in all the treatment groups compared to baseline. The CPT induced arterial stiffness was counteracted by treatment with single dose and multiple doses of aqueous and hydro-alcoholic extracts *Tinospora cordifolia*. Treatment with single doses of aqueous and hydro-alcoholic extracts *Tinospora cordifolia* produced a mild increase in cardiac output during 45^0 & 60^0 phases of the tilt. Treatment with multiple doses of aqueous and hydro-alcoholic extracts *Tinospora cordifolia* increased cardiac output at 60^0 tilt (Day 1) and at 45^0 & 60^0 phases of the tilt (Day 11).

Conclusion: Treatment with single and multiple doses of *Tinospora cordifolia* produced remarkable changes in the cardiovascular profile. Further studies in larger number of subjects and in patients with cardiovascular diseases are needed to confirm these effects.

Keywords: *Tinospora cordifolia*, Cardiotonic, Cold pressor test.

INTRODUCTION

Tinospora cordifolia commonly named as "Guduchi" in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude[1-3]. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters[4]. A variety of constituents have been isolated from Tinospora cordifolia plant and their structures were elucidated. They belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides[5]. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties[6-8] like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immuno-modulatory and anti-neoplastic activities.

However, there are no studies evaluating the effects of *Tinospora cordifolia* in healthy human subjects. Hence, this study was undertaken to evaluate the cardiovascular and pharmacodynamic effects of single and multiple doses of *Tinospora cordifolia* in healthy human male subjects.

METHODOLOGY

The study is a randomized double blind single and multiple dose study done in the Department of Clinical Pharmacology and Therapeutics. The study was approved by the institutional ethics committee and all subjects gave written informed consent prior to participation in the study. Healthy male subjects were screened according to the inclusion and exclusion criteria of the study protocol and all vital parameters and lab safety parameters were performed one week prior to study initiation.

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. The baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling of subjects in all the groups were performed after an overnight fast, using non-invasive methods. The various cardiac profiling parameters that were assessed include - Brachial-Ankle Pulse Wave Velocity (baPWV cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI %) (Micro medical Pulse Tracer Gallingham, Kent, UK), Aortic Augmentation Pressure (AP mmHg), Pulse Pressure (PP mmHg), Aortic Augmentation Index (AiX %) and Sub Endocardial Viability Ratio (SEVR %) (Sphygmocor®) before and after cold pressor test (CPT). Blood pressure and pulse rate were taken before, during and after CPT. Then, tilt table test was performed and blood pressure, basal Impedance (ohms), cardiac output(L/min), cardiac index(L/min/ m^2), stroke volume(ml/beat), stroke volume index(ml/beat/ m^2), systemic vascular resistance (dyne.sec/cm^5), systemic vascular resistance index(dyne.sec/cm^5/ m^2), left ventricular ejection time(ms), pulse rate(bpm), velocity index(/1000sec) and central velocity pressure(mmHg) were measured at 0°, 45°, 60° and again at baseline 0° tilt using L&T Nivomon monitor. Pretreatment blood samples were drawn for assessment of safety parameters.

In the single dose study, pretreatment blood samples were drawn for estimation of biomarkers of endothelial function i.e. highly sensitive C-reactive protein (hsCRP), Malondialdehyde (MDA) and Nitric oxide (NO) and platelet aggregation test. First group of six subjects were randomized of whom four subjects received four capsules of active medication i.e. aqueous extract of Tinospora cordifolia 250mg (TC-1) and two subjects received four capsules of placebo. In the second group, four subjects received four capsules hydro-alcoholic extract of Tinospora cordifolia 250mg (TC-2) and two subjects received four capsules of placebo according to prior randomization schedule. At 3 hrs of post treatment blood samples were drawn for assessment of biomarkers and platelet aggregation study, then all procedures were repeated as done at baseline and all the same parameters mentioned above were recorded.

In the single dose groups, the subject's vital parameters were recorded before and at hourly intervals up to 6 hrs then at 8, 12 and 24 hrs of post treatment. The lab safety parameters were measured at 24 hours post administration of study medication. Any adverse drug reaction (ADR) reported was recorded in case report form. Subjects were discharged from the clinical research unit after all vital parameters were found to be normal 24 hours post treatment.

In multiple dose study, pretreatment blood samples were drawn for estimation of biomarkers of endothelial function and platelet aggregation test on day 1. In the first group of six subjects, four subjects received two capsules of TC-1 twice daily and two subjects received two capsules of placebo twice daily. In the second group, four subjects received two capsules of TC-2 twice daily and two subjects received two capsules of placebo twice daily according to prior randomization schedule. Then the subjects were asked to continue the study medication for next 10 days. The subjects were housed in a temperature and humidity controlled room in the clinical research unit again on day 10. After an overnight fast, baseline measurements of vital parameters were recorded and all the procedures done on day 1 were repeated on day 11. Any adverse drug reaction (ADR) reported was recorded in case report form.

RESULTS

Six volunteers each were enrolled and randomized in the study groups. All subjects completed the study uneventfully. The demographic characteristics of the study groups were homogenous in nature, as shown in Table 1.

Effect of treatments on vital parameters with Cold Pressor test

In all the study groups, cold pressor test (CPT) increased systolic, diastolic blood pressure (SBP, DBP) and pulse rate (PR) at 30 sec during the test from baseline, whereas after 1 min and 10 min of CPT, SBP, DBP & PR were within normal limits. At 3hrs post treatment in all groups, there was increase in SBP, DBP & PR at 30 sec during CPT which were however much lower than that compared to pre-treatment values. In the placebo group, after 3hrs of treatment, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

The cold pressor test produced arterial stiffness at baseline and the same is evidenced by an increase in baPWV, RI, AP, PP and AIx and also as a decrease in values of SEVR in all treatment and placebo groups.

Treatment with *Tinospora cordifolia* decreased baPWV in all the treatment groups. However the changes in other pharmacodynamic parameters recorded

during cold pressor test were not found to be remarkable. The CPT induced arterial stiffness was counteracted by treatment with *Tinospora cordifolia* and there was decrease in baPWV, RI, AP, PP, AIx and increase in SEVR in TC-1, TC-2 single dose and TC-1, TC-2 multiple dose groups both at Day 1 & Day 11. There were no remarkable changes in the placebo group.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters

Tilt table test per se did not produce any remarkable change in SBP, DBP, PR and cardiac output at different degrees of tilt in any of the study groups. Treatment with single dose of TC-1 and TC-2 produced a mild increase in cardiac output during 45^0 & 60^0 phases of the tilt. Treatment with multiple doses of TC-1 and TC-2 increased cardiac output at 60^0 tilt (Day 1) and at 45^0 & 60^0 phases of the tilt (Day 11).

Significant inhibition in platelet aggregation was seen with TC-1 and TC-2 single dose and TC-1 and TC-2 multiple dose on day 1 and day 11. However, inhibition was more on day 11 than on day 1. Placebo treatment did not produce any change in the platelet aggregation test.

Adverse Events

All subjects tolerated both treatments and procedures well. No subjects developed any adverse drug reaction. Study was completed uneventful. There was no remarkable change in any of the hematological, biochemical safety lab parameters with either treatment.

DISCUSSION

This was a placebo-controlled, phase I dose ranging study evaluating the safety and efficacy of single doses of TC-1 and TC-2 single dose and multiple doses on cardiovascular profile in healthy human male volunteers.

Tinospora cordifolia has been studied for various conditions in preclinical studies such as hyperlipidemia[9], diabetes[10,11], anti-oxidant[12,13] and cardio-protective activity[14].

In our study, cold pressor test (CPT) was performed to induce arterial stiffness which manifested as an increase in systolic and diastolic blood pressure and pulse rate. Treatment with *Tinospora cordifolia* resulted in lower increments in SBP, DBP & PR, at 30 sec during CPT compared to pretreatment CPT increments of the same parameters, thus attenuating the cardiovascular effect of CPT & protecting against arterial stiffness. This effect of *Tinospora cordifolia* may be ascribed to its cardioprotective properties of vasodilatation and anti-oxidant effects.

Further, CPT induced arterial stiffness was also evaluated with changes in pharmacodynamic parameters before and after CPT. Treatment with *aqueous and hydro-alcoholic extracts of Tinospora cordifolia* for 10 days counteracted the CPT induced arterial stiffness by decreasing baPWV, RI, AP, PP, AIx and increase in SEVR compared to baseline. This effect was seen with both single and multiple doses of *Tinospora cordifolia*, with the effect being more prominent in multiple dose groups. This indicates that multiple dose administration of *Tinospora cordifolia* protects against the cardiovascular effects induced by cold pressor test.

The role vascular endothelium has been studied in relation to arterial stiffness and it was demonstrated that an intact and functional vascular endothelium is essential to maintain vascular tone thus countering any arterial stiffness[15]. *Tinospora cordifolia* promotes the function of intact vascular endothelium by virtue of its anti-oxidant activity.

The cardiac function was also assessed with tilt table test. Treatment with

both single and multiple doses of *Tinospora cordifolia* did not alter the vital parameters except for a mild increase in cardiac output at 45 and 60 degree tilt, which was comparable in both the groups. This effect may be due to the cardiotonic activity[16] of *Tinospora cordifolia.*

The effect of *Tinospora cordifolia* on platelet aggregation was evaluated using agonists- ADP & Collagen. Inhibition of platelet aggregation was seen with single and multiple doses of TC-1 and TC-2. However, this effect was much higher with the multiple dose treatment suggesting a dose-dependent inhibition of platelet aggregation by *Tinospora cordifolia*. Viswanatha et al[17] demonstrated the in vitro anti-platelet effect of *Tinospora cordifolia in* combination with other herbal preparations. The mechanism of anti-platelet aggregatory effect is considered to be associated with the inhibition of one of the common mediator/pathways involved in ADP and/or by stabilizing the platelet membrane.

All the treatments were safe and well tolerated and no adverse effects were reported in all of the treatment groups.

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Conflicts of Interest

The authors report no conflict of interest.

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Table 1. Demographic Data

	Total No.	Age (yrs)	Weight (Kg)	BMI (Kg/m^2)
TC-1 Single dose Group				
TC-1 Group	4	31.50±4.79	61.50±5.38	22.84±0.78
Placebo	2	34.00±5.65	62.10±3.25	23.08±0.81
TC-2 Single dose Group				
TC-2 Group	4	30.25±2.75	65.48±4.13	23.00±0.88
Placebo	2	30.50±0.71	63.30±4.10	22.88±0.60
TC-1 Multiple dose Group				
TC-1 Group	4	30.00±3.16	65.18±1.77	23.82±0.74
Placebo	2	33.50±2.12	61.05±2.75	22.49±0.01
TC-2 Multiple dose Group				
TC-2 Group	4	31.25±2.50	62.90±4.75	22.69±0.66
Placebo	2	32.50±3.53	65.60±6.36	23.31±1.28

Table 2A. Effect of CPT on pharmacodynamic parameters at baseline – Single dose group

	baPWV (cm/s)	ABI	Reflection Index (RI %)	AP(mmHg)	PP(mmHg)	AIX (%)	SEVR (%)
TC-1 Single dose Group							
TC-1 Before CPT	1150±0.00	1.14±0.00	71.50±2.13	8.00±1.41	23.50±3.10	131.50±5.50	139.80±10.11
Placebo Before CPT	1188±53.03	1.10±0.01	65.65±2.33	8.50±0.70	24.50±0.70	128.50±3.53	145.50±9.19
TC-1 After CPT	1244±8.48	1.10±0.00	77.23±4.70	11.75±1.70	30.00±2.58	145.30±5.56	128.50±3.78
Placebo After CPT	1300±0.00	1.11±0.01	70.65±2.33	12.00±1.41	32.00±1.41	145.00±4.24	140.00±12.73
TC-2 Single dose Group							
TC-2 Before CPT	1157±9.19	1.12±0.01	69.80±3.73	9.00±2.00	25.50±4.43	126.80±1.89	144.00±7.87
Placebo Before CPT	1150±70.71	1.11±0.03	69.95±0.91	7.50±0.70	25.00±5.65	126.50±0.70	149.00±7.07
TC-2 After CPT	1225±35.36	1.11±0.01	74.38±2.39	12.00±1.82	35.25±3.09	142.50±5.91	135.30±6.44
Placebo After CPT	1238±53.03	1.09±00.00	72.30±0.99	12.50±0.70	31.50±2.12	149.50±2.12	136.00±4.24

Table 2B. Effect of CPT on pharmacodynamic parameters at baseline– Multiple dose groups

	baPWV (cm/s)	ABI	Reflection Index (RI %)	AP(mmHg)	PP(mmHg)	AIX (%)	SEVR (%)
TC-1 Multiple Dose Group: Day 1							
TC-1 Before CPT	1175±35.36	1.13±0.01	71.38±33.10	8.25±1.25	23.75±3.59	130.0±4.89	133.3±6.50
TC-1 After CPT	1257±26.16	1.11±0.05	75.25±4.03	11.25±1.70	30.25±2.98	141.3±4.78	135.5±4.72
Placebo Before CPT	1200±35.36	1.13±0.04	72.95±0.91	7.50±2.12	22.50±0.70	131.5±10.61	131.0±4.24
Placebo After CPT	1263±17.68	1.10±0	78.80±0.70	12.50±0.70	30.50±0.70	140.0±2.82	127.0±5.67
TC-1 Multiple Dose Group: Day 11							
TC-1 Before CPT	1157±9.19	1.12±0.01	71.20±1.47	8.0±1.82	21.25±2.36	130.0±7.61	140.3±2.75
TC-1 After CPT	1132±26.16	1.11±0.03	69.15±1.88	7.50±1.29	20.25±0.95	128.5±8.34	142.3±10.18
Placebo Before CPT	1200±35.36	1.14±0.02	72.15±0.21	7.50±0.70	23.0±1.41	133.5±0.70	144.0±2.82
Placebo After CPT	1263 ±17.68	1.13±0.02	80.80±0.70	13.50±0.70	31.50±0.70	142.0±1.41	133.0±12.73
TC-2 Multiple Dose Group: Day 1							
TC-2 Before CPT	1182±9.19	1.13±0.00	69.15±3.28	7.50±0.57	22.25±1.50	130.8±4.99	142.3±6.99
TC-2 After CPT	1250±0.0	1.132±0.02	74.88±5.05	12.0±1.63	28.0±0.81	142.0±3.55	132.3±8.22
Placebo Before CPT	1163±53.03	1.12±0.014	67.15±1.20	6.50±0.70	23.0±0.0	121.0±4.24	141.5±7.77
Placebo After CPT	1225±35.36	1.15±0.02	70.80±0.28	9.0±0.0	30.50±3.53	126.5±3.53	134.5±2.12
TC-2 Multiple Dose Group: Day 11							
TC-2 Before CPT	1157±9.19	1.12±0.00	71.23±1.35	8.50±1.29	23.00±2.16	128.0±1.41	135.0±6.05
TC-2 After CPT	1107±26.16	1.13±0.00	68.73±1.43	7.50±1.29	21.00±2.16	125.0±2.44	137.5±5.68
Placebo Before CPT	1175±0.0	1.14±0.0	70.80±1.69	7.00±1.41	23.0±1.41	125.5±2.12	135.5±2.12
Placebo After CPT	1250±35.36	1.15±0.0	77.50±2.12	11.00±1.41	29.00±0.0	135.0±1.41	130.5±4.95

Table 2C. Effect of treatments on pharmacodynamic parameters during CPT – Single dose group

	baPWV (cm/s)	ABI	Reflection Index (RI %)	AP(mmHg)	PP(mmHg)	AIX (%)	SEVR (%)
TC-1 Single dose Group							
TC-1 Before CPT	1207±44.55	1.15 ±1.01	68.38±3.19	8.5±1.29	22.5±0.57	129.3±6.94	143.0±6.0
Placebo Before CPT	1213±53.03	1.13±0.07	67.30±3.81	10.5±2.12	24.5±2.12	136.5±0.70	137.5±16.26
TC-1 After CPT	1169±26.87	1.20 ±0.08	65.73±2.48	6.75 ±1.70	18.50 ±1.29	125.3±8.18	148.80±6.70
Placebo After CPT	1250±35.36	1.11±0. 04	73.65±0.91	14.00±1.41	32.50±2.12	146.5±0.70	128.5±6.36
TC-2 Single dose Group							
TC-2 Before CPT	1150±35.36	1.11±0.015	72.23±1.29	7.50±1.91	21.00±4.96	127.3±5.37	136.8±7.411
Placebo Before CPT	1213±53.03	1.14±0.01	72.65±2.33	9.0±0.0	25.0±2.82	130.0±1.41	143.0±5.65
TC-2 After CPT	1107±9.19	1.14±0.00	68.05±1.28	6.75±0.95	17.50±3.41	121.8±3.09	142.0±8.32
Placebo After CPT	1300±70.71	1.15±0.28	79.80±1.13	13.00±1.41	31.50±2.12	150.5±4.95	130.5±0.70

Table 2D. Effect of treatments on pharmacodynamic parameters during CPT – Multiple dose group

	baPWV (cm/s)	ABI	Reflection Index (RI %)	AP(mmHg)	PP(mmHg)	AIX (%)	SEVR (%)
TC-1 Multiple Dose Group: Day 1							
TC-1 Before CPT	1175±0.0	1.11±0.01	70.75±1.70	7.75±0.95	21.0±3.55	124.8±2.21	132.3±9.53
TC-1 After CPT	1138±53.03	1.08±0.0	68.73±1.50	6.50±1.29	18.25±2.87	120.5±1.29	136.8±9.97
Placebo Before CPT	1188±17.68	1.15±0.02	71.50±2.12	9.50±0.70	22.50±0.70	127.5±2.12	137.5±2.12
Placebo After CPT	1250±35.36	1.16±0.06	79.45±3.04	13.50±0.70	35.00±2.82	138.5±0.70	146.5±3.53
TC-1 Multiple Dose Group: Day 11							
TC-1 Before CPT	1163±17.68	1.13±0.01	69.23±0.97	8.75±1.70	21.75±1.50	127.0±6.05	141.3±5.56
TC-1 After CPT	1107±26.16	1.16±0.02	66.33±1.24	7.0±0.81	19.75±1.89	122.5±4.20	145.5±7.55
Placebo Before CPT	1213±17.68	1.13±0.0	72.50±2.12	8.50±0.70	23.5±0.70	138.0±1.41	143.0±7.07
Placebo After CPT	1250±35.36	1.16±0.0	79.90±0.14	13.0±1.41	31.50±2.12	150.5±2.12	132.5±0.70
TC-2 Multiple Dose Group: Day 1							
TC-2 Before CPT	1207±9.19	1.12±0.007	69.75±1.50	8.00±1.82	22.5±1.29	133.0±5.88	145.3±4.64
TC-2 After CPT	1163±0.00	1.125±0.00	66.90±2.45	6.0±1.41	19.25±0.95	125.3±5.56	156.8±5.56
Placebo Before CPT	1200±35.36	1.12±0.03	69.45±0.21	7.0±0.0	24.50±0.70	121.5±6.36	135.0±2.82
Placebo After CPT	1288±17.68	1.13±0.02	72.50±0.70	9.0±1.41	32.0±4.24	131.0±9.89	131.5±0.70
TC-2 Multiple Dose Group: Day 11							
TC-2 Before CPT	1119±8.48	1.15±0.02	69.48±0.76	8.25±0.50	22.50±3.10	127.3±0.95	137.8±4.78
TC-2 After CPT	1051±17.68	1.11±0.04	65.33±1.42	5.75±0.50	18.75±0.95	118.3±1.70	147.8±5.37
Placebo Before CPT	1188±53.03	1.14±0.01	71.00±2.82	9.0±0.0	25.50±4.95	128.0±1.41	135.0±1.41
Placebo After CPT	1288±53.03	1.11±0.0	76.65±3.74	12.50±0.70	30.00±2.82	134.0±0.0	130.5±0.70

Table 3. Effect of Treatments on Platelet aggregation test

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
TC-1 Single dose Group			
TC-1	64.75±2.53	62.88±5.55	4.24±3.19
Placebo	55.75±1.76	58.25±0.35	Nil
TC-2 Single dose Group			
TC-2	65.63±3.56	62.63±3.90	4.74±0.98
Placebo	58.75±2.47	58.50±1.41	0.80±1.13
TC-1 Multiple dose Group Day 1			
TC-1	64.13±8.10	61.25±7.14	3.98±1.87
Placebo	61.50±0.70	63.50±0.00	NIL
TC-1 Multiple dose Group Day 11			
TC-1	60.0±6.96	53.63±7.43	9.26±3.67
Placebo	58.50±2.12	61.75±7.42	NIL
TC-2 Multiple dose Group Day 1			
TC-2	67.88±2.95	61.25±6.27	6.25±1.17
Placebo	61.75±5.30	63.75±6.01	NIL
TC-2 Multiple dose Group Day 11			
TC-2	66.00±2.55	57.88±2.13	12.75±1.041
Placebo	62.00±2.12	64.00±3.53	NIL