Project Report

Evaluation of Cardiovascular and Pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial function in healthy human male subjects

Study IV: 2000mg Single Dose

Clinical trial site

Nizam's Institute of Medical Sciences
Department of Clinical Pharmacology and Therapeutics
Punjagutta, Hyderabad, Andhra Pradesh
India

Principal - Investigator

Dr. P.Usha Rani

Co-Investigator
Dr. M.U.R.Naidu
Dr. I.V.Sravanti
(Ayurvedic Physician)
Trial Co-ordinators
Nishat Fatima
N.Muralidhar
Study Nurse
Rita Salomi

Sponsor

Ministry of AYUSH, AYUSH Bhawan
B-Block, GPO Complex, INA, New Delhi-110023

Terminalia arjuna 2000mg single dose study

Title: Evaluation of Cardiovascular pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Introduction:

Terminalia arjuna (TA) is a medicinal plant used as a cardiotonic in ayurveda. Its stem bark possesses glycosides, large quantities of flavonoids, tannins and minerals. Flavonoids have been detected to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiotonic. The presence of potent antioxidant constituents which causes improvement in endothelial dysfunction seen in coronary artery disease and heart failure. Thus making Terminalia arjuna unique amongst currently used medicinal plants. Experimental studies have revealed its bark exerting significant inotropic and hypotensive effect, increasing coronary artery flow and protecting myocardium against ischemic damage. However, dose response studies in healthy human subjects were not reported. Therefore *Terminalia arjuna* can be exploited for its therapeutic potential in CAD and related cardiovascular disorders.

Therefore the present study was undertaken to evaluate the effect of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects and further study its probable mechanism of action.

Methodology:

The study is a randomized double blind single 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. Six subjects were screened and randomized to the two treatments. Two subjects received placebo while 4 subjects received active medication of *Terminalia arjuna* as per prior randomization schedule.

Single dose study

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. After an overnight fast, baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling were performed using non-invasive methods. The various cardiac profiling parameters were assessed using the following instruments - Brachial-Ankle Pulse Wave Velocity (cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI%) (Micromedical Pulse Tracer Gallingham, Kent, UK), Aortic Augmentation Pressure (mmHg), Plus Pressure (mmHg), Aortic Augmentation Index (%) and Sub Endocardial Viability Ratio (%)(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, Body Surface Area (m^2),

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. However the Cardiac Output is more significant among the other parameters recorded with Nivomon monitor.

Pretreatment blood samples were drawn for estimation of biomarkers of endothelial function. The study medication was then administered as per the randomization schedule (either four capsules of *Terminalia arjuna* 500mg or four capsules of Placebo) with 240 ml of water.

At 3 hrs of post treatment blood samples were drawn for assessment of biomarkers and then all procedures were repeated as done at baseline and all the same parameters mentioned above were recorded.

The subject's vital parameters were recorded before and at hourly intervals upto 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. The safety lab reports were evaluated once they were available.

OBSERVATIONS:

A total of 6 volunteers were enrolled and randomised. Four subjects received single dose of *Terminalia arjuna* 2000mg and two subjects received identical placebo and all subjects completed the study uneventfully. Detailed demographic characteristics of the two study groups are shown in Table 1.

Table1: Demographic Data

Parameter	***Terminalia arjuna***	**Placebo**
Total No.	4	2
Age	32.0±2.16	31.0±0.0
Gender (M)	4	2
Weight (Kg)	64.50±4.20	65.15±7.70
BMI (Kg/m^2)	24.28±1.24	25.16±3.41

Effect of both treatments on Vital parameters with Cold Pressor test:

Table 2 –Showing effect of *Terminalia Arjuna* on Vital parameters before, during and after Cold Pressor test (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	115.0±1.15	75.50±1.91	76.50±1.00	113.0±1.15	76.50±1.00	75.00±2.0
During (30 Sec) CPT	141.0±3.16	89.50±2.38	85.00±1.15	132.5±3.41	86.25±1.25	82.25±1.7
1 min after CPT	116.5±1.00	80.50±1.00	82.50±1.00	116.5±1.00	80.50±1.00	82.00±2.8
10 min after CPT	113.0±1.15	75.50±1.00	75.50±1.91	112.0±1.63	75.00±1.15	75.50±1.0

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 2000 mg single dose of *Terminalia arjuna*, there was an increase in systolic and diastolic blood pressure and pulse rate which were however much lower than that compared to pretreatment values.

Table 3 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test (n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	114.0±5.65	73.0±1.41	74.00±2.82	113.0±1.41	77.0±1.41	73.0±1.41
During (30Sec) CPT	145.0±4.24	89.0±1.41	86.50±0.70	143.5±2.12	91.0±2.82	87.5±2.12
1min after CPT	117.0±4.24	77.0±1.41	79.50±4.95	114.0±0.0	80.0±0.0	82.0±2.82
10 min after CPT	114.0±2.82	73.0±1.41	76.00±2.82	112.0±0.0	75.0±1.41	75.0±1.41

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in systolic blood pressure, diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline:

Table 4 -Showing the effect of Cold Pressor test at baseline in *Terminalia arjuna* group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1169±8.48	1225±0.00
ABI	1.08±0.03	1.11±0.03
Reflection Index (RI%)	65.98±4.62	71.80±4.58
AP(mmHg)	8.00±0.81	12.50±1.29
PP(mmHg)	23.25±0.95	30.25±2.21
AIX (%)	131.5±3.69	144.0±3.74
SEVR (%)	155.5±7.93	148.3±7.50

Table 5 –Showing effect of on Cold Pressor test at baseline in Placebo group (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1188.0±53.03	1288.0±17.68
ABI	1.09±0.91	1.12±0.00
Reflection Index (RI%)	68.45±1.62	73.50±2.12
AP (mmHg)	8.0±0.0	11.50±0.70
PP(mmHg)	25.00±1.41	33.50±0.70
AIX (%)	136.5±6.36	141.5±3.53
SEVR (%)	171.0±9.89	163.5±3.53

As seen from the above tables 4 and 5 showing the effect of cold pressor test per se in both treatment groups at baseline. Cold pressor test produces arterial stiffness and the same is evidenced by an increase in baPWV, RI, AP, PP and AIx and also as a decrease in values of SEVR.

Effect of both treatments on Pharmacodynamic parameters during Cold Pressor test

Table 6 -Showing effect of *Terminalia arjuna* 2000 mg single dose on pharmacodynamic parameters during Cold Pressor test (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1200±35.36	1144±43.84
ABI	1.13±0.02	1.10±0.02
Reflection Index (RI%)	67.40±2.96	62.50±2.38
AP(mmHg)	7.75±0.95	6.00±0.81
PP(mmHg)	23.25±1.89	19.00±1.15
AIX (%)	136.0±11.78	130.8±12.63
SEVR (%)	155.8±6.60	162.5±10.75

As observed from the above table, cold pressor test per se did not produce changes in ABI, AP, PP, baPWV, RI and SEVR .The values indicate that the cold pressor induced arterial stiffness was counteracted by treatment with *Terminalia arjuna* and there was decrease in baPWV, RI, AP, PP, AIx and increase in SEVR compared to baseline

Table 7 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1163±53.03	1250±35.36
ABI	1.06±0.01	1.11±0.01
Reflection Index (RI%)	70.10±4.95	73.45±4.03
AP (mmHg)	7.50±0.70	12.00±1.41
PP(mmHg)	24.50±2.12	30.50±0.70
AIX (%)	132.5±0.70	140.5±2.12
SEVR (%)	161.5±17.68	148.0±0.0

As seen from the above table, treatment with placebo did not alter the arterial stiffness produced by cold pressor test and the same is reflected by an increase in baPWV, RI, AP, PP, AIx and decrease in SEVR.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters

Table 8 –Showing effect of *Terminalia Arjuna* on Tilt Table Test (n=4)

	Pretreatment				Post Treatment			
Parameter	**SBP**	**DBP**	**PR**	**CO**	**SBP**	**DBP**	**PR**	**CO**
Basal1(0^o)	115.0±2.58	74.5±1.0	73.5±1.91	3.60±0.34	114.5±1.0	75.5±1.9	75.0±2.6	3.67±0.4
At 45^o	120.0±1.63	82.0±1.6	84.0±2.82	3.47±0.34	119.0±1.1	82.5±1.0	85.0±2.0	3.62±0.4
At 60^o	117.5±1.91	82.0±1.6	82.2±2.87	3.32±0.22	116.5±1.0	80.5±2.5	83.0±2.0	3.57±0.2
Basal2(0^o)	113.5±1.00	74.0±0.0	74.5±1.00	3.77±0.34	112.5±1.0	74.0±1.6	74.0±1.6	3.77±0.4

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with *Terminalia arjuna* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^o and 60^o a mild increase in cardiac output was observed compared to baseline.

Figure 1 shows the mean percent change in cardiac output on treatment with *Terminalia arjuna* on Tilt Table test. All values are compared to baseline.

Figure 1

Mean Percent change in Cardiac output with Terminalia arjuna on Tilt Table Test

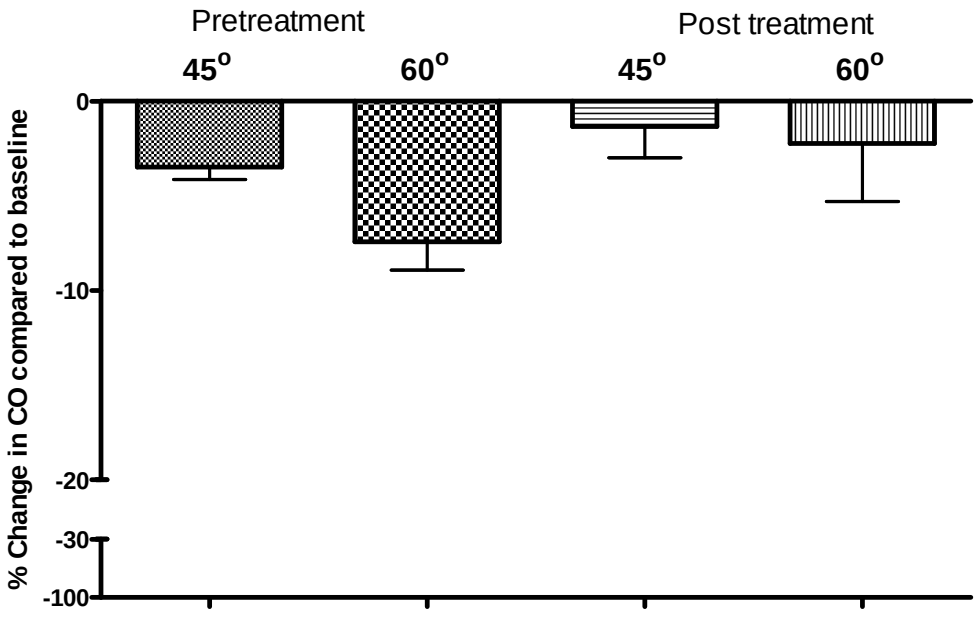


Table 9 -Showing effect of Placebo on Tilt Table Test (n=2)

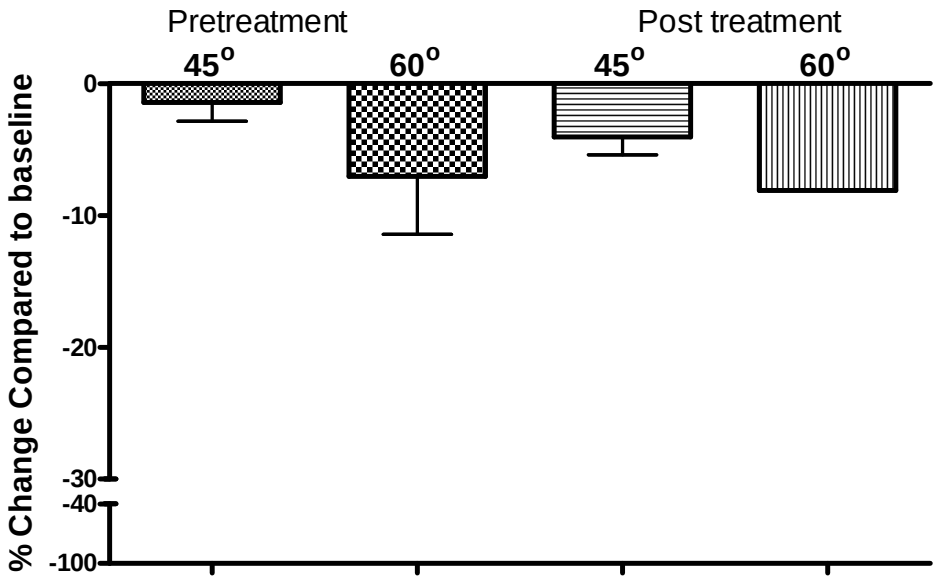
	Pretreatment				Post Treatment			
Parameter	**SBP**	**DBP**	**PR**	**CO**	**SBP**	**DBP**	**PR**	**CO**
Basal1(0^o)	113.0±1.41	73.0±1.41	76.0±0.0	3.65±0.21	112.0±0.0	77.0±1.41	73.5±6.36	3.70±0.0
At 45^o	119.0±4.24	80.0±2.82	83.0±1.41	3.6±0.28	116.0±2.82	83.0±1.41	83.0±1.41	3.55±0.1
At 60^o	117.0±4.24	79.0±1.41	80.0±2.82	3.4±0.42	113.0±4.24	81.0±1.41	80.0±2.82	3.40±0.0
Basal2(0^o)	114.0±2.82	74.0±0.0	76.0±2.82	3.6±0.14	110.0±0.0	74.0±0.0	73.0±1.41	3.80±0.0

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 2 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.

Figure 2

Mean Percent Change in Cardiac output with Placebo on Tilt Table Test



Effect of Treatments on Biomarkers:

Table 10 -Showing effect of *Terminalia arjuna* on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
High sensitivity CRP(mg/L)	1.07±0.22	0.96±0.17
Malondialdehyde (nm/ml)	3.31±0.34	2.69±0.52
Nitric oxide (µMol/L)	30.48±2.13	34.09±2.05

As observed from the above table, treatment with *Terminalia arjuna* produced a decrease in highly sensitive C-reactive protein, malondialdehyde and increase in nitric oxide levels compared to baseline. The post treatment values of biomarkers indicate that treatment with *Terminalia arjuna* improved antioxidant status.

Table 11 -Showing effect of Placebo on biomarkers (n=2)

Parameter	Pretreatment	Post treatment
High sensitivity CRP(mg/L)	1.10±0.25	1.11±0.20
Malondialdehyde (nm/ml)	3.17±0.28	3.29±0.33
Nitric oxide (µMol/L)	33.0±3.24	32.83±2.77

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Effect of Treatments on Platelet aggregation test:

Table 12 -Showing effect of *Terminalia arjuna* on Inhibition of Platelet Aggregation (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	65.63±7.01	63.38±9.12	4.81±3.95

As seen from the above table, *Terminalia arjuna* produced remarkable changes in inhibition of platelet aggregation.

Table 13 -Showing effect of Placebo on Inhibition of Platelet Aggregation (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	69.25±8.13	71.65±9.40	NIL

As seen from the above table, Placebo treatment did not produce changes in platelet aggregation test performed.

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.

Inference:

Treatment with *Terminalia arjuna* produced remarkable changes in AIx, AP, PP and ABI on cold pressor test. The active treatment also showed mild decrease in pharmacodynamic parameters such as baPWV and reflection index on cold pressor test. Treatment with single dose of *Terminalia arjuna* 2000mg produced a mild increase in cardiac output at 45^{o} and 60^{o} of tilt compared to baseline. There was slight increase in nitric oxide and reduction in malondialdehyde and hsCRP levels compared with baseline in *Terminalia arjuna* group. Reduction in platelet aggregation was also recorded in the active group. It can be concluded from the present study that *Terminalia arjuna* 2000 mg single dose was found to be safe and well tolerated without any adverse event. Studies are needed to be conducted to assess tolerability and also with multiple dose of the test product for safety and efficacy.

Glossary:

TA=*Terminalia arjuna*

SBP=Systolic Blood Pressure (mmHg)

DBP=Diastolic Blood Pressure (mmHg)

PR=Pulse Rate (bpm)

baPWV= Brachial-Ankle Pulse Wave Velocity (cm/s) (Using ABI Colins, Komaki, Japan**)**

ABI= Ankle-Brachial Index (Using ABI Colins, Komaki, Japan**)**

RI =Reflection Index (%) (Using Micromedical Pulse Tracer Gallingham, Kent, UK)

AP=Aortic Augmentation Pressure (mmHg) (Using Sphygmocor® Atcor Medical Pvt Ltd Sydney, Australia)

PP=Pulse Pressure (mmHg) (Using Sphygmocor®)

AIX=Aortic Augmentation Index (%) (Using Sphygmocor®)

SEVR=Sub Endocardial Viability Ratio (%) (Using Sphygmocor®)

BMI = Body Mass Index (Kg/m^2)

CPT= Cold Pressor Test

CO= Cardiac Output (L /min)(L&T Nivomon Monitor)

AYUSH STUDY REPORT 2013

Evaluation of Cardiovascular and Pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial function in healthy human male subjects

Study V: 1500mg Multiple Dose

Clinical trial site

Nizam's Institute of Medical Sciences
Department of Clinical Pharmacology and Therapeutics
Punjagutta, Hyderabad, Andhra Pradesh
India

Principal - Investigator

Dr. Dr.P.Usha Rani

Co-Investigator

Dr. M.U.R.Naidu
Dr. I.V.Sravanti
(Ayurvedic Physician)

Trial Co-ordinators

Nishat Fatima
N.Muralidhar

Study Nurse

Rita Salomi

Sponsor

Central Council for Research in Ayurveda & Siddha
Department of AYUSH
Ministry of Health and Family Welfare
Government of India

Multiple dose study of *Terminalia arjuna* 1500mg Report

Title: Evaluation of Cardiovascular pharmacodynamic effects of *Terminalia Arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects-Multiple dose (1500mg/day- i.e.,500mg TID) study.

Introduction:

Terminalia arjuna (TA) is a medicinal plant used as a cardiotonic in ayurveda. Its stem bark possesses glycosides, large quantities of flavonoids, tannins and minerals. Flavonoids have been detected to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiotonic. The presence of potent antioxidant constituents which causes improvement in endothelial dysfunction seen in coronary artery disease and heart failure. Thus making *Terminalia arjuna* unique amongst currently used medicinal plants. Experimental studies have revealed its bark exerting significant inotropic and hypotensive effect, increasing coronary artery flow and protecting myocardium against ischemic damage. However, dose response studies in healthy human subjects were not reported. Therefore *Terminalia arjuna* can be exploited for its therapeutic potential in CAD and related cardiovascular disorders.

Therefore the present study was undertaken to evaluate the effect of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects and further study its probable mechanism of action.

Methodology:

The study was a randomized double blind single dose (day1) and multiple dose study (day 11) of *Terminalia arjuna* 1500mg per day, conducted in the Department of Clinical Pharmacology and Therapeutics, NIMS, Hyderabad. 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. Six subjects were screened and randomized to the two treatments. Two subjects received placebo while 4 subjects received active medication of *Terminalia arjuna* 1500mg as per prior randomization schedule.

Single dose study(Day1)

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. After an overnight fast, baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling were performed using non-invasive methods. The various cardiac profiling parameters were assessed using the following instruments - Brachial-Ankle Pulse Wave Velocity (cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI%) (Micromedical Pulse Tracer Gallingham, Kent, UK), Aortic Augmentation Pressure (mmHg), Plus Pressure (mmHg), Aortic Augmentation Index (%) and Sub Endocardial Viability Ratio (%)(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°, $60^\circ$and again at baseline 0° tilt using L&T Nivomon monitor. However the change in Cardiac Output was more significant among the other parameters recorded with Nivomon monitor.

Pretreatment blood samples were drawn for estimation of biomarkers of endothelial function. The study medication was then administered as per the randomization schedule (either three capsules of *Terminalia arjuna* 500mg or three capsules of Placebo) with 240 ml of water.

At 3 hrs of post treatment blood samples were drawn for assessment of biomarkers and then all procedures were repeated as done at baseline and all the parameters mentioned above were recorded. The subject's vital parameters were recorded before and at hourly intervals upto 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. The safety lab reports were evaluated once they were available.

Then the subjects were asked to continue the study medication for next 10 days (each capsule three times a day). The subjects were again 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.

OBSERVATIONS:

A total of 6 volunteers were enrolled and randomised. Four subjects received single dose of *Terminalia arjuna* 1500mg and 2 subjects received identical placebo for 10 days and all subjects completed the study uneventfully. Detailed demographic characteristics of the two study groups are shown in Table 1.

Table1: Demographic Data

Parameter	Terminalia arjuna	Placebo
Total No.	4	2
Age	33.75±2.50	31.50±0.70
Gender (M)	4	2
Weight (Kg)	66.75±1.70	63.50±0.70
BMI (Kg/m^2)	24.20±1.37	23.55±0.63

Effect of both treatments on vital parameters with Cold Pressor test on Day 1:

Table 2 –Showing effect of *Terminalia arjuna* on Vital parameters before, during and after Cold Pressor test (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	115.0±2.58	75.00±2.58	76.50±1.91	114.5±2.51	75.00±2.0	74.00±1.63
During (30 Sec) CPT	137.0±2.58	87.75±3.50	85.25±2.98	130.8±2.75	83.00±1.15	82.00±2.16
1 min after CPT	117.5±1.91	77.50±4.43	77.50±5.26	117.0±5.03	79.5±3.41	81.5±1.00
10 min after CPT	114.0±3.65	74.50±1.00	76.00±2.82	114.5±2.51	74.50±3.0	77.00±2.58

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 1500 mg single dose of *Terminalia arjuna*, there was an increase in systolic and diastolic blood pressure and pulse rate which were however much lower than that compared to pretreatment values.

Table 3 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test (n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	114.0±0.0	75.0±1.41	76.0±0.0	113.0±1.41	74.0±0.0	76.0±0.0
During (30Sec) CPT	143.0±4.24	89.5±0.70	86.50±0.7	144.0±5.65	88.0±1.41	87.0±1.41
1min after CPT	117.0±1.41	77.0±1.41	78.0±0.0	117.0±1.41	78.0±5.65	80.0±0.0
10 min after CPT	114.0±2.82	75.0±1.41	59.0±21.21	115.0±1.41	75.0±1.41	76.0±2.82

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in systolic blood pressure, diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline on Day 1:

Table 4 -Showing the effect of Cold Pressor test at baseline in *Terminalia arjuna* group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1119.0±79.20	1238.0±0.0
ABI	1.09±0.02	1.12±0.01
Reflection Index (RI%)	71.73±1.17	75.05±1.60
AP(mmHg)	9.50±3.10	13.75±1.70
PP(mmHg)	23.25±2.63	28.50±2.08
AIX (%)	133.8±6.55	146.0±1.82
SEVR (%)	153.0±10.86	149.5±9.88

Table 5 –Showing effect of on Cold Pressor test at baseline in Placebo group (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1138±17.68	1300±35.36
ABI	1.13±0.0	1.09±0.00
Reflection Index (RI%)	73.15±4.03	78.65±1.90
AP (mmHg)	9.50±3.53	11.50±3.53
PP(mmHg)	22.0±1.41	26.0±2.82
AIX (%)	132.5±4.95	143.0±1.41
SEVR (%)	151.5±3.53	148.0±5.65

As seen from the above tables 4 and 5 showing the effect of cold pressor test per se in both treatment groups at baseline. Cold pressor test produces arterial stiffness and the same is evidenced by an increase in baPWV, RI, AP, PP and Aix and also as a decrease in values of SEVR.

Effect of both treatments on Pharmacodynamic parameters during Cold Pressor test on Day 1

Table 6 -Showing effect of *Terminalia arjuna* 1500 mg single dose on pharmacodynamic parameters during Cold Pressor test (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1207±9.92	1150±35.36
ABI	1.13±0.01	1.15±0.03
Reflection Index (RI%)	72.55±1.18	69.80±2.46
AP(mmHg)	6.75±0.95	5.25±1.78
PP(mmHg)	24.25±2.63	21.50±3.41
AIX (%)	124.5±5.68	120.5± 2.08
SEVR (%)	144.3±0.86	161.0±6.68

As observed from the above table, treatment with *Terminalia arjuna* decreased the arterial stiffness produced by cold pressor test. The same is evidenced by a decrease in baPWV, RI, augmentation index and increase in sub-endocardial viability ratio.

Table 7 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1113±17.68	1225±70.71
ABI	1.11±0.03	1.08±0.01
Reflection Index (RI%)	71.50±0.70	79.00±1.41
AP (mmHg)	7.00±0.0	11.50±2.12
PP(mmHg)	22.50±0.70	27.50±3.53
AIX (%)	129.5±2.12	140.0±2.82
SEVR (%)	143.5±0.70	134.5±17.68

As seen from the above table, treatment with placebo did not alter the arterial stiffness produced by cold pressor test and the same is reflected by an increase in baPWV, RI, AP, PP, AIx and decrease in SEVR.

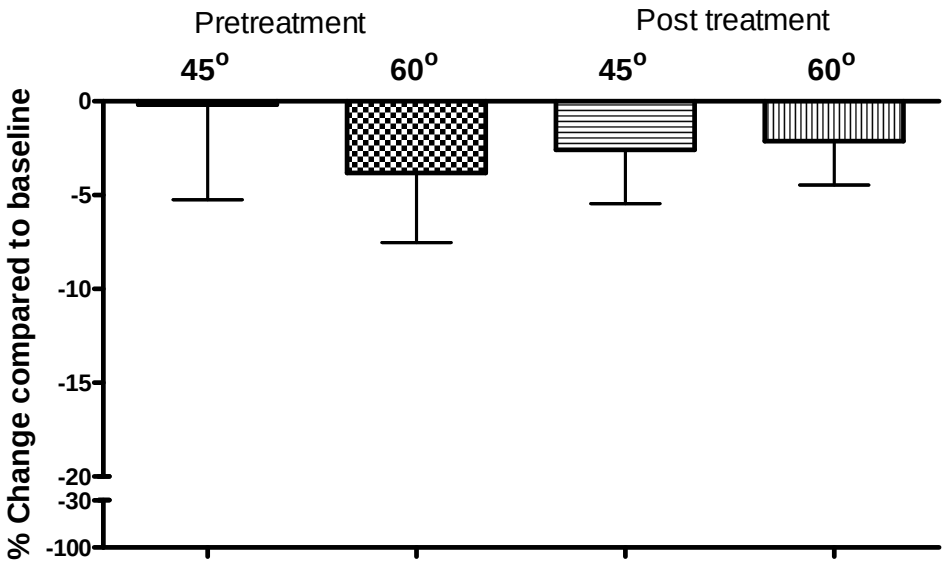
Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters on Day1

Table 8 –Showing effect of Terminalia Arjuna on Tilt Table Test on Day 1 (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	114.5±1.9	74.50±1.9	76.0±2.8	3.55±0.3	115.5±1.91	75.00±1.15	76.50±1.91	3.57±0.20
At 45^o	116.0±4.9	82.00±2.3	81.0±5.0	3.52±0.1	117.5±5.26	80.50±4.43	84.50±2.51	3.47±0.09
At 60^o	116.5±5.0	80.00±2.3	79.5±3.4	3.40±0.0	116.5±5.26	78.00±4.89	79.50±3.00	3.50±0.28
Basal2(0^o)	114.5±2.5	73.50±1.9	74.5±1.9	3.55±0.3	113.5±2.51	73.50±1.91	76.00±1.63	3.72±0.26

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with *Terminalia arjuna* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 60^o a mild increase in cardiac output was observed compared to baseline.

Figure 1 shows the mean percent change in cardiac output on treatment with *Terminalia Arjuna* on Tilt Table test on Day1. All values are compared to baseline.

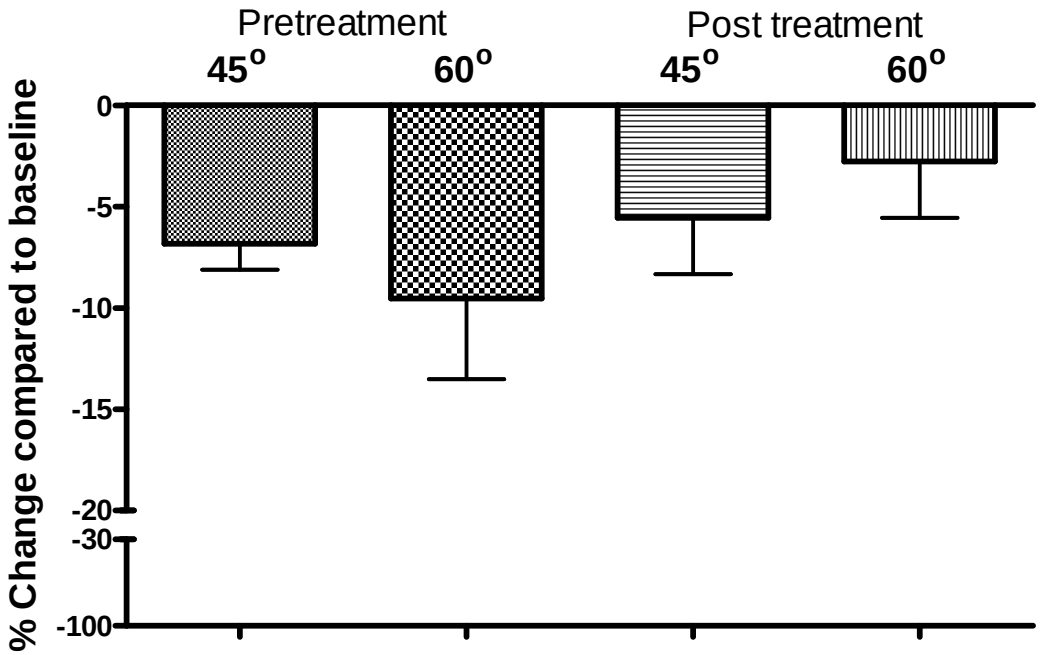


9 -Showing effect of Placebo on Tilt Table Test on Day1 (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	118.0±0.0	74.0±0.0	75.0±1.41	3.6±0.1	115.0±1.41	77.0±1.4	77.0±1.4	3.6±0.0
At 45^o	115.0±7.1	83.0±1.4	85.0±1.41	3.4±0.0	117.0±4.24	84.0±0.0	82.0±0.0	3.4±0.1
At 60^o	111.0±9.9	80.0±2.8	79.0±4.24	3.3±0.1	114.0±2.82	77.0±4.2	77.0±1.4	3.5±0.1
Basal2(0^o)	113.0±1.4	76.0±0.0	73.0±1.41	3.6±0.1	113.0±1.41	75.0±1.4	74.0±0.0	3.7±0.1

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 2 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test on Day1. All values are compared to baseline.



Effect of Treatments on Biomarkers Day1:

Table 10 -Showing effect of *Terminalia arjuna* on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.17±0.11	1.02±0.12
Malondialdehyde (nm/ml)	3.13±0.24	2.95±0.40
Nitric oxide (µMol/L)	32.91±3.09	35.29±3.96

As observed from the above table, treatment with *Terminalia arjuna* showed remarkable changes in HsCRP and malondialdehyde levels on day 1. A mild increase in nitric oxide levels was also observed compared with baseline.

Table 11 -Showing effect of Placebo on biomarkers (n=2)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.31±0.09	1.22±0.0
Malondialdehyde (nm/ml)	3.13±0.12	3.16±0.14
Nitric oxide (µMol/L)	34.42±2.02	34.75±1.88

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline on day 1.

Effect of Treatments on Platelet aggregation test on Day 1:

Table 12 -Showing effect of *Terminalia arjuna* on Inhibition of Platelet Aggregation on Day 1(n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	70.50±2.12	73.38±2.13	Nil

Table 13 -Showing effect of Placebo on Inhibition of Platelet Aggregation on Day 1 (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	67.75±1.76	69.75 ±2.47	Nil

As seen from the above tables, treatment with *Terminalia arjuna* and Placebo did not produce remarkable changes in inhibition of platelet aggregation.

.

Multiple dose study-(Day 11)

Effect of both treatments on vital parameters with Cold Pressor test on Day 11:

Table 14 –Showing effect of *Terminalia arjuna* on Vital parameters before, during and after Cold Pressor test (CPT) on day 11 (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	115.5±1.91	74.5±1.91	75.0±1.15	113.0±3.4	75.5±1.0	74.0±0.0
During (30 Sec) CPT	134.5±3.00	88.7±0.95	85.0±1.15	124.0±4.32	81.5±2.51	80.5±1.3
1 min after CPT	118.0±1.63	80.0±1.63	80.0±2.82	113.0±2.58	77.0±4.76	78.5±1.0
10 min after CPT	113.5±1.91	75.5±1.0	75.0±2.58	112.0±1.63	75.0±1.15	74.0±2.8

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec) compared to baseline. Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 1500 mg single dose of *Terminalia arjuna*, the increase in SBP, DBP and PR produced with CPT was much less than that compared to the values at baseline. This indicates that treatment with *Terminalia arjuna* attenuates the cardiovascular effects of cold pressor test and protects against the arterial stiffness induced by CPT.

Table 15 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test on day 11(n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	116.0±2.82	75.0±1.41	74.0±2.82	115.0±4.24	76.0±0.0	76.0±2.82
During (30Sec) CPT	141.5±3.53	89.5±0.70	86.0±0.0	142.0±2.82	90.0±2.82	83.0±1.41
1min after CPT	117.0±1.41	75.0±1.41	76.0±2.8	117.0±1.41	77.0±4.24	76.0±0.0
10 min after CPT	114.0±0.0	77.0±1.41	73.0±1.41	112.0±2.82	73.0±1.41	73.0±1.41

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in systolic blood pressure, diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline (Pretreatment values) on Day 11:

Table 16 -Showing the effect of Cold Pressor test at baseline in *Terminalia arjuna* group on day 11(n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1113±17.68	1057±26.16
ABI	1.13±0.0	1.14±0.20
Reflection Index (RI%)	70.55±4.47	68.23±5.51
AP(mmHg)	7.50±1.73	5.00±0.81
PP(mmHg)	22.25±0.95	19.50±1.29
AIX (%)	130.8±4.99	121.3±2.5
SEVR (%)	142.8±4.11	151.3±9.5

As seen from the above table, cold pressor test per se did not produce changes in ABI, AP, PP, baPWV, RI and SEVR . The values indicate that the cold pressor induced arterial stiffness was counteracted by treatment with *Terminalia arjuna* and there was decrease in baPWV, RI, AP, PP, AIx and increase in SEVR compared to baseline (Day 1).

Table 17 –Showing effect of on Cold Pressor test at baseline in Placebo group on Day 11(n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1150 ±106.1	1238 ±123.7
ABI	1.13±0.0	1.15±0.0
Reflection Index (RI%)	68.50±0.70	73.00± 1.41
AP (mmHg)	7.50±0.70	12.50±0.70
PP(mmHg)	23.0±1.41	30.00±2.82
AIX (%)	130.5±2.12	140.0±1.41
SEVR (%)	137.5±2.12	133.5±6.36

As seen from the above table, cold pressor test per se produced an increase in baPWV, PP, AIx and decrease in SEVR.

Effect of both treatments on Pharmacodynamic parameters during Cold Pressor test on Day 11

Table 18 -Showing effect of *Terminalia arjuna* 1500 mg multiple dose on pharmacodynamic parameters during Cold Pressor test on Day 11(n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1107 ±61.52	1032± 44.55
ABI	1.13±0.0	1.15±0.0
Reflection Index (RI%)	71.65±1.98	67.9±2.45
AP(mmHg)	7.75±1.70	5.50±1.29
PP(mmHg)	20.25±1.50	18.25±1.25
AIX (%)	127.5±8.22	119.5±7.55
SEVR (%)	133.3±10.69	146.3±4.03

As observed from the above table, treatment with *Terminalia arjuna* produced a remarkable decrease in baPWV, RI, AP, PP, AIx and an increase in sub-endocardial viability ratio. This indicates that multiple dose administration of Terminalia arjuna protects against the cardiovascular effects induced by cold pressor test.

Table 19 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor on Day 11(n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1150 ± 35.36	1213± 88.39
ABI	1.19 ±0.00	1.14± 0.01
Reflection Index (RI%)	70.8±2.54	74.95±0.91
AP (mmHg)	8.50±0.70	12.50±0.70
PP(mmHg)	20.50±2.12	28.50±0.70
AIX (%)	131.0±4.24	145.5±4.95
SEVR (%)	150.0±2.82	147.5±0.70

As seen from the above table, placebo did not produce any remarkable changes in the pharmacodynamic parameters recorded during cold pressor test.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters on Day11

Table 20 –Showing effect of *Terminalia arjuna* on Tilt Table Test on Day 11 (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	114.5±2.51	74.0±1.63	76.0±1.63	3.6±0.24	116.5±3.0	75.5±1.0	74.0±1.63	3.8±0.12
At 45^o	118.0±5.65	82.0±1.63	84.5±2.51	3.6±0.14	119.0±3.83	83.5±3.0	82.5±3.41	3.7±0.12
At 60^o	113.0±6.63	80.5±2.51	82.5±3.0	3.5±0.17	115.5±5.26	78.0±4.2	80.0±4.89	3.6±0.21
Basal2(0^o)	114.5±1.91	74.0±1.63	75.5±1.91	3.7±0.25	115.0±1.15	72.5±1.0	74.0±1.63	3.9±0.18

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with *Terminalia arjuna* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^o and 60^o a mild increase in cardiac output was observed compared to baseline.

Figure 3 shows the mean percent change in cardiac output on treatment with *Terminalia arjuna* on Tilt Table test. All values are compared to baseline.

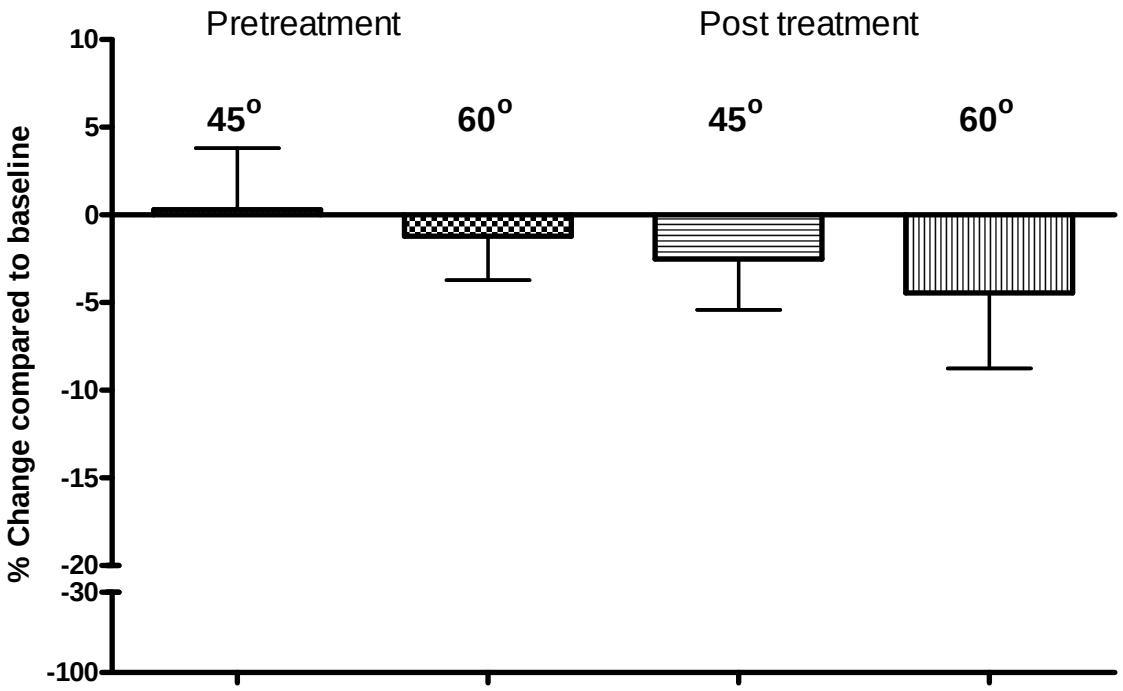
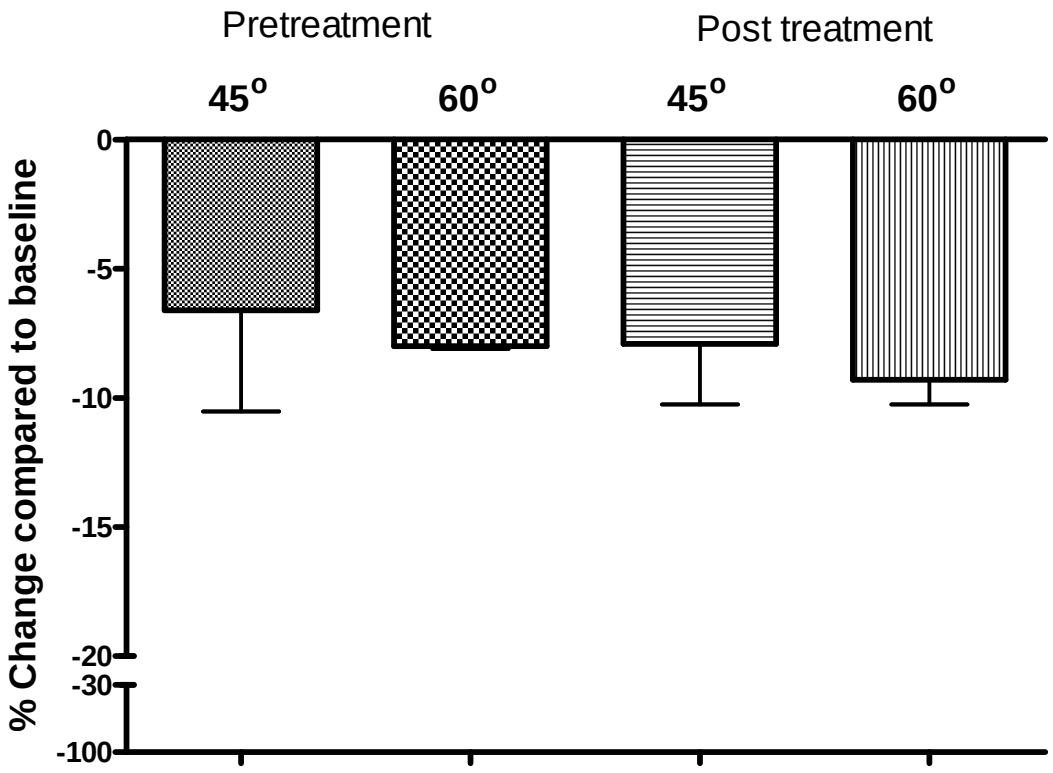


Table 21 -Showing effect of Placebo on Tilt Table Test on Day 11 (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	114.0±2.82	75.0±1.41	78.0±0.0	3.75±0.07	116.0±0.0	74.0±2.8	77.0±1.4	3.75±0.21
At 45^o	119.0±1.41	83.0±1.41	84.5±3.53	3.50±0.14	116.0±2.82	82.0±0.0	84.0±0.0	3.45±0.07
At 60^o	117.0±1.41	80.0±2.82	78.0±5.65	3.45±0.07	116.0±0.0	81.0±4.2	78.0±5.6	3.40±0.14
Basal2(0^o)	113.0±1.41	75.0±4.24	76.0±0.0	3.75±0.21	113.0±1.41	74.0±0.0	74.0±2.8	3.80±0.14

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 4 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers on Day 11:

Table 22 -Showing effect of *Terminalia arjuna* on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.07±0.10	0.82±0.06
Malondialdehyde (nm/ml)	3.11±0.25	2.45±0.41
Nitric oxide (µMol/L)	34.17±3.51	40.11±2.76

As observed from the above table, treatment with *Terminalia arjuna* produced a decrease in highly sensitive C-reactive protein, malondialdehyde and increase in nitric oxide levels compared to baseline.

Table 23 -Showing effect of Placebo on biomarkers (n=2)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.30± 0.09	1.32± 0.10
Malondialdehyde (nm/ml)	3.21± 0.09	3.27± 0.16
Nitric oxide (µMol/L)	34.51± 2.11	34.49± 1.56

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Effect of Treatments on Platelet aggregation test on Day 11:

Table 24-Showing effect of *Terminalia arjuna* on Inhibition of Platelet Aggregation on Day 11 (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	69.25±2.90	58.0±3.24	16.28±1.60

As seen from the above table, *Terminalia arjuna* produced slight changes in inhibition of platelet aggregation.

Table 25 -Showing effect of Placebo on Inhibition of Platelet Aggregation on Day 11 (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	70.25±3.18	69.75±7.42	1.80±2.54

As seen from the above table, Placebo did not produce changes in inhibition of platelet aggregation.

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.

Inference:

Treatment with *Terminalia arjuna* produced remarkable changes in AIx, AP, PP and ABI on cold pressor test. The active treatment also showed mild decrease in pharmacodynamic parameter such as baPWV and reflection index on cold pressor test. Ten days of treatment with *Terminalia arjuna* showed mild increase in nitric oxide levels and reduction in malondialdehyde and hsCRP levels compared with baseline. Multiple dose (10 days) treatment with *Terminalia arjuna* produced slight increase in cardiac output compared to baseline (Day1). On day 1 (baseline) there was no change in platelet aggregation test. However, treatment with *Terminalia arjuna* produced remarkable change in inhibition of platelet aggregation on day11. All subjects tolerated both the treatments and procedures well. No subject 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 compared to baseline.

Glossary:

TA=*Terminalia arjuna*

SBP=Systolic Blood Pressure (mmHg)

DBP=Diastolic Blood Pressure (mmHg)

PR=Pulse Rate (bpm)

baPWV= Brachial-Ankle Pulse Wave Velocity (cm/s) (Using ABI Colins, Komaki, Japan**)**

ABI= Ankle-Brachial Index (Using ABI Colins, Komaki, Japan**)**

RI =Reflection Index (%) (Using Micromedical Pulse Tracer Gallingham, Kent, UK)

AP=Aortic Augmentation Pressure (mmHg) (Using Sphygmocor® Atcor Medical Pvt Ltd Sydney, Australia)

PP=Pulse Pressure (mmHg) (Using Sphygmocor®)

AIX=Aortic Augmentation Index (%) (Using Sphygmocor®)

SEVR=Sub Endocardial Viability Ratio (%) (Using Sphygmocor®)

BMI = Body Mass Index (Kg/m^2)

CPT= Cold Pressor Test

CO= Cardiac Output (L /min)(L&T Nivomon Monitor)

AYUSH STUDY REPORT 2013

Evaluation of Cardiovascular and Pharmacodynamic effects of *Tinospora cordifolia* TC-1 (Aqueous extract) with special reference to arterial stiffness and endothelial function in healthy human male subjects

Study VI: 1000mg (TC-1) Single Dose

Clinical trial site

Department of Clinical Pharmacology and Therapeutics
Nizam's Institute of Medical Sciences
Punjagutta, Hyderabad, Andhra Pradesh, India.

Principal - Investigator

Dr.P.Usha Rani

Co-Investigator

Dr. M.U.R.Naidu
Dr. I.V.Sravanti
(Ayurvedic Physician)

Trial Co-ordinators

Nishat Fatima
N.Muralidhar

Study Nurse

Rita Salomi

Sponsor

Central Council for Research in Ayurveda & Siddha
Department of AYUSH
Ministry of Health and Family Welfare
Government of India

1000mg Single dose study of aqueous extract of *Tinospora cordifolia*(TC-1)

Title: Evaluation of Cardiovascular pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Sub title: Evaluation of Cardiovascular pharmacodynamic effects of 1000mg single dose of *Tinospora cordifolia*(TC-1) with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

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. In racemes or racemose panicles, the male flowers are clustered and female are. The flowering season expands over summers and winters. A variety of active components solitary derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities.

Therefore the present study was undertaken to evaluate the effect of *Tinospora cordifolia* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Methodology:

The study was a randomized double blind single 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. Six subjects were randomized to the two treatments. Two subjects received placebo while 4 subjects received active medication of aqueous extract of *Tinospora cordifolia* (TC-1) as per prior randomization schedule.

Single dose study

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. After an overnight fast, baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling were performed using non-invasive methods. The various cardiac profiling parameters were assessed using the following instruments - Brachial-Ankle Pulse Wave Velocity (cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI%) (Micromedical Pulse Tracer Gallingham, Kent, UK), Aortic Augmentation Pressure (mmHg), Pulse Pressure (mmHg), Aortic Augmentation Index (%) and Sub Endocardial Viability Ratio (%)(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, Body Surface Area (m^2), 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. However the Cardiac Output is more significant among the other parameters recorded with Nivomon monitor.

Pretreatment blood samples were drawn for estimation of biomarkers of endothelial function and platelet aggregation test. The study medication was then administered as per the randomization schedule (either four capsules of aqueous extract of *Tinospora cordifolia* 250mg (TC-1) or four capsules of Placebo) with 240 ml of water.

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.

The subject's vital parameters were recorded before and at hourly intervals upto 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. The safety lab reports were evaluated once they were available.

OBSERVATIONS:

A total of 7 volunteers were enrolled and 6 were randomised. One subject was excluded due to high SGOT levels. Four subjects received single dose of *Tinospora cordifolia* 1000mg (TC-1) and 2 subjects received identical placebo and all subjects completed the study uneventfully. Detailed demographic characteristics of the two study groups are shown in Table 1.

Table1: Demographic Data

Parameter	***Tinospora cordifolia***	**Placebo**
Total No.	4	2
Age	31.50±4.79	34.0±5.65
Gender (M)	4	2
Weight (Kg)	61.50± 5.38	62.10 ±3.25
BMI (Kg/m^2)	22.84±0.78	23.08±0.81

Effect of both treatments on vital parameters with Cold Pressor test:

Table 2 –Showing effect of *Tinospora cordifolia* (TC-1) on Vital parameters before, during and after Cold Pressor test (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	113.0±1.15	75.5±1.0	74.0±1.6	112.5±1.0	75.5±1.9	75.0±1.1
During (30 Sec) CPT	141.0±1.82	89.7±4.0	86.0±2.0	136.0±2.2	86.0±0.8	84.5±1.3
1 min after CPT	115.0±2.58	77.7±3.5	80.0±1.6	115.0±2.0	77.0±3.4	79.5±3.8
10 min after CPT	113.0±1.15	74.5±1.0	74.5±1.9	112.5±2.5	75.5±1.9	73.5±1.9

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 1000 mg single dose of *Tinospora cordifolia*, there was an increase in systolic and diastolic blood pressure and pulse rate which were however much lower than that compared to pretreatment values.

Table 3 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test (n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	114.0±2.82	75.0±1.4	74.0±2.82	112.0±2.8	74.0±2.8	74.0±0.0
During (30Sec) CPT	142.0±1.41	89.5±2.1	87.0±1.41	140.5±2.1	91.0±4.2	87.5±0.7
1min after CPT	115.0±4.24	76.0±2.8	78.0±5.65	116.0±2.8	79.0±1.4	82.0±0.0
10 min after CPT	114.0±2.82	75.0±4.2	74.50±2.1	112.0±0.0	74.0±0.0	74.0±2.8

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline:

Table 4 -Showing the effect of Cold Pressor test at baseline in *Tinospora cordifolia* (TC-1) group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1150±0.0	1244±8.48
ABI	1.14±0.0	1.10±0.0
Reflection Index (RI%)	71.5±2.13	77.23±4.70
AP(mmHg)	8.0±1.41	11.75±1.70
PP(mmHg)	23.5±3.10	30.0±2.58
AIX (%)	131.5±5.50	145.3±5.56
SEVR (%)	139.8±10.11	128.5±3.78

Table 5 –Showing effect of on Cold Pressor test at baseline in Placebo group (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1188±53.03	1300±0.00
ABI	1.10±0.01	1.11±0.01
Reflection Index (RI%)	65.65±2.33	70.65±2.33
AP (mmHg)	8.50±0.70	12.0±1.41
PP(mmHg)	24.5±0.70	32.0±1.41
AIX (%)	128.5±3.53	145.0± 4.24
SEVR (%)	145.5±9.192	140.0±12.73

As seen from the above tables 4 and 5 showing the effect of cold pressor test per se in both treatment groups at baseline. Cold pressor test produces arterial stiffness and the same is evidenced by an increase in baPWV, RI, AP, PP and AIx and also as a decrease in values of SEVR.

Effect of Both Treatments on Pharmacodynamic parameters during Cold Pressor test

Table 6 -Showing effect of *Tinospora cordifolia* 1000 mg (TC-1) single dose on pharmacodynamic parameters during Cold Pressor test (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1207±44.55	1169±26.87
ABI	1.15 ±1.01	1.20 ±0.08
Reflection Index (RI%)	68.38±3.19	65.73±2.48
AP(mmHg)	8.5±1.29	6.75 ±1.70
PP(mmHg)	22.5±0.57	18.50 ± 1.29
AIX (%)	129.3±6.94	125.3±8.18
SEVR (%)	143.0±6.0	148.80±6.70

As observed from the above table, treatment with *Tinospora cordifolia* decreased the arterial stiffness produced by cold pressor test. The same is evidenced by a decrease in baPWV, RI, augmentation index and increase in sub-endocardial viability ratio.

Table 7 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1213±53.03	1250±35.36
ABI	1.13±0.07	1.11±0.04
Reflection Index (RI%)	67.30±3.81	73.65±0.91
AP (mmHg)	10.5±2.12	14.00±1.41
PP(mmHg)	24.5±2.12	32.50±2.12
AIX (%)	136.5±0.70	146.5±0.70
SEVR (%)	137.5±16.26	128.5±6.36

As seen from the above table, treatment with placebo did not alter the arterial stiffness produced by cold pressor test and the same is reflected by an increase in baPWV, RI, AP, PP, AIx and decrease in SEVR.

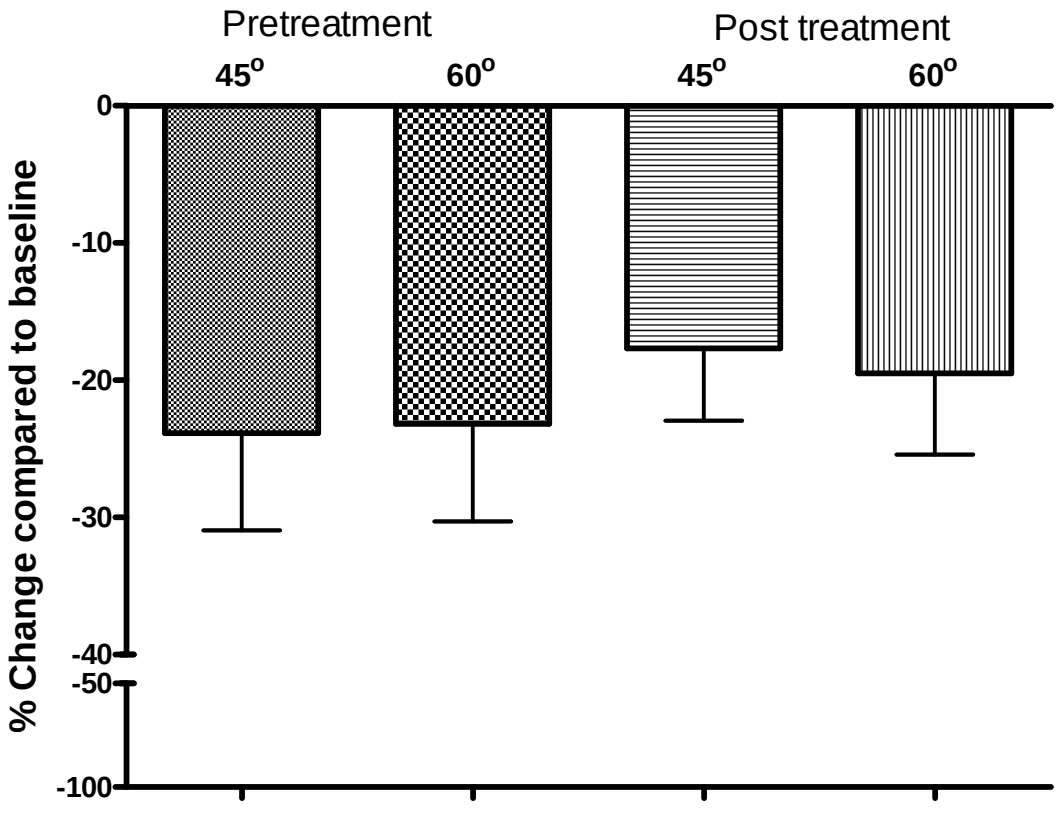
Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters

Table 8 –Showing effect of *Tinospora cordifolia* (TC-1) on Tilt Table Test (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	112.5±1.0	75.5±1.0	74.5±2.5	4.35±0.4	112.5±2.5	76.5±1.9	73.5±1.0	4.2±0.1
At 45^o	117.0±4.8	83.0±2.0	84.5±1.9	3.27±0.4	115.0±3.8	84.0±0.0	82.5±1.0	3.4±0.3
At 60^o	110.5±3.4	79.0±4.2	82.5±2.5	3.30±0.3	114.5±1.9	80.5±1.9	81.5±1.9	3.3±0.4
Basal2(0^o)	113.0±2.0	74.0±1.6	74.0±1.6	4.25±0.4	114.0±1.6	74.5±1.0	74.0±1.6	4.2±0.2

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with *Tinospora cordifolia* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^o a mild increase in cardiac output was observed compared to baseline.

Figure 1 shows the mean percent change in cardiac output on treatment with *Tinospora cordifolia* (TC-1) on Tilt Table test. All values are compared to baseline.

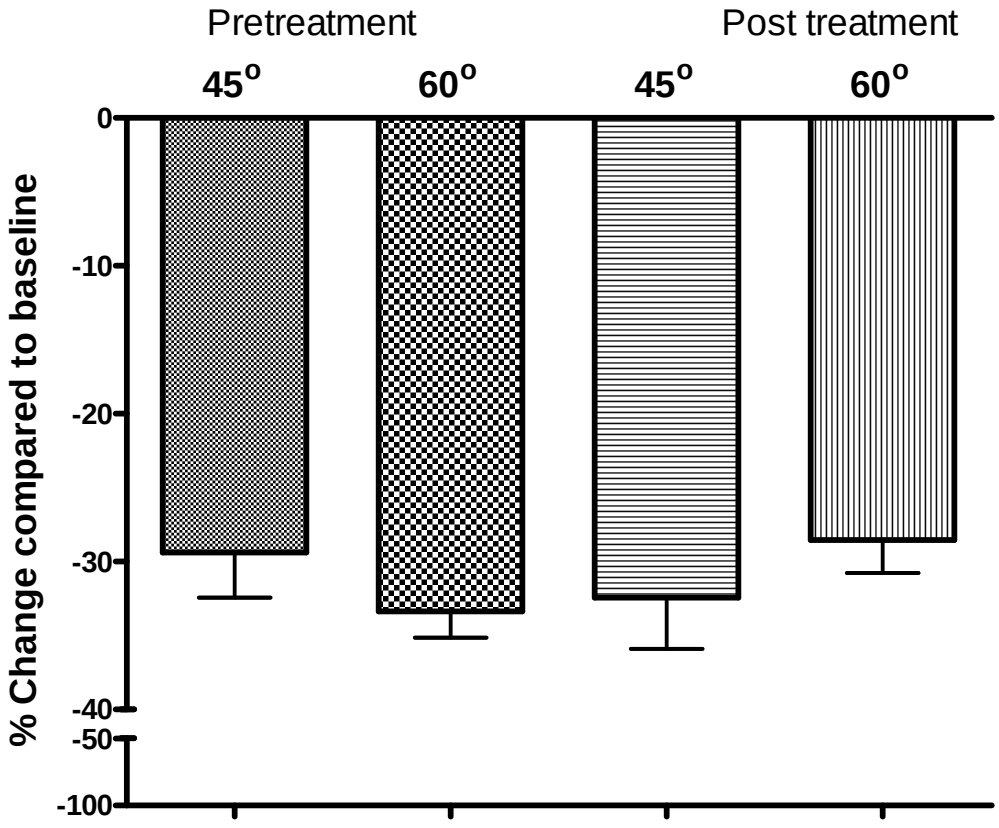


9 -Showing effect of Placebo on Tilt Table Test (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	111.0±1.4	74.0±2.8	74.0±0.0	3.75±0.07	112.0±0.0	75.0±1.41	73.0±1.41	3.85±0.07
At 45^o	111.0±1.4	82.0±0.0	84.0±0.0	2.65±0.21	117.0±1.4	83.0±1.41	83.0±1.41	2.60±0.14
At 60^o	114.0±0.0	82.0±2.8	84.0±2.8	2.50±0.14	112.0±2.8	84.0±0.0	84.0±2.82	2.75±0.07
Basal2(0^o)	112.0±0.0	75.0±1.4	73.0±1.4	3.85±0.21	113.0±1.4	75.0±1.41	76.0±2.82	3.90±0.0

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 2 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers:

Table 10 -Showing effect of *Tinospora cordifolia* on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.07±0.15	1.02±0.10
Malondialdehyde (nm/ml)	3.06±0.17	2.96±0.21
Nitric oxide (µMol/L)	33.84±1.57	35.29±2.13

As observed from the above table, treatment with *Tinospora cordifolia* produced a mild decrease in highly sensitive C-reactive protein, malondialdehyde levels and increase in nitric oxide levels compared to baseline.

Table 11 -Showing effect of Placebo on biomarkers (n=2)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.18±0.06	1.17±0.11
Malondialdehyde (nm/ml)	3.10±0.12	3.07±0.02
Nitric oxide (µMol/L)	29.89±0.38	31.47±1.07

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Effect of Treatments on Platelet aggregation test:

Table 12 -Showing effect of *Tinospora cordifolia* on Inhibition of Platelet Aggregation (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	64.75±2.53	62.88±5.55	4.24±3.19

As seen from the above table, *Tinospora cordifolia* produced changes in inhibition of platelet aggregation.

Table 13 -Showing effect of Placebo on Inhibition of Platelet Aggregation (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	55.75±1.76	58.25±0.35	Nil

As seen from the above table, Placebo treatment did not produce changes in platelet aggregation test performed.

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 treatments.

Inference:

Treatment with aqueous extract of *Tinospora cordifolia* (TC-1) produced decrease in AIx, AP, PP and ABI on cold pressor test. The active treatment also showed mild reduction in pharmacodynamic parameters such as baPWV and reflection index on cold pressor test. A slight increase in cardiac output was observed at 45^{o} on tilt table test compared to baseline in *Tinospora cordifolia* group. Treatment with Tinospora cordifolia showed mild increase in nitric oxide and reduction in malondialdehyde and hsCRP levels compared with baseline. Treatment with 1000 mg of *Tinospora cordifolia* produced inhibition of platelet aggregation. It can be concluded from the present study that 1000 mg of aqueous extract of *Tinospora cordifolia* was found to be safe and well tolerated without any adverse event. There was no significant change in any of the pharmacodynamic parameters tested with single dose of aqueous extract of *Tinospora cordifolia* 1000 mg (TC-1). Studies are needed to be conducted to assess the tolerability and also with multiple dose of the test product for safety and efficacy.

Glossary:

TC-1= Aquoeus extract of *Tinospora cordifolia*

SBP=Systolic Blood Pressure (mmHg)

DBP=Diastolic Blood Pressure (mmHg)

PR=Pulse Rate (bpm)

baPWV= Brachial-Ankle Pulse Wave Velocity (cm/s) (Using ABI Colins, Komaki, Japan**)**

ABI= Ankle-Brachial Index (Using ABI Colins, Komaki, Japan**)**

RI =Reflection Index (%) (Using Micromedical Pulse Tracer Gallingham, Kent, UK)

AP=Aortic Augmentation Pressure (mmHg) (Using Sphygmocor® Atcor Medical Pvt Ltd Sydney, Australia)

PP=Pulse Pressure (mmHg) (Using Sphygmocor®)

AIX=Aortic Augmentation Index (%) (Using Sphygmocor®)

SEVR=Sub Endocardial Viability Ratio (%) (Using Sphygmocor®)

BMI = Body Mass Index (Kg/m^2)

CPT= Cold Pressor Test

CO= Cardiac Output (L /min)(L&T Nivomon Monitor)

AYUSH STUDY REPORT 2013

Evaluation of Cardiovascular and Pharmacodynamic effects of *Tinospora cordifolia* TC-1 (Aqueous extract) with special reference to arterial stiffness and endothelial function in healthy human male subjects

Study VII: 1000mg (TC-1) Multiple Dose

Clinical trial site

Department of Clinical Pharmacology and Therapeutics
Nizam's Institute of Medical Sciences
Punjagutta, Hyderabad, Andhra Pradesh, India.

Principal - Investigator

Dr.P.Usha Rani

Co-Investigator
Dr. M.U.R.Naidu
Dr. I.V.Sravanti
(Ayurvedic Physician)
Trial Co-ordinators
Nishat Fatima
N.Muralidhar
Study Nurse
Rita Salomi

Sponsor

Central Council for Research in Ayurveda & Siddha
Department of AYUSH
Ministry of Health and Family Welfare
Government of India

1000mg multiple dose study of aqueous extract of *Tinospora cordifolia*(TC-1)

Title: Evaluation of Cardiovascular pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Sub title: Evaluation of Cardiovascular pharmacodynamic effects of 1000mg multiple dose of *Tinospora cordifolia*(TC-1) with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

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. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities.

Therefore the present study was undertaken to evaluate the effect of *Tinospora cordifolia* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Methodology:

The study was a randomized double blind single 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. Six subjects were randomized to the two treatments. Two subjects received placebo while 4 subjects received active medication of aqueous extract of *Tinospora cordifolia* (TC-1) as per prior randomization schedule.

Single dose study

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. After an overnight fast, baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling were performed using non-invasive methods. The various cardiac profiling parameters were assessed using the following instruments - Brachial-Ankle Pulse Wave Velocity (cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI%) (Micromedical Pulse Tracer Gallingham, Kent, UK),

Aortic Augmentation Pressure (mmHg), Pulse Pressure (mmHg), Aortic Augmentation Index (%) and Sub Endocardial Viability Ratio (%)(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, Body Surface Area (m^2), 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. However the Cardiac Output is more significant among the other parameters recorded with Nivomon monitor.

Pretreatment blood samples were drawn for estimation of biomarkers of endothelial function and platelet aggregation test. The study medication was then administered as per the randomization schedule (either four capsules of aqueous extract of *Tinospora cordifolia* 250mg (TC-1) or four capsules of Placebo) with 240 ml of water.

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.

The subject's vital parameters were recorded before and at hourly intervals upto 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. The safety lab reports were evaluated once they were available.

Then the subjects were asked to continue the study medication for next 10 days(2 capsules two times a day). 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 day11.

OBSERVATIONS:

A total of 7 volunteers were enrolled and 6 were randomised. One subject was excluded due to high total bilirubin and blood urea levels. Four subjects received single dose of *Tinospora cordifolia* 1000mg (TC-1) and 2 subjects received identical placebo and all subjects completed the study uneventfully. Detailed demographic characteristics of the two study groups are shown in Table 1.

Table1: Demographic Data

Parameter	*Tinospora cordifolia*	Placebo
Total No.	4	2
Age	30.00±3.16	33.50±2.12
Gender (M)	4	2
Weight (Kg)	65.18±1.77	61.05±2.75
BMI (Kg/m^2)	23.82±0.74	22.49±0.01

Effect of both treatments on vital parameters with Cold Pressor test on Day1:

Table 2 –Showing effect of *Tinospora cordifolia* (TC-1) on Vital parameters before, during and after Cold Pressor test (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	111.5±1.91	75.5±1.00	75.50±1.9	111.5±1.00	75.5±1.0	74.0±0.0
During (30 Sec) CPT	142.3±1.70	89.5±1.29	87.25±1.9	139.5±1.29	87.5±1.29	85.5±1.29
1 min after CPT	115.0±2.58	81.0±3.46	80.75±1.5	114.0±1.63	78.5±4.43	81.0±2.0
10 min after CPT	112.5±1.00	74.0±1.63	76.5±2.51	113.0±2.58	74.5±1.00	73.5±3.0

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 1000 mg single dose of *Tinospora cordifolia*, there was an increase in systolic and diastolic blood pressure and pulse rate which were however much lower than that compared to pretreatment values.

Table 3 -Showing effect of Placebo on vital parameters before, during and after Cold Pressor test (n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	113.0±1.41	75.0±1.41	75.0±1.41	113.0±1.4	74.0±2.82	78.0±0.0
During (30Sec) CPT	143.5±3.53	92.0±2.82	88.5±0.70	142.5±0.7	91.5±0.7	87.0±1.41
1min after CPT	115.0±1.41	75.0±1.41	78.0±0.0	115.0±4.2	78.0±0.0	78.0±5.65
10 min after CPT	112.0±0.0	78.0±0.0	73.0±1.41	114.0±2.8	75.0±1.41	73.0±1.41

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment

with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline on Day1 :

Table 4 -Showing the effect of Cold Pressor test at baseline in *Tinospora cordifolia* (TC-1) group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1175±35.36	1257±26.16
ABI	1.13±0.01	1.11±0.05
Reflection Index (RI%)	71.38±33.10	75.25±4.03
AP(mmHg)	8.25±1.25	11.25±1.70
PP(mmHg)	23.75±3.59	30.25±2.98
AIX (%)	130.0±4.89	141.3±4.78
SEVR (%)	133.3±6.50	135.5±4.72

Table 5 –Showing effect of on Cold Pressor test at baseline in Placebo group (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1200±35.36	1263±17.68
ABI	1.13±0.04	1.10±0.02
Reflection Index (RI%)	72.95±0.91	78.80±0.70
AP (mmHg)	7.50±2.12	12.50±0.70
PP(mmHg)	22.50±0.70	30.50±0.70
AIX (%)	131.5±10.61	140.0±2.82
SEVR (%)	131.0±4.24	127.0±5.67

As seen from the above tables 4 and 5 showing the effect of cold pressor test per se in both treatment groups at baseline. Cold pressor test produces arterial stiffness and the same is evidenced by an increase in baPWV, RI, AP, PP and AIx and also as a decrease in values of SEVR.

Effect of both treatments on Pharmacodynamic parameters during Cold Pressor test on Day1:

Table 6 -Showing effect of *Tinospora cordifolia* 1000 mg (TC-1) single dose on pharmacodynamic parameters during Cold Pressor test (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1175±0.0	1138±53.03
ABI	1.11±0.01	1.08±0.0
Reflection Index (RI%)	70.75±1.70	68.73±1.50
AP(mmHg)	7.75±0.95	6.50±1.29
PP(mmHg)	21.0±3.55	18.25±2.87
AIX (%)	124.8±2.21	120.5±1.29
SEVR (%)	132.3±9.53	136.8±9.97

As observed from the above table, treatment with *Tinospora cordifolia* decreased the arterial stiffness produced by cold pressor test. The same is evidenced by a decrease in baPWV, RI, augmentation index and increase in sub-endocardial viability ratio.

Table 7 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1188±17.68	1250±35.36
ABI	1.15±0.02	1.16±0.06
Reflection Index (RI%)	71.50±2.12	79.45±3.04
AP (mmHg)	9.50±0.70	13.50±0.70
PP(mmHg)	22.50±0.70	35.00±2.82
AIX (%)	127.5±2.12	138.5±0.70
SEVR (%)	137.5±2.12	146.5±3.53

As seen from the above table, treatment with placebo did not alter the arterial stiffness produced by cold pressor test and the same is reflected by an increase in baPWV, RI, AP, PP, AIx and decrease in SEVR.

Table 8 –Showing effect of *Tinospora cordifolia* (TC-1) on Tilt Table Test (n=4) on Day1

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	114.0±1.6	76.0±2.3	74.5±1.0	4.32±0.41	113.0±1.1	76.5±1.0	75.0±1.1	4.27±0.41
At 45^o	115.5±5.2	83.5±1.0	82.5±1.0	3.67±0.35	117.5±1.9	82.0±0.0	83.2±0.5	3.27±0.26
At 60^o	113.0±2.6	81.0±1.1	80.5±2.4	3.47±0.23	113.5±5.5	78.0±4.9	79.5±3.8	3.50±0.34
Basal2(0^o)	112.0±1.6	75.5±1.0	73.5±1.9	4.35±0.44	112.5±1.0	74.0±1.6	74.0±2.3	4.22±0.41

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with *Tinospora cordifolia* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 60^o a mild increase in cardiac output was observed compared to baseline.

Figure 1 shows the mean percent change in cardiac output on treatment with *Tinospora cordifolia* (TC-1) on Tilt Table test. All values are compared to baseline.

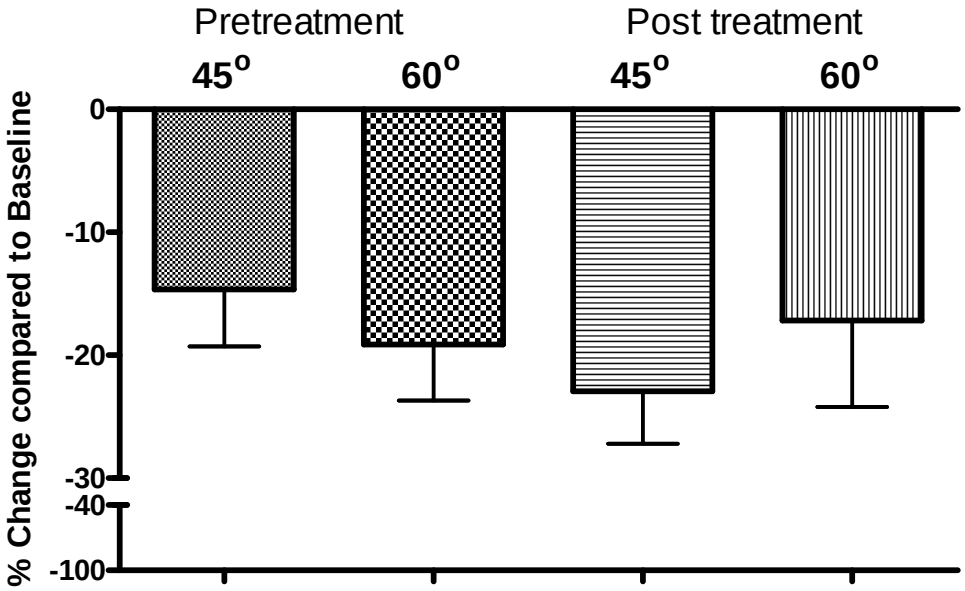
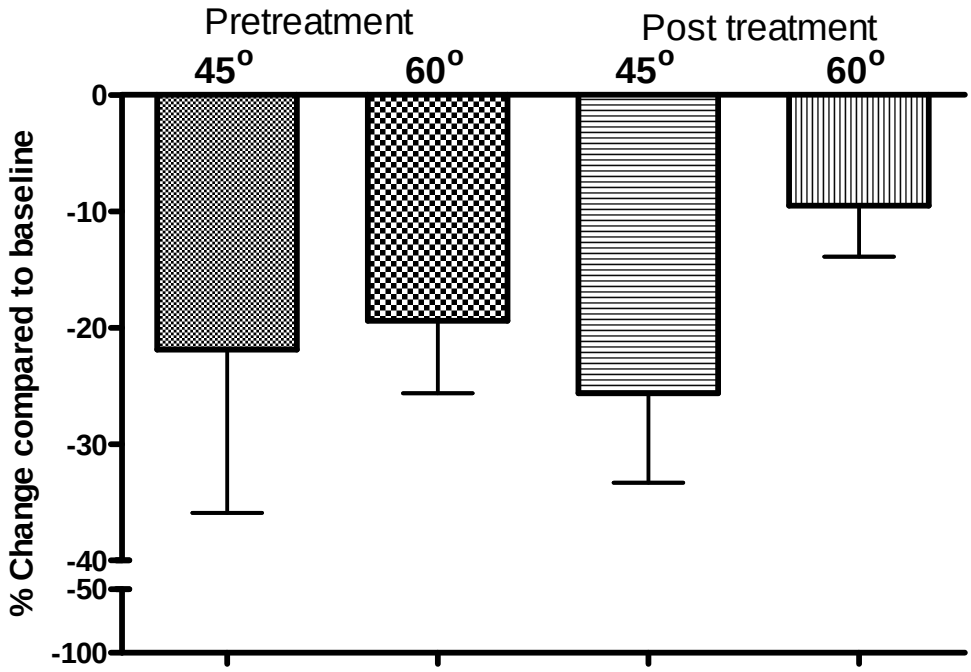


Table 9 -Showing effect of Placebo on Tilt Table Test (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	113.0±1.4	76.0±2.8	73.0±1.4	3.8±0.1	113.0±1.4	75.0±1.4	76.0±2.8	3.75±0.2
At 45^o	120.0±0.0	82.0±0.0	83.5±0.7	3.0±0.7	122.0±0.0	83.0±1.4	82.5±0.7	2.80±0.6
At 60^o	116.0±2.8	83.0±1.4	84.0±0.3	3.1±0.3	117.0±1.4	81.0±1.4	80.0±0.0	3.40±0.4
Basal2(0^o)	113.0±.23	75.0±1.4	76.0±2.8	3.7±0.1	115.0±1.4	72.0±0.0	73.0±1.4	3.85±0.1

As observed from the above table, Tilt table test did not produce changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 2 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers on Day1:

Table 10 -Showing effect of *Tinospora cordifolia* on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.12±0.052	1.05±0.055
Malondialdehyde (nm/ml)	3.15±0.25	2.82±0.37
Nitric oxide (µMol/L)	33.38±2.28	35.02±2.52

As observed from the above table, treatment with *Tinospora cordifolia* produced a mild decrease in highly sensitive C-reactive protein, malondialdehyde levels and increase in nitric oxide levels compared to baseline.

Table 11 -Showing effect of Placebo on biomarkers (n=2)

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.08±0.29	1.09±0.33
Malondialdehyde (nm/ml)	3.52±0.14	3.69±0.16
Nitric oxide (µMol/L)	29.47±2.04	29.87±1.61

Effect of Treatments on Platelet aggregation test on Day1:

Table 12 -Showing effect of *Tinospora cordifolia* on Inhibition of Platelet Aggregation on day 1(n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	64.13±8.10	61.25±7.14	3.98±1.87

As seen from the above table, *Tinospora cordifolia* produced change in inhibition of platelet aggregation.

Table 13 -Showing effect of Placebo on Inhibition of Platelet Aggregation day1 (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	61.50±0.70	63.50±0.0	Nil

As seen from the above table, Placebo treatment did not produce changes in platelet aggregation test performed.

Multiple dose study-(Day 11)

Effect of both treatments on vital parameters with Cold Pressor test on Day 11:

Table 14 –Showing effect of *Tinospora cordifolia* on Vital parameters before, during and after Cold Pressor test on day 11 (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	113.5±3.4	75.5±1.0	76.0±2.3	112.5±2.5	75.5±1.0	75.0±2.0
During (30 Sec) CPT	139.8±1.7	90.0±3.2	88.2±1.7	136.3±2.6	87.2±0.9	85.2±0.9
1 min after CPT	114.5±3.0	79.5±3.0	79.5±1.0	114.0±1.6	77.0±2.0	76.5±3.0
10 min after CPT	113.5±1.9	74.5±1.0	75.0±1.1	113.0±2.6	72.7±1.5	74.0±2.3

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be

within normal limits. At 3hrs post treatment with 1000 mg single dose of *Tinospora cordifolia*, the increase in SBP, DBP and PR produced with CPT was much less than that compared to the values at baseline. This indicates that treatment with aqueous extract of *Tinospora cordifolia* attenuates the cardiovascular effects of cold pressor test and protects against the arterial stiffness induced by CPT.

Table 15 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test on Day 11(n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	112.0±0.0	77.0±1.4	77.0±1.4	114.0±2.8	76.0±2.8	73.0±1.4
During (30Sec) CPT	141.5±0.7	88.5±0.7	86.5±0.7	141.0±1.4	90.0±1.4	87.5±0.7
1min after CPT	112.0±2.8	77.0±4.2	79.0±4.2	115.0±4.2	76.0±0.0	83.0±1.4
10 min after CPT	115.0±1.4	73.5±0.7	73.0±1.4	112.0±2.8	74.0±2.8	76.0±2.8

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in systolic blood pressure, diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline (Pretreatment values) on Day 11:

Table 16 -Showing the effect of Cold Pressor test at baseline in *Tinospora cordifolia* group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1157±9.19	1132±26.16
ABI	1.12±0.01	1.11±0.03
Reflection Index (RI%)	71.20±1.47	69.15±1.88
AP(mmHg)	8.0±1.82	7.50±1.29
PP(mmHg)	21.25±2.36	20.25±0.95
AIX (%)	130.0±7.61	128.5±8.34
SEVR (%)	140.3±2.75	142.3±10.18

As seen from the above table, cold pressor test per se did not produce changes in ABI, AP, PP, baPWV, RI and SEVR . The values indicate that the cold pressor induced arterial stiffness was counteracted by treatment with *Tinospora cordifolia* and there was decrease in baPWV, RI, AP, PP, AIx and increase in SEVR compared to baseline (Day1).

Table 17 –Showing effect of on Cold Pressor test at baseline in Placebo group on Day11(n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1200±35.36	1263 ±17.68
ABI	1.14±0.02	1.13±0.02
Reflection Index (RI%)	72.15±0.21	80.80±0.70
AP (mmHg)	7.50±0.70	13.50±0.70
PP(mmHg)	23.0±1.41	31.50±0.70
AIX (%)	133.5±0.70	142.0±1.41
SEVR (%)	144.0±2.82	133.0±12.73

As seen from the above table, cold pressor test per se produced an increase in baPWV, PP, AIx and decrease in SEVR.

Effect of both treatments on Pharmacodynamic parameters during Cold Pressor test on Day 11

Table 18 -Showing effect of *Tinospora cordifolia* 1000 mg multiple dose on pharmacodynamic parameters during Cold Pressor test on Day 11(n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1163±17.68	1107±26.16
ABI	1.13±0.01	1.16±0.02
Reflection Index (RI%)	69.23±0.97	66.33±1.24
AP(mmHg)	8.75±1.70	7.0±0.81
PP(mmHg)	21.75±1.50	19.75±1.89
AIX (%)	127.0±6.05	122.5±4.20
SEVR (%)	141.3±5.56	145.5±7.55

As observed from the above table, treatment with *Tinospora cordifolia* produced a remarkable decrease in baPWV, RI, AP, PP, AIx and an increase in sub-endocardial viability ratio. This indicates that multiple dose administration of *Tinospora cordifolia* protects against the cardiovascular effects induced by cold pressor test.

Table 19 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor on Day 11(n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1213±17.68	1250±35.36
ABI	1.13±0.0	1.16±0.0
Reflection Index (RI%)	72.50±2.12	79.90±0.14
AP (mmHg)	8.50±0.70	13.0±1.41
PP(mmHg)	23.5±0.70	31.50±2.12
AIX (%)	138.0±1.41	150.5±2.12
SEVR (%)	143.0±7.07	132.5±0.70

As seen from the above table, placebo did not produce any remarkable changes in the pharmacodynamic parameters recorded during cold pressor test.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters on Day11

Table 20 –Showing effect *of Tinospora cordifolia* on Tilt Table Test on Day 11 (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	113.5±1.9	75.501.0	77.2±4.3	4.70±0.08	112.5±2.51	77.0±1.1	75.0±2.0	4.67±0.12
At 45^o	117.0±4.8	82.0±0.0	84.2±1.9	3.32±0.23	118.5±5.26	81.2±1.5	82.7±1.9	3.87±0.60
At 60^o	114.0±2.8	79.2±4.1	81.5±1.0	3.50±0.34	114.0±4.61	79.2±3.0	80.7±2.7	3.95±0.66
Basal2(0^o)	113.0±1.1	75.5±2.5	73.7±1.7	4.70±0.08	113.0±1.15	73.5±1.0	75.5±2.5	4.52±0.17

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with *Tinospora cordifolia* did

not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^{o} and 60^{o} an increase in cardiac output was observed compared to baseline.

Figure 3 shows the mean percent change in cardiac output on treatment with *Tinospora cordifolia* on Tilt Table test. All values are compared to baseline.

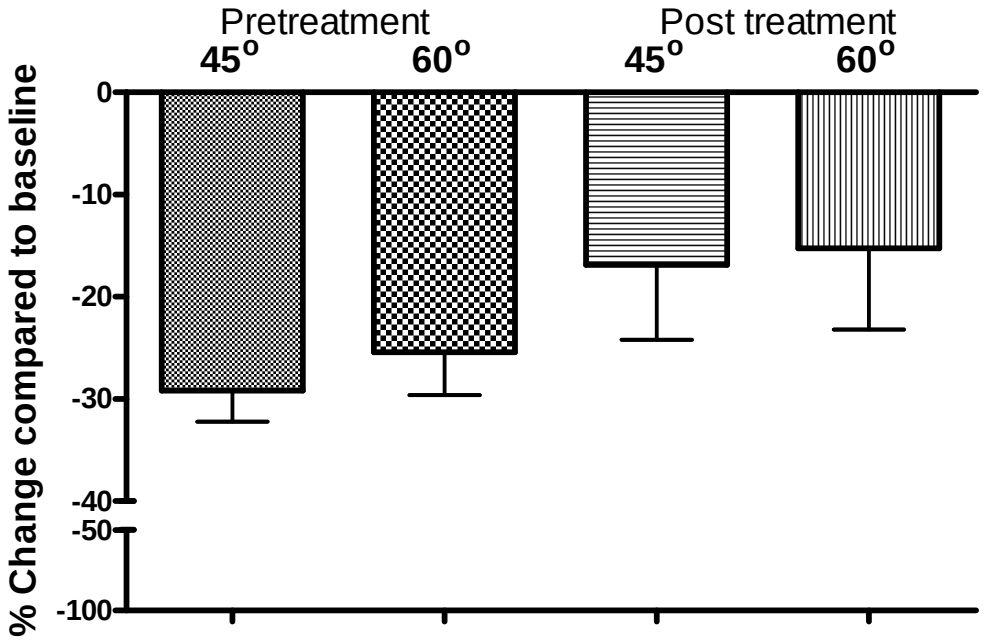
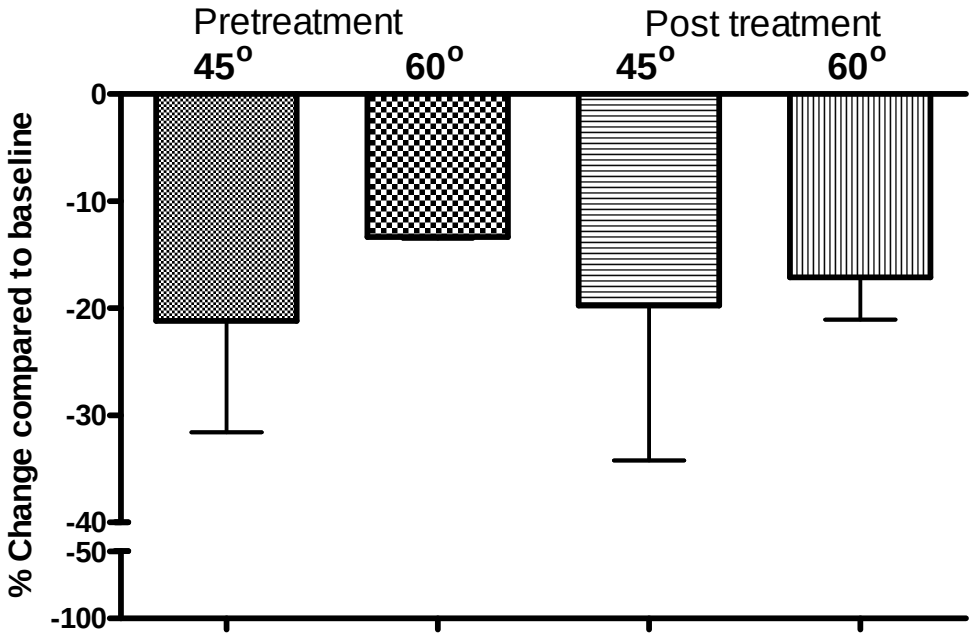


Table 21 -Showing effect of Placebo on Tilt Table Test on Day 11 (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^{o})	112.0±2.8	76.0±0.0	74.0±0.0	3.75±0.1	114.0±0.0	75.0±1.4	77.0±1.4	3.80±0.0
At 45^{o}	119.0±1.4	86.0±2.8	85.0±1.4	2.95±0.5	121.0±1.4	82.0±0.0	84.0±0.0	3.05±0.8
At 60^{o}	115.0±4.2	81.0±1.4	85.0±1.4	3.25±0.1	120.0±2.8	81.0±1.4	82.0±0.0	3.15±0.2
Basal2(0^{o})	115.0±1.4	75.0±1.4	77.0±1.4	3.85±0.1	116.0±0.0	76.00±2.8	75.0±1.4	3.95±0.2

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 4 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers on Day 11:

Table 22 -Showing effect of *Tinospora cordifolia* on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.10±0.020	0.99±0.026
Malondialdehyde (nm/ml)	3.33±0.25	2.72±0.27
Nitric oxide (µMol/L)	32.55±1.81	35.28±1.06

As observed from the above table, treatment with *Tinospora cordifolia* produced a slight decrease in HsCRP, malondialdehyde and increase in nitric oxide levels compared to baseline.

Table 23 -Showing effect of Placebo on biomarkers (n=2)

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.05±0.34	1.12±0.28
Malondialdehyde (nm/ml)	3.62±0.15	3.81±0.12
Nitric oxide (µMol/L)	30.07±1.63	30.44±1.17

Effect of Treatments on Platelet aggregation test on Day 11:

Table 24-Showing effect of *Tinospora cordifolia* on Inhibition of Platelet aggregation on Day 11 (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	60.0±6.96	53.63±7.43	9.26±3.67

As seen from the above table, *Tinospora cordifolia* produced changes in inhibition of platelet aggregation.

Table 25 -Showing effect of Placebo on Inhibition of Platelet aggregation on Day 11 (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	58.50±2.12	61.75±7.42	NIL

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 treatments.

Inference:

Treatment with aqueous extract of *Tinospora cordifolia* (TC-1) produced decrease in AIx, AP, and PP on cold pressor test. The active treatment also showed mild reduction in pharmacodynamic parameters such as baPWV and reflection index on cold pressor test. Ten days of treatment with *Tinospora cordifolia* showed slight increase in cardiac output compared to baseline (day 1). Multiple dose (10 days) administration with *Tinospora cordifolia* showed mild increase in nitric oxide and reduction in malondialdehyde and hsCRP levels compared with baseline. Treatment with 1000 mg of *Tinospora cordifolia* produced remarkable change in inhibition of platelet aggregation on day 11. It can be concluded from the present study that 1000 mg of aqueous extract of *Tinospora cordifolia* was found to be safe and well tolerated without any adverse event. There was slight change in the pharmacodynamic parameters tested with single and multiple doses of aqueous extract of *Tinospora cordifolia* 1000 mg (TC-1).However more studies are needed to assess the tolerability, safety and efficacy of the test product and also compare the effect with that of hydro-alcoholic extract.

Glossary:

TC-1 = Aqueous extract of *Tinospora cordifolia*

SBP=Systolic Blood Pressure (mmHg)

DBP=Diastolic Blood Pressure (mmHg)

PR=Pulse Rate (bpm)

baPWV= Brachial-Ankle Pulse Wave Velocity (cm/s) (Using ABI Colins, Komaki, Japan**)**

ABI= Ankle-Brachial Index (Using ABI Colins, Komaki, Japan**)**

RI =Reflection Index (%) (Using Micromedical Pulse Tracer Gallingham, Kent, UK)

AP=Aortic Augmentation Pressure (mmHg) (Using Sphygmocor® Atcor Medical Pvt Ltd Sydney, Australia)

PP=Pulse Pressure (mmHg) (Using Sphygmocor®)

AIX=Aortic Augmentation Index (%) (Using Sphygmocor®)

SEVR=Sub Endocardial Viability Ratio (%) (Using Sphygmocor®)

BMI = Body Mass Index (Kg/m^2)

CPT= Cold Pressor Test

CO= Cardiac Output (L /min)(L&T Nivomon Monitor)

AYUSH STUDY REPORT 2013

Evaluation of Cardiovascular and Pharmacodynamic effects of *Tinospora cordifolia* TC-2 (Hydro-alcoholic extract) with special reference to arterial stiffness and endothelial function in healthy human male subjects

Study VIII: 1000mg (TC-2) Single Dose

Clinical trial site

Department of Clinical Pharmacology and Therapeutics
Nizam's Institute of Medical Sciences
Punjagutta, Hyderabad, Andhra Pradesh, India.

Principal - Investigator

Dr.P.Usha Rani

Co-Investigator

Dr. M.U.R.Naidu
Dr. I.V.Sravanti
(Ayurvedic Physician)

Trial Co-ordinators

Nishat Fatima
N.Muralidhar

Study Nurse

Rita Salomi

Sponsor

Central Council for Research in Ayurveda & Siddha
Department of AYUSH
Ministry of Health and Family Welfare
Government of India.

1000mg Single dose study of hydro-alcoholic extract of *Tinospora cordifolia*(TC-2)

Title: Evaluation of Cardiovascular pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Sub title: Evaluation of Cardiovascular pharmacodynamic effects of 1000mg single dose of *Tinospora cordifolia*(TC-2) with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

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. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. Singh et al, in their study demonstrated the antioxidant activity of hydro-alcoholic extract of *Tinospora cordifolia* in albino mice (50 and 100 mg/kg body wt./day for 2 weeks).

Therefore the present study was undertaken to evaluate the effect of *Tinospora cordifolia* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Methodology:

The study was a randomized double blind single 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. Six subjects were randomized to the two treatments. Two subjects received placebo while 4 subjects received active medication of hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) as per prior randomization schedule.

Single dose study

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. After an overnight fast, baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling were performed using non-invasive

methods. The various cardiac profiling parameters were assessed using the following instruments - Brachial-Ankle Pulse Wave Velocity (cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI%) (Micromedical Pulse Tracer Gallingham, Kent, UK), Aortic Augmentation Pressure (mmHg), Pulse Pressure (mmHg), Aortic Augmentation Index (%) and Sub Endocardial Viability Ratio (%) (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, Body Surface Area (m^2), 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°, $60^\circ$and again at baseline 0° tilt using L&T Nivomon monitor. However the Cardiac Output is more significant among the other parameters recorded with Nivomon monitor.

Pretreatment blood samples were drawn for estimation of biomarkers of endothelial function and platelet aggregation test. The study medication was then administered as per the randomization schedule (either four capsules of hydro-alcoholic extract of *Tinospora cordifolia* 250mg (TC-2) or four capsules of Placebo) with 240 ml of water.

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.

The subject's vital parameters were recorded before and at hourly intervals upto 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. The safety lab reports were evaluated once they were available.

OBSERVATIONS:

A total of 7 volunteers were enrolled and 6 were randomised. One subject was excluded due to high total bilirubin levels. Four subjects received single dose of hydro-alcoholic extract of *Tinospora cordifolia* 1000mg (TC-2) and 2 subjects received identical placebo and all subjects completed the study uneventfully. Detailed demographic characteristics of the two study groups are shown in Table 1.

Table1: Demographic Data

Parameter	***Tinospora cordifolia***	**Placebo**
Total No.	4	2
Age	30.25±2.75	30.50±0.71
Gender (M)	4	2
Weight (Kg)	65.48± 4.13	63.30 ±4.10

BMI (Kg/m^2)	23.00±0.88	22.88±0.60

Effect of both treatments on vital parameters with Cold Pressor test:

Table 2 –Showing effect of *Tinospora cordifolia* (TC-2) on Vital parameters before, during and after Cold Pressor test (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	110.5±1.9	75.5±1.0	74.5±2.5	110.5±1.9	74.0±0.0	74.5±3.0
During (30 Sec) CPT	140.8±1.7	88.2±1.7	86.5±1.3	133.5±1.3	85.2±2.2	83.5±1.0
1 min after CPT	113.5±3.4	80.2±3.9	81.0±2.6	113.0±1.1	78.5±1.9	79.5±3.0
10 min after CPT	112.5±1.0	74.0±1.6	73.5±1.9	111.5±1.9	76.5±1.0	74.0±0.0

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with hydro-alcoholic extract 1000 mg single dose of *Tinospora cordifolia*, there was an increase in systolic and diastolic blood pressure and pulse rate which were however much lower than that compared to pretreatment values.

Table 3 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test (n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	110.5±2.1	74.0±2.8	73.0±1.4	111.0±1.4	75.0±4.2	75.0±1.4
During (30Sec) CPT	142.5±0.7	90.0±2.8	88.0±1.4	141.5±2.1	91.0±1.4	87.5±2.1
1min after CPT	111.5±3.5	83.5±0.7	84.0±2.8	111.5±3.5	80.0±2.8	80.0±0.0
10 min after CPT	111.0±1.4	74.0±0.0	74.0±2.8	112.0±0.0	74.0±0.0	74.0±2.8

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline:

Table 4 -Showing the effect of Cold Pressor test at baseline in *Tinospora cordifolia* (TC-2) group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1157±9.19	1225±35.36
ABI	1.12±0.01	1.11±0.017
Reflection Index (RI%)	69.80±3.73	74.38±2.39
AP(mmHg)	9.0±2.0	12.0±1.82
PP(mmHg)	25.50±4.43	35.25±3.09
AIX (%)	126.8±1.89	142.5±5.91
SEVR (%)	144.0±7.87	135.3±6.44

Table 5 –Showing effect of on Cold Pressor test at baseline in Placebo group (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1150±70.71	1238±53.03
ABI	1.11±0.03	1.09±0.00
Reflection Index (RI%)	69.95±0.91	72.30±0.99
AP (mmHg)	7.50±0.70	12.50±0.70
PP(mmHg)	25.0±5.65	31.50±2.12
AIX (%)	126.5±0.70	149.5±2.12
SEVR (%)	149.0±7.07	136.0±4.24

As seen from the above tables 4 and 5 showing the effect of cold pressor test per se in both treatment groups at baseline. Cold pressor test produces arterial stiffness and the same is evidenced by an increase in baPWV, RI, AP, PP and AIx and also as a decrease in values of SEVR.

Effect of both treatments on Pharmacodynamic parameters during Cold Pressor test

Table 6 -Showing effect of *Tinospora cordifolia* 1000 mg (TC-2) single dose on pharmacodynamic parameters during Cold Pressor test (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1150±35.36	1107±9.19
ABI	1.11±0.015	1.14±0.00
Reflection Index (RI%)	72.23±1.29	68.05±1.28
AP(mmHg)	7.50±1.91	6.75±0.95
PP(mmHg)	21.00±4.96	17.50±3.41
AIX (%)	127.3±5.37	121.8±3.09
SEVR (%)	136.8±7.411	142.0±8.32

As observed from the above table, treatment with hydro-alcoholic extract of Tinospora cordifolia (TC-2) decreased the arterial stiffness produced by cold pressor test. The same is evidenced by a decrease in baPWV, RI, augmentation index and increase in sub-endocardial viability ratio.

Table 7 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1213±53.03	1300±70.71
ABI	1.14±0.01	1.15±0.28
Reflection Index (RI%)	72.65±2.33	79.80±1.13
AP (mmHg)	9.0±0.0	13.00±1.41
PP(mmHg)	25.0±2.82	31.50±2.12
AIX (%)	130.0±1.41	150.5±4.95
SEVR (%)	143.0±5.65	130.5±0.70

As seen from the above table, placebo did not produce any remarkable changes in the pharmacodynamic parameters recorded during cold pressor test.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters

Table 8 –Showing effect of Tinospora cordifolia (TC-2) on Tilt Table Test (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	112.0±2.8	74.0±1.6	76.0±1.6	3.57±0.3	111.0±1.1	75.0±1.1	72.5±1.0	3.80±0.4
At 45^o	114.5±3.0	82.2±0.5	84.5±1.0	3.35±0.2	111.0±2.6	83.5±1.0	83.7±1.5	3.57±0.3
At 60^o	110.5±1.0	80.5±2.5	82.2±0.5	3.17±0.2	113.0±2.6	82.5±1.0	80.7±1.5	3.35±0.3
Basal2(0^o)	113.5±1.9	75.0±2.6	74.5±1.0	3.55±0.3	113.5±1.0	76.5±5.2	74.0±1.6	3.85±0.3

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, and DBP at different degrees of tilt. There was increase in PR recorded at 45 and 60 degrees of tilt. Treatment with hydro-alcoholic extract of Tinospora cordifolia did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^o and 60^o a mild increase in cardiac output was observed compared to baseline.

Figure 1 shows the mean percent change in cardiac output on treatment with Tinospora cordifolia (TC-2) on Tilt Table test. All values are compared to baseline.

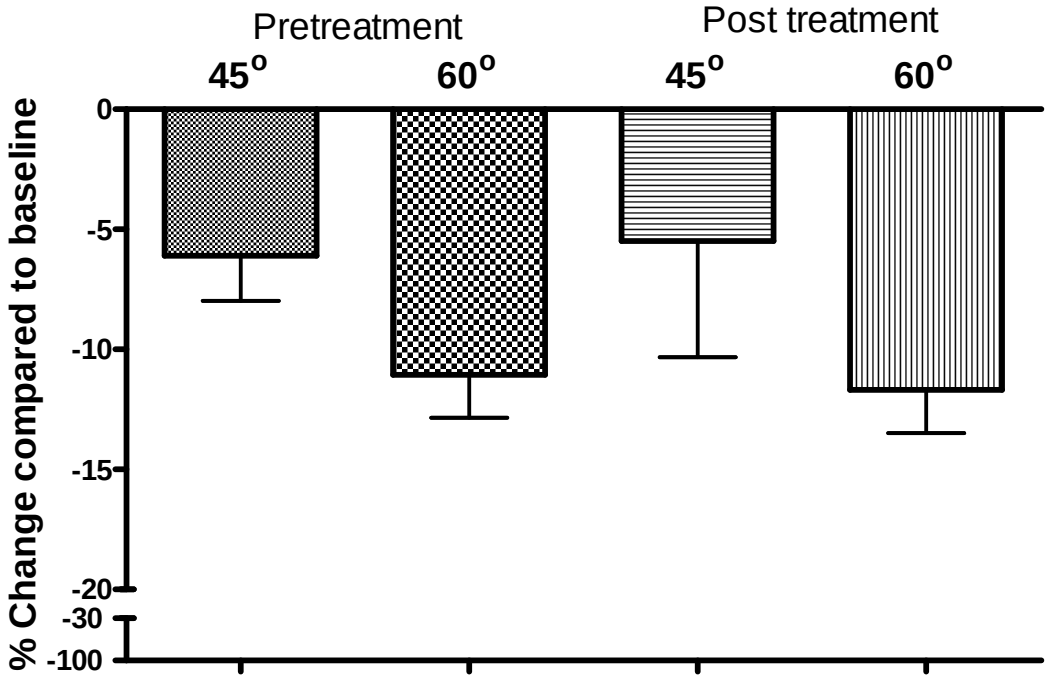
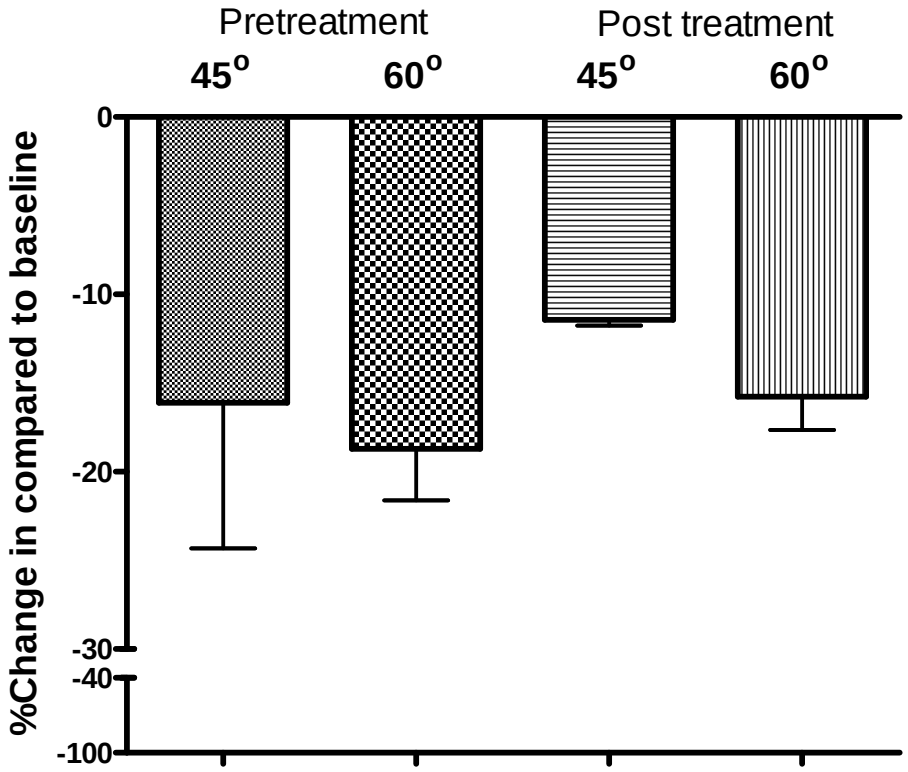


Table 9 -Showing effect of Placebo on Tilt Table Test (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	110.0±2.8	75.0±1.4	73.0±1.4	3.75±0.07	112.0±0.0	76.0±0.0	76.0±2.8	3.50±0.1
At 45^o	114.0±5.6	83.0±1.4	83.5±0.7	3.15±0.49	110.0±0.0	83.0±1.4	81.0±4.2	3.10±0.1
At 60^o	115.0±1.4	84.0±0.0	84.5±2.1	3.05±0.21	111.0±4.2	80.0±2.8	78.5±6.3	2.95±0.2
Basal2(0^o)	112.0±0.0	75.0±1.4	76.0±2.8	3.65±0.07	112.0±2.8	74.0±0.0	76.0±0.0	3.25±0.5

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 2 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers:

Table 10 -Showing effect of *Tinospora cordifolia* (TC-2) on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.09±0.03	0.97±0.06
Malondialdehyde (nm/ml)	3.06±0.25	2.71±0.26
Nitric oxide (µMol/L)	31.84±3.15	34.48±3.04

As observed from the above table, treatment with *Tinospora cordifolia* produced a decrease in HsCRP, malondialdehyde and increase in nitric oxide levels compared to baseline.

Table 11 -Showing effect of Placebo on biomarkers (n=2)

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.53±0.39	1.58±0.36
Malondialdehyde (nm/ml)	3.34±0.39	3.42±0.38
Nitric oxide (µMol/L)	28.29±0.17	28.71±0.12

Effect of Treatments on Platelet aggregation test:

Table 12 -Showing effect of *Tinospora cordifolia* (TC-2) on Inhibition of Platelet Aggregation (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	65.63±3.56	62.63±3.90	4.75±0.98

As seen from the above table, Tinospora cordifolia produced changes in inhibition of platelet aggregation.

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition

Mean±SD	58.75±2.47	58.50±1.41	0.80±1.13

Table 13 -Showing effect of Placebo on Inhibition of Platelet Aggregation (n=2)

As seen from the above table, Placebo treatment did not produce changes in platelet aggregation test performed.

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 treatments.

Inference:

Treatment with hydro-alcoholic extract of *Tinospora cordifolia* 1000 mg (TC-2) produced a decrease in AIx, AP and PP on cold pressor test. The active treatment also showed mild reduction in pharmacodynamic parameters such as baPWV and reflection index on cold pressor test. A slight increase in cardiac output was observed at 45^{o} and 60^{o} on tilt table test compared to baseline in *Tinospora cordifolia* group. Treatment with *Tinospora cordifolia* (TC-2) showed mild increase in nitric oxide and reduction in malondialdehyde and hsCRP levels compared with baseline. Treatment with 1000 mg of Tinospora cordifolia (TC-2) produced inhibition of platelet aggregation. It can be concluded from the present study that 1000 mg of hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) was found to be safe and well tolerated without any adverse event. There was no significant change in any of the pharmacodynamic parameters tested with single dose of hydro-alcoholic extract of *Tinospora cordifolia* 1000 mg (TC-2). Basing on this present study it can be inferred that TC-2 produced apparently better response than TC1 on parameters of arterial stiffness and endothelial dysfunction. Thus, further, studies are needed to be conducted in patients to assess the therapeutic potential of this formulation.

Glossary:

TC-2 = Hydro-alcoholic extract of *Tinospora cordifolia*

SBP=Systolic Blood Pressure (mmHg)

DBP=Diastolic Blood Pressure (mmHg)

PR=Pulse Rate (bpm)

baPWV= Brachial-Ankle Pulse Wave Velocity (cm/s) (Using ABI Colins, Komaki, Japan**)**

ABI= Ankle-Brachial Index (Using ABI Colins, Komaki, Japan**)**

RI =Reflection Index (%) (Using Micromedical Pulse Tracer Gallingham, Kent, UK)

AP=Aortic Augmentation Pressure (mmHg) (Using Sphygmocor® Atcor Medical Pvt Ltd Sydney, Australia)

PP=Pulse Pressure (mmHg) (Using Sphygmocor®)

AIX=Aortic Augmentation Index (%) (Using Sphygmocor®)

SEVR=Sub Endocardial Viability Ratio (%) (Using Sphygmocor®)

BMI = Body Mass Index (Kg/m^2)

CPT= Cold Pressor Test

CO= Cardiac Output (L /min)(L&T Nivomon Monitor)

AYUSH STUDY REPORT 2013

Evaluation of Cardiovascular and Pharmacodynamic effects of *Tinospora cordifolia* TC-2 (Hydro-alcoholic extract) with special reference to arterial stiffness and endothelial function in healthy human male subjects

Study IX: 1000mg (TC-2) Multiple Dose

Clinical trial site

Department of Clinical Pharmacology and Therapeutics
Nizam's Institute of Medical Sciences
Punjagutta, Hyderabad, Andhra Pradesh, India.

Principal - Investigator

Dr.P.Usha Rani

Co-Investigator
Dr. M.U.R.Naidu
Dr. I.V.Sravanti
(Ayurvedic Physician)
Trial Co-ordinators
Nishat Fatima
N.Muralidhar
Study Nurse
Rita Salomi

Sponsor

Central Council for Research in Ayurveda & Siddha
Department of AYUSH
Ministry of Health and Family Welfare
Government of India.

1000mg Multiple dose study of hydro-alcoholic extract of *Tinospora cordifolia*(TC-2)

Title: Evaluation of Cardiovascular pharmacodynamic effects of *Terminalia arjuna* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Sub title: Evaluation of Cardiovascular pharmacodynamic effects of 1000mg multiple dose of *Tinospora cordifolia*(TC-2) with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

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. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. Singh et al, in their study demonstrated the antioxidant activity of hydro-alcoholic extract of *Tinospora cordifolia* in albino mice (50 and 100 mg/kg body wt./day for 2 weeks).

Therefore the present study was undertaken to evaluate the effect of *Tinospora cordifolia* with special reference to arterial stiffness and endothelial dysfunction in healthy human male subjects.

Methodology:

The study was a randomized double blind single dose (day1) and multiple dose study (day 11) of hydro-alcoholic extract of *Tinospora cordifolia* 1000mg (TC-2) per day, conducted in the Department of Clinical Pharmacology and Therapeutics, NIMS,Hyderabad. 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. Six subjects were randomized to the two treatments. Two subjects received placebo while 4 subjects received active medication of hydro-alcoholic extract of *Tinospora cordifolia* 1000mg (TC-2) as per prior randomization schedule.

Single dose study (Day 1)

The subjects were housed in a temperature and humidity controlled room in the clinical research unit. After an overnight fast, baseline measurements of vital parameters and pharmacodynamic assessment of cardiac profiling were performed using non-invasive

methods. The various cardiac profiling parameters were assessed using the following instruments - Brachial-Ankle Pulse Wave Velocity (cm/s) and Ankle-Brachial Index (ABI-Colin), Reflection Index (RI%) (Micromedical Pulse Tracer Gallingham, Kent, UK), Aortic Augmentation Pressure (mmHg), Pulse Pressure (mmHg), Aortic Augmentation Index (%) and Sub Endocardial Viability Ratio (%) (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, Body Surface Area (m^2), 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°, $60^\circ$and again at baseline 0° tilt using L&T Nivomon monitor. However the Cardiac Output is more significant among the other parameters recorded with Nivomon monitor.

Pretreatment blood samples were drawn for estimation of biomarkers of endothelial function and platelet aggregation test. The study medication was then administered as per the randomization schedule (either four capsules of hydro-alcoholic extract of *Tinospora cordifolia* 250mg (TC-2) or four capsules of Placebo) with 240 ml of water.

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.

The subject's vital parameters were recorded before and at hourly intervals upto 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. The safety lab reports were evaluated once they were available.

Then the subjects were asked to continue the study medication for next 10 days (2 capsules two times a day). 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.

OBSERVATIONS:

A total of 7 volunteers were enrolled and 6 were randomised. One subject was excluded due to high SGPT and Alkaline phosphatase levels. Four subjects received single dose of hydro-alcoholic extract of *Tinospora cordifolia* 1000mg (TC-2) and 2 subjects received identical placebo and all subjects completed the study uneventfully. Detailed demographic characteristics of the two study groups are shown in Table 1.

Table1: Demographic Data

Parameter	*Tinospora cordifolia*	Placebo
Total No.	4	2
Age	31.25±2.5	32.50±3.53
Gender (M)	4	2
Weight (Kg)	62.90±4.75	65.60±6.36
BMI (Kg/m^2)	22.69±0.66	23.31±1.28

Effect of both treatments on vital parameters with Cold Pressor test on Day 1:

Table 2 –Showing effect of *Tinospora cordifolia* (TC-2) on Vital parameters before, during and after Cold Pressor test (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	113.0±2.0	73.5±1.0	74.5±1.9	111.5±1.9	75.0±1.1	73.0±1.1
During (30 Sec) CPT	139.8±1.7	89.2±1.9	87.0±1.4	133.8±1.7	86.5±1.9	85.7±1.2
1 min after CPT	116.0±6.9	81.0±2.0	80.2±2.1	112.0±3.3	77.5±1.9	80.5±1.9
10 min after CPT	114.0±1.6	74.0±2.8	74.0±1.6	112.5±2.5	74.0±1.6	74.5±1.9

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 1000 mg single dose of *Tinospora cordifolia*, the increase in SBP, DBP and PR produced with CPT was much less than that compared to the values at baseline. This indicates that treatment with hydro-alcoholic extract of *Tinospora cordifolia* attenuates the cardiovascular effects of cold pressor test and protects against the arterial stiffness induced by CPT.

Table 3 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test (n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	114.0±2.8	75.0±1.4	74.5±0.7	111.0±1.4	73.0±1.4	73.0±1.4
During (30Sec) CPT	139.5±2.1	88.0±1.4	86.0±1.4	140.0±2.8	88.5±0.7	85.0±1.4
1min after CPT	112.0±0.0	79.0±7.1	81.5±0.7	115.0±1.4	77.0±1.4	80.0±2.8
10 min after CPT	115.0±1.4	74.0±2.8	74.0±2.8	113.0±1.4	73.0±1.4	74.0±2.8

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline on Day 1:

Table 4 -Showing the effect of Cold Pressor test at baseline in *Tinospora cordifolia* (TC-2) group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1182±9.19	1250±0.0
ABI	1.13±0.00	1.132±0.02
Reflection Index (RI%)	69.15±3.28	74.88±5.05
AP(mmHg)	7.50±0.57	12.0±1.63
PP(mmHg)	22.25±1.50	28.0±0.81
AIX (%)	130.8±4.99	142.0±3.55
SEVR (%)	142.3±6.99	132.3±8.22

Table 5 –Showing effect of on Cold Pressor test at baseline in Placebo group (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1163±53.03	1225±35.36
ABI	1.12±0.014	1.15±0.02
Reflection Index (RI%)	67.15±1.20	70.80±0.28
AP (mmHg)	6.50±0.70	9.0±0.0
PP(mmHg)	23.0±0.0	30.50±3.53
AIX (%)	121.0±4.24	126.5±3.53
SEVR (%)	141.5±7.77	134.5±2.12

As seen from the above tables 4 and 5 showing the effect of cold pressor test per se in both treatment groups at baseline. Cold pressor test produces arterial stiffness and the same is evidenced by an increase in baPWV, RI, AP, PP and AIx and also as a decrease in values of SEVR.

Effect of Both Treatments on Pharmacodynamic parameters during Cold Pressor test on Day1

Table 6 -Showing effect of *Tinospora cordifolia* 1000 mg (TC-2) single dose on pharmacodynamic parameters during Cold Pressor test (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1207±9.19	1163±0.00
ABI	1.12±0.007	1.125±0.00
Reflection Index (RI%)	69.75±1.50	66.90±2.45
AP(mmHg)	8.00±1.82	6.0±1.41
PP(mmHg)	22.5±1.29	19.25±0.95
AIX (%)	133.0±5.88	125.3±5.56
SEVR (%)	145.3±4.64	156.8±5.56

As observed from the above table, treatment with hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) decreased the arterial stiffness produced by cold pressor test. The same is evidenced by a decrease in baPWV, RI, augmentation index and increase in sub-endocardial viability ratio.

Table 7 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor (n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1200±35.36	1288±17.68
ABI	1.12±0.03	1.13±0.02
Reflection Index (RI%)	69.45±0.21	72.50±0.70
AP (mmHg)	7.0±0.0	9.0±1.41
PP(mmHg)	24.50±0.70	32.0±4.24
AIX (%)	121.5±6.36	131.0±9.89
SEVR (%)	135.0±2.82	131.5±0.70

As seen from the above table, placebo did not produce any remarkable changes in the pharmacodynamic parameters recorded during cold pressor test.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters on Day 1

Table 8 –Showing effect of *Tinospora cordifolia* (TC-2) on Tilt Table Test (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	112.0±1.6	75.5±1.9	74.5±1.9	3.5±0.2	112.0±1.6	74.0±1.6	76.5±1.9	3.8±0.3
At 45^o	114.0±3.3	83.5±1.0	82.5±1.0	3.3±0.2	112.0±1.6	83.0±1.1	85.0±1.8	3.7±0.4
At 60^o	109.0±1.1	82.5±1.0	81.2±1.9	3.2±0.3	112.5±1.9	82.7±0.9	84.2±1.2	3.6±0.4
Basal2(0^o)	113.0±2.0	73.0±2.0	73.5±1.0	3.7±0.3	114.5±1.9	72.5±1.0	74.5±1.0	3.9±0.3

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, and DBP at different degrees of tilt. There was increase in PR recorded at 45 and 60 degrees of tilt. Treatment with hydro-alcoholic extract of *Tinospora cordifolia* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^o and 60^o an increase in cardiac output was observed compared to baseline.

Figure 1 shows the mean percent change in cardiac output on treatment with *Tinospora cordifolia* (TC-2) on Tilt Table test. All values are compared to baseline.

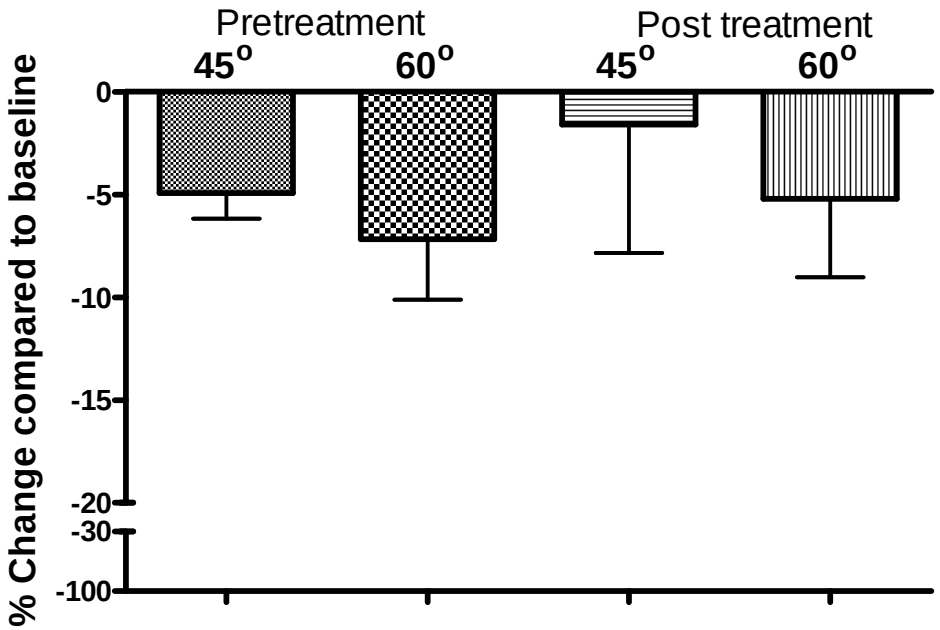
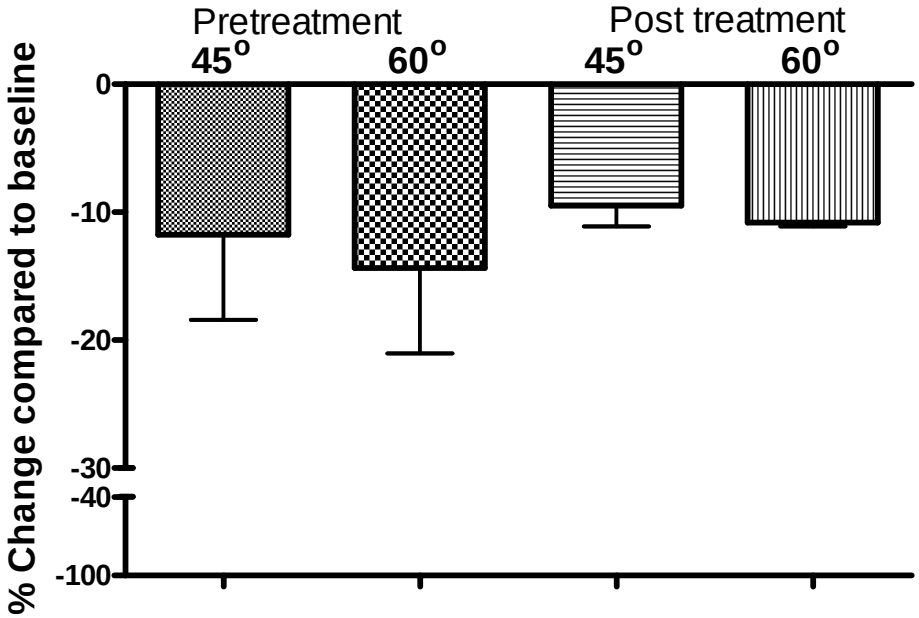


Table 9 -Showing effect of Placebo on Tilt Table Test (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	110.0±2.8	73.0±1.4	75.0±1.4	3.85±0.1	111.0±1.4	74.0±0.0	72.0±0.0	3.70±0.1
At 45^o	116.0±8.5	84.0±0.0	82.5±0.7	3.40±0.4	116.0±5.6	83.0±1.4	83.0±0.0	3.35±0.2
At 60^o	113.0±1.4	79.0±4.2	80.0±0.0	3.30±0.4	113.0±7.1	78.0±8.5	83.0±1.4	3.30±0.1
Basal2(0^o)	113.0±1.4	75.0±1.4	73.0±1.4	3.90±0.3	114.0±0.0	76.0±0.0	75.0±1.4	3.85±0.1

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 2 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers on Day 1:

Table 10 -Showing effect of *Tinospora cordifolia* (TC-2) on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.14±0.04	1.005±0.05
Malondialdehyde (nm/ml)	3.17±0.08	2.70±0.19
Nitric oxide (µMol/L)	30.20±1.29	33.49±0.90

As observed from the above table, treatment with *Tinospora cordifolia* produced a decrease in HsCRP, malondialdehyde and increase in nitric oxide levels compared to baseline.

Table 11 -Showing effect of Placebo on biomarkers (n=2)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.42±0.01	1.44±0.02
Malondialdehyde (nm/ml)	3.19±0.23	3.28±0.16
Nitric oxide (µMol/L)	33.59±1.17	31.91±1.31

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Effect of Treatments on Platelet aggregation test on Day 1:

Table 12 -Showing effect of *Tinospora cordifolia* (TC-2) on Inhibition of Platelet Aggregation (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	67.88±2.95	61.25±6.27	6.25±1.17

As seen from the above table, *Tinospora cordifolia* produced changes in inhibition of platelet aggregation.

Table 13 -Showing effect of Placebo on Inhibition of Platelet Aggregation (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	61.75±5.30	63.75±6.01	NIL

As seen from the above table, Placebo treatment did not produce changes in platelet aggregation test performed.

Multiple dose study-(Day 11)

Effect of both treatments on vital parameters with Cold Pressor test on Day 11:

Table 14 –Showing effect of *Tinospora cordifolia* (TC-2) on Vital parameters before, during and after Cold Pressor test on day 11 (n=4)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	113.5±1.9	73.5±1.0	76.5±1.0	111.0±1.1	75.0±1.1	74.5±1.0
During (30 Sec) CPT	138.5±1.3	86.7±0.9	85.7±2.4	131.5±1.3	82.7±0.9	81.5±1.3
1 min after CPT	115.5±1.9	79.0±3.5	78.7±3.8	113.5±3.4	80.5±4.3	78.7±3.6
10 min after CPT	112.5±1.9	72.5±1.0	74.0±1.6	114.5±1.0	74.0±1.6	73.0±1.1

As observed from the above table, there was an increase in systolic, diastolic blood pressure and pulse rate from baseline to during cold pressor test (i.e., 30 sec). Whereas after 1min and 10min of CPT, the above mentioned vital parameters appeared to be within normal limits. At 3hrs post treatment with 1000 mg single dose of *Tinospora cordifolia*, the increase in SBP, DBP and PR produced with CPT was much less than that compared to the values at baseline. This indicates that treatment with hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) attenuates the cardiovascular effects of cold pressor test and protects against the arterial stiffness induced by CPT.

Table 15 -Showing effect of Placebo on Vital parameters before, during and after Cold Pressor test on Day 11(n=2)

	Pretreatment			Post Treatment		
Parameter	SBP	DBP	PR	SBP	DBP	PR
Before CPT	113.0±1.4	73.0±1.4	75.0±1.4	111.0±1.4	74.0±2.8	74.0±0.0
During (30Sec) CPT	140.5±2.1	87.0±1.4	87.5±0.7	140.0±1.4	88.5±0.7	85.5±0.7
1min after CPT	111.0±1.4	79.5±4.9	79.0±7.1	113.0±1.4	78.0±0.0	81.0±1.4
10 min after CPT	116.0±2.8	73.0±1.4	72.5±0.7	113.0±1.4	74.0±2.8	74.0±2.8

It can be seen from the above table, that there was an increase from baseline during cold pressor test (i.e., 30 sec) in systolic blood pressure, diastolic blood pressure and pulse rate which returned to near normal values by 1min after completion of CPT. However after 3hrs of post treatment with Placebo, cold pressor test did not produce any remarkable changes in the vital parameters from baseline to during 30sec, after 1min and 10min of CPT.

Effect of Cold pressor test on pharmacodynamic parameters in both treatment groups at baseline on Day 11:

Table 16 -Showing the effect of Cold Pressor test at baseline in *Tinospora cordifolia* (TC-2) group (n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1157±9.19	1107±26.16
ABI	1.12±0.00	1.13±0.00
Reflection Index (RI%)	71.23±1.35	68.73±1.43
AP(mmHg)	8.50±1.29	7.50±1.29
PP(mmHg)	23.00±2.16	21.00±2.16
AIX (%)	128.0±1.41	125.0±2.44
SEVR (%)	135.0±6.05	137.5±5.68

As seen from the above table, cold pressor test per se did not produce changes in ABI, AP, PP, baPWV, RI and SEVR . The values indicate that the cold pressor induced arterial stiffness was counteracted by treatment with hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) and there was decrease in baPWV, RI, AP, PP, AIx and increase in SEVR compared to baseline (Day1).

Table 17 –Showing effect of on Cold Pressor test at baseline in Placebo group on Day 11(n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1175±0.0	1250±35.36
ABI	1.14±0.0	1.15±0.0
Reflection Index (RI%)	70.80±1.69	77.50±2.12
AP (mmHg)	7.00±1.41	11.00±1.41
PP(mmHg)	23.0±1.41	29.00±0.0
AIX (%)	125.5±2.12	135.0±1.41
SEVR (%)	135.5±2.12	130.5±4.95

As seen from the above table, cold pressor test per se produced an increase in baPWV, PP, AIx and decrease in SEVR.

Effect of Both Treatments on Pharmacodynamic parameters during Cold Pressor test on Day 11

Table 18 -Showing effect of *Tinospora cordifolia* 1000 mg (TC-2) multiple dose on pharmacodynamic parameters during Cold Pressor test on Day 11(n=4)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1119±8.48	1051±17.68
ABI	1.15±0.02	1.11±0.04
Reflection Index (RI%)	69.48±0.76	65.33±1.42
AP(mmHg)	8.25±0.50	5.75±0.50
PP(mmHg)	22.50±3.10	18.75±0.95
AIX (%)	127.3±0.95	118.3±1.70
SEVR (%)	137.8±4.78	147.8±5.37

As observed from the above table, treatment with *Tinospora cordifolia* produced a remarkable decrease in baPWV, RI, AP, PP, AIx and an increase in sub-endocardial viability ratio. This indicates that multiple dose administration of hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) protects against the cardiovascular effects induced by cold pressor test.

Table 19 –Showing effect of Placebo pharmacodynamic parameters during Cold Pressor on Day 11(n=2)

Parameter	Before Cold Pressor	After Cold Pressor
baPWV(cm/s)	1188±53.03	1288±53.03
ABI	1.14±0.01	1.11±0.0
Reflection Index (RI%)	71.00±2.82	76.65±3.74
AP (mmHg)	9.0±0.0	12.50±0.70
PP(mmHg)	25.50±4.95	30.00±2.82
AIX (%)	128.0±1.41	134.0±0.0
SEVR (%)	135.0±1.41	130.5±0.70

As seen from the above table, placebo did not produce any remarkable changes in the pharmacodynamic parameters recorded during cold pressor test.

Effect of Treatments on Tilt Table Test on Various Pharmacodynamic parameters on Day11

Table 20 –Showing effect of *Tinospora cordifolia* (TC-2) on Tilt Table Test on Day 11 (n=4)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	113.5±1.91	74.5±1.9	75.0±1.1	3.77±0.3	113.5±3.0	75.0±1.1	75.0±1.1	3.95±0.34
At 45^o	112.5±4.43	83.0±1.1	82.7±1.9	3.42±0.3	114.0±3.3	82.5±1.0	84.7±1.5	3.60±0.14
At 60^o	111.0±1.15	81.2±2.2	81.7±2.8	3.4±0.0	112.0±2.3	80.0±3.6	82.2±0.5	3.52±0.12
Basal2(0^o)	113.0±1.15	74.5±2.5	74.5±1.9	3.77±0.4	114.0±1.6	72.5±1.0	72.7±0.9	3.92±0.49

It can be seen from the above table, that Tilt table test per se did not produce any change in SBP, DBP and PR at different degrees of tilt. Treatment with hydro-alcoholic extract of *Tinospora cordifolia* did not alter any of the vital parameters in the subjects during all phases of the tilt, except at 45^o and 60^o an increase in cardiac output was observed compared to baseline.

Figure 3 shows the mean percent change in cardiac output on treatment with *Tinospora cordifolia* on Tilt Table test. All values are compared to baseline.

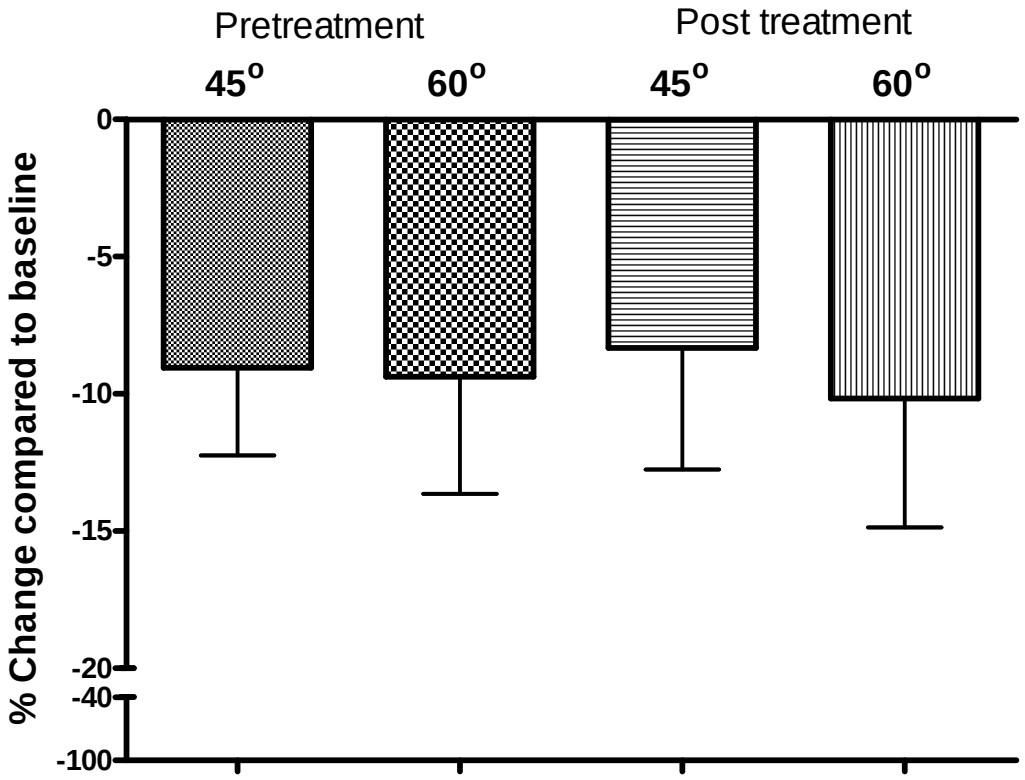
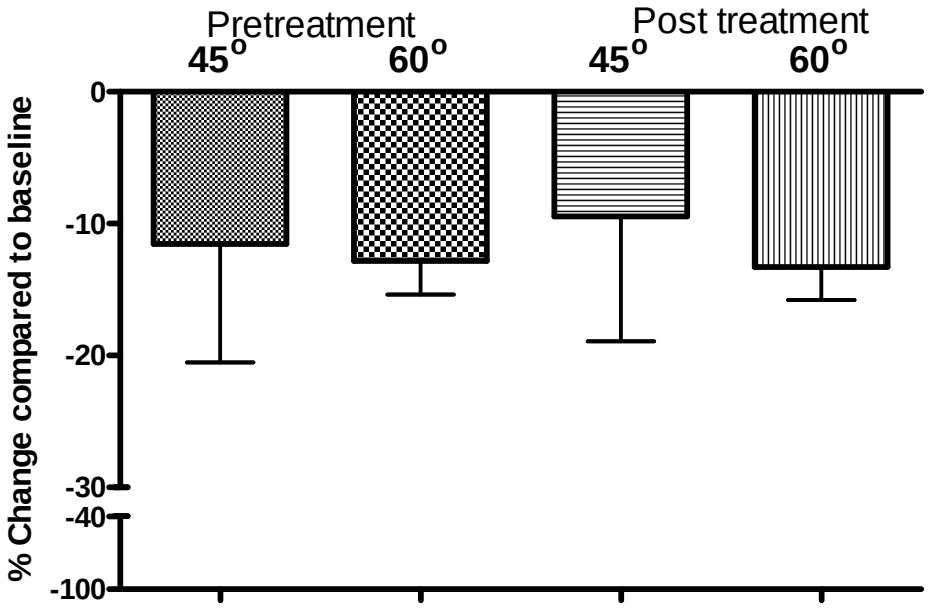


Table 21 -Showing effect of Placebo on Tilt Table Test on Day 11 (n=2)

	Pretreatment				Post Treatment			
Parameter	SBP	DBP	PR	CO	SBP	DBP	PR	CO
Basal1(0^o)	113.0±1.4	73.0±1.4	75.0±1.4	3.90±0.0	110.0±0.0	73.0±1.4	74.0±2.8	3.75±0.1
At 45^o	112.0±5.6	84.0±0.0	82.5±0.7	3.45±0.5	113.0±1.4	84.0±0.0	83.0±1.4	3.40±0.6
At 60^o	114.0±2.8	82.5±0.7	82.0±2.8	3.4±0.14	111.0±1.4	80.5±3.5	82.5±2.1	3.25±0.1
Basal2(0^o)	115.0±4.2	75.0±1.4	72.0±0.0	3.55±0.1	115.0±1.4	74.0±0.0	74.0±2.8	3.70±0.0

As observed from the above table, Tilt table test did not produce any changes in cardiac output at different angles of tilt. Further, placebo produced similar effect as seen at baseline on SBP, DBP, PR and cardiac output at 45 and 60 degrees of tilt.

Figure 4 shows the mean percent change in cardiac output on treatment with Placebo on Tilt Table test.



Effect of Treatments on Biomarkers on Day 11:

Table 22 -Showing effect of *Tinospora cordifolia* (TC-2) on biomarkers (n=4)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.10±0.01	0.93±0.03
Malondialdehyde (nm/ml)	3.22±0.08	2.57±0.24
Nitric oxide (µMol/L)	31.55±2.29	36.43±2.27

As observed from the above table, treatment with *Tinospora cordifolia* produced a slight decrease in HsCRP, malondialdehyde and increase in nitric oxide levels compared to baseline.

Table 23 -Showing effect of Placebo on biomarkers (n=2)

Parameter	Pretreatment	Post treatment
Highly sensitive CRP(mg/L)	1.44±0.02	1.47±0.08
Malondialdehyde (nm/ml)	3.28±0.16	3.34±0.18
Nitric oxide (µMol/L)	32.56±0.39	33.07±0.60

As seen from the above table, placebo did not produce any remarkable changes in the biomarkers of oxidative stress compared to baseline.

Effect of Treatments on Platelet aggregation test on Day 11:

Table 24-Showing effect of *Tinospora cordifolia* (TC-2) on Inhibition of Platelet aggregation on Day 11 (n=4)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	66.00±2.55	57.88±2.13	12.75±1.041

As seen from the above table, *Tinospora cordifolia* slight changes in inhibition of platelet aggregation.

Table 25 -Showing effect of Placebo on Inhibition of Platelet aggregation on Day 11 (n=2)

	% Platelet Aggregation (ADP)		
	Pretreatment	Post Treatment	% Inhibition
Mean±SD	62.00±2.12	64.00±3.53	NIL

As seen from the above table, Placebo treatment did not produce changes in platelet aggregation test performed.

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 treatments.

Inference:

Treatment with hydro-alcoholic extract of *Tinospora cordifolia* 1000mg (TC-2) produced a decrease in AIx, AP and PP on cold pressor test. The active treatment also showed mild reduction in pharmacodynamic parameters such as baPWV and reflection index on cold pressor test. A slight increase in cardiac output was observed at 45^{o} and 60^{o} on tilt table test compared to baseline in *Tinospora cordifolia* group. Treatment with *Tinospora cordifolia* (TC-2) showed mild increase in nitric oxide and reduction in malondialdehyde and hsCRP levels compared with baseline. Treatment with 1000 mg of *Tinospora cordifolia* (TC-2) produced mild change in inhibition of platelet aggregation on day 11. It can be concluded from the present study that 1000 mg of hydro-alcoholic extract of *Tinospora cordifolia* (TC-2) was found to be safe and well tolerated without any adverse event. There was no significant change in any of the pharmacodynamic parameters tested with single dose of hydro-alcoholic extract of *Tinospora cordifolia* 1000 mg (TC-2). Basing on this present study it can be inferred that TC2 produced apparently better response than TC1 on parameters of arterial stiffness and endothelial dysfunction. Thus, further, studies are needed to be conducted in patients to assess the therapeutic potential of this formulation.

Glossary:

TC-2= Hydro-alcoholic extract of *Tinospora cordifolia*

SBP=Systolic Blood Pressure (mmHg)

DBP=Diastolic Blood Pressure (mmHg)

PR=Pulse Rate (bpm)

baPWV= Brachial-Ankle Pulse Wave Velocity (cm/s) (Using ABI Colins, Komaki, Japan**)**

ABI= Ankle-Brachial Index (Using ABI Colins, Komaki, Japan**)**

RI =Reflection Index (%) (Using Micromedical Pulse Tracer Gallingham, Kent, UK)

AP=Aortic Augmentation Pressure (mmHg) (Using Sphygmocor® Atcor Medical Pvt Ltd Sydney, Australia)

PP=Pulse Pressure (mmHg) (Using Sphygmocor®)

AIX=Aortic Augmentation Index (%) (Using Sphygmocor®)

SEVR=Sub Endocardial Viability Ratio (%) (Using Sphygmocor®)

BMI = Body Mass Index (Kg/m^2)

CPT= Cold Pressor Test

CO= Cardiac Output (L /min)(L&T Nivomon Monitor)