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A STUDY OF CARDIOVASCULAR AND ANTIMICROBIAL EFFECTS OF *TINOSPORA CORDIFOLIA*

J. Archana* and M.P. Kusuma

RBVRR Women's college of pharmacy, Barkathpura, Hyderabad-500027, India

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Correspondence to Author:

Jorige Archana

Asst. professor
RBVRR Women's college of pharmacy
Barkathpura, Hyderabad-500027, India

E-mail:archana_jarc@yahoo.com

ABSTRACT

Tinospora cordifolia is known for a wide range of medicinal properties. In this study, cardiovascular and antimicrobial properties of aqueous and ethanolic extracts of *Tinospora cordifolia* were evaluated. Dose dependent negative ionotropic and chronotropic effects were observed with both aqueous and ethanolic extracts. The effects were antagonized by atropine indicating involvement of muscarinic receptors. Maximum antimicrobial activity was found with ethanolic extract of *Tinospora cordifolia* (15mm) against *Pseudomonas aeruginosa*. The organism showed resistance to aqueous extract giving an inhibition zone of 0.3mm. The data suggest that *Tinospora cordifolia* could be of benefit in arrhythmias and microbial infections.

INTRODUCTION: Plants are invaluable sources of pharmaceutical products¹. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat variety of diseases².

Tinospora cordifolia which is commonly known as Gudichi is a large glabrous, deciduous climbing shrub belonging to family Menispermaceae³. It has been reported to possess antiallergic, anti-inflammatory, antioxidant⁴, immunomodulatory, hepatoprotective, antipyretic⁵, antidiabetic⁶ and anti hyperlipidemic properties⁷. It has been reported to be useful in urinary diseases, syphilis, skin diseases, bronchitis⁸, and allergic rhinitis⁹ and known to promote longevity and increased body resistance against various diseases¹⁰. It has been traditionally used as antispasmodic and antiarrhoeal agent¹¹.

MATERIALS AND METHODS:

Plant material: Fresh stem and leaves of *Tinospora*

cordifolia were collected from the botanical garden of our college.

Procurement of bacterial strain: Pure cultures of test organism *Pseudomonas aeruginosa* (ATCC 2668) were purchased from MTCC, Chandigarh. Viability of the culture was maintained on nutrient agar by periodic transfers.

Procurement of Chemicals: Atropine sulphate (Harson Laboratories)

Preparation of Aqueous extract: Fresh stem and leaves of *Tinospora cordifolia* were shed dried at room temperature and powdered mechanically.



The powder (100gm) was allowed to soak for 3 days in 1000ml of distilled water with normal regular stirring. The suspension was filtered using Whatmann no. 1 filter paper and the resulting solution was concentrated on water bath. The concentrated solution was dried under normal tube light. Completely dried extract was collected by scraping with spatula, and then stored in cool and dry place. The extract was dissolved in distilled water to obtain appropriate concentration of 0.1mg/ml, 0.2mg/ml, 0.5mg/ml and 1mg/ml.

Preparation of Ethanolic extract: 100gm of powder was soaked in ethanol (50%) for 48 hr and the solution was then dried at 40°C in oven for 36hr. The dried extract was dissolved in distilled water to make 0.1mg/ml, 0.2mg/ml, 0.5mg/ml and 1mg/ml concentration solution.

Isolated perfused frog heart preparation: The frog of species *Rana tigrina* was pithed and pinned it to the frog board. A midline incision was given on the abdomen, the pectoral girdle was removed and the heart was exposed. The pericardium was carefully removed and put a few drops of frog ringer over the heart. The inferior venacava was traced, a thread was placed around it and a small cut was given in order to insert the venous cannula. The cannula was inserted in the vein and thread was tied to assure the cannula in place which is in turn connected to a saline bottle containing frog ringer solution.

A small cut in one of the aorta was given for the ringer to come out. Heart was isolated and attached to the stand with moderate flow of ringer. A thin pin hook was passed through the tip off the ventricle and with the help of a fine thread attached to the hook it was tied to the free limb of Sterling's heart lever which was fixed to stand. A proper tension was adjusted by altering the height of the lever. The normal heart rate was noted^{12, 13}. The effect of various doses of ethonolic and aqueous extracts of *Tinospora cordifolia* were recorded before as well as after atropine. The effect of *Tinospora cordifolia* was studied for heart rate and myocardial force of contraction.

Agar diffusion assay: Anti microbial screening was done using agar well diffusion methods. For this 25ml of sterile nutrient agar was poured in sterile

autoclaved petri plates. Before pouring, 100 µl of activated culture of bacteria was added and then allowed to solidify completely. The wells were prepared with the help of sterile 10mm diameter cork-borer.

The 100µl of plant extract from stock solution were poured into the wells. The plates were sealed with plasticine and transferred to refrigerator to diffuse out for 30mins. The plates were then incubated in the incubator at 37°C for 24hrs. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well, were recorded¹⁴.

Antibiotic susceptibility of selected bacterial strains: Susceptibility of selected bacterial strain was done against the standard antibiotic of Streptomycin (0.375gm/ml). Twenty ml of sterilized nutrient agar seeded with activated bacterial culture was poured in petri dish, allowed to solidify and paper disc containing antibiotic was placed gently on surface by pointed forceps. The plates were sealed with plasticine and kept in the incubator at 37°C for 48hrs.

RESULTS AND DISCUSSION: The results of chronotropic and ionotropic effect of aqueous extract and ethanolic extracts were given in **Tables 1 & 2**. Data obtained from various groups were pooled and expressed as mean \pm SEM. The difference between control, aqueous and ethanolic extracts in absence and in presence of atropine was analyzed statistically using students T test. HR and FC are mentioned in terms of percentages. Both aqueous and ethanolic extracts showed dose dependent negative chronotropic and negative ionotropic effect.

Negative chronotropic and negative inotropic effects were maximum with 1mg/ml of ethanolic extract. As the dose of aqueous and ethanolic extracts were increased heart rate and force of contraction decreased which indicates that maximum inhibition is observed at high doses. Ethanolic extract produced very significant negative chronotropic and ionotropic effects and these effects were maximum with 1mg/ml of ethanolic extract. Negative chronotropic and ionotropic effects were blocked by atropine which determines the involvement of muscarinic receptors.

Maximum antimicrobial activity was found with 1mg/ml of ethanolic extract of *Tinospora cordifolia* (15mm) against *Pseudomonas aeruginosa*. The organism showed resistance to aqueous extract giving an inhibition zone of 0.3mm (Table 3). This could be because of phospholipids membrane in addition to

inner peptidoglycan layer, which makes the cell more impermeable for exogenous molecules (Fig.1). When antibiotic susceptibility test was conducted for Streptomycin it was found to be susceptible showing an inhibition of 17mm (Fig. 1).

TABLE 1.EFFECT OF *TINOSPORA CORDIFOLIA* EXTRACT ON HEART RATE OF ISOLATED FROG HEART

Conc. (mg/ml)	Aqueous extract		Aqueous extract +Atropine		Alcoholic extract		Alcoholic extract +Atropine	
	HR	%HR	HR	%HR	HR	%HR	HR	%HR
0.1	29.2±1.36*	83.42±2.08	34.6±1.4	98.9±0.67	31.2±1.59	89.09±2.51	31.4±1.39	89.7±1.41
0.2	27.0±1.79**	76.89±3.05	35±1.05	100.1±0.74	29.2±1.39*	83.54±3.05	30.6±1.21*	82.43±1.8
0.5	19.2±1.11***	54.83±2.23	33.2±0.91	95.1±1.52	24.8±1.46**	70.98±3.8	28.8±1.46*	82.28±1.49
1	14.8±1.32***	41.99±2.5	36.2±0.98*	88.05±2.07	5.4±0.68***	15.22±1.49	28±1.33**	80.00±1.92

Basal value: HR = Heart Rate =35±1.48. Values are expressed as mean ± standard error mean (n=5) *P<0.05; **P<0.01; ***P<0.001

TABLE 2.EFFECT OF *TINOSPORA CORDIFOLIA* EXTRACT ON FORCE OF CONTRACTION OF ISOLATED FROG HEART.

Conc in (mg/ml)	Aqueous extract		Aqueous extract + Atropine		Alcoholic extract		Alcoholic extract + Atropine	
	FC	%FC	FC	%FC	FC	%FC	FC	%FC
0.1	3.2±0.37	72±2.95	4.1±0.44	96±3.29	3.2±0.37	74±2.39	4.2±0.33	100±1.79
0.2	2.1±0.24**	50±3.1	4.0±0.35	96±0.93	2.2±0.37**	52±3.73	4.2±0.34	101.2±1.2
0.5	1.14±0.27***	24.4±3.26	37±0.42	88±2.56	0.72±0.18***	16.14±3.00	3.9±0.28	94±1.07
1	0.84±0.18***	18.9±2.07	37±0.53	89±3.6	0.02±0.02***	0.005±0.009	4.0±0.34	95±2.24

Basal value: FC-Force of contraction - 4.2±0.37. Values are expressed as mean ± standard error mean (n=5) *P<0.05; **P<0.01; ***P<0.001



FIG. 1: COMPARISON OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF *TINOSPORA CORDIFOLIA* AND STREPTOMYCIN

CONCLUSION: The extract of *Tinospora cordifolia* possesses antibacterial and negative chronotropic and ionotropic effects. Like the known parasympathomimetic drugs these drugs may be useful in cardiovascular disorders like arrhythmia. The biochemical role of the aqueous and ethanolic extracts can be investigated in future in animals like mice and rats which may explore their cholinomimetic or anticholinesterase activity in brain and neuromuscular junction and can have potential benefit in the

treatment of Alzheimer's disease and *Myasthenia gravis*. These findings would pave way for evolution more drugs.

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