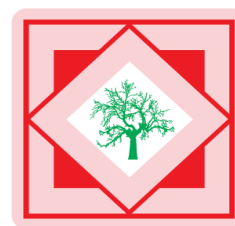




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Comparative study of cardiac action in infected and non-infected leaves of *Ocimum tenuiflorum*

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ABSTRACT

Ocimum tenuiflorum had many traditional uses reported and it is widely available. *Asperigillus Niger* fermentation is "generally regarded as safe" (GRAS) by the United States Food and Drug Administration. In the present study leaves of *Ocimum tenuiflorum* infected with *Asperigillus niger* were used to produce positive inotropic and chronotropic effects in frog's heart whereas only positive inotropic effect was reported in normal leaves. This is due to biotransformation which is confirmed from preliminary phytochemical tests. This study concludes after infection of normal leaves shows the presence of steroids and amino acids might be reason for activity.

Keywords: Heart, *Ocimum tenuiflorum*, *Asperigillus niger*, leaves, Biotransformation.

INTRODUCTION

Cardio vascular disease incurs a greater economical constraint than any other illness especially in the developing countries. It is the most common cause of death by the year 2020. The risk factors for heart disease are family history, sex, increased lipid levels, diabetes mellitus, hypertension, obesity, and cigarette smoking. There is overwhelming evidence indicating hypercholesterolemia and other lipid abnormalities as major risk factors in the development of atherosclerosis and coronary heart disease. Therefore, cardiovascular disease becomes a very common problem in the affluent societies related to their life style [1]. In developed countries, coronary vascular diseases now constitute the principal cause of human mortality. Not surprisingly, therefore, this is an intensive research not entirely devoted to treatment, but also to prevention of these diseases. Many factors affect the complex regulation of the heart and there is a large group of drugs which will affect the hearts performance, often in manner not directly associated with the heart muscle itself. However, therapeutic use of the drugs for the treatment of the failing heart is confined to small group of glycosides that act directly on the heart muscle [2]. Cardio vascular disorder leads to variety of pathological changes including endothelial, pulmonary, hepatic, renal, endocrine, skeletal muscle abnormalities and the state of multiorgan impairment [3]. Cardiac glycosides and catecholamines have been used as the main therapeutic drugs in the treatment of coronary artery disease [4]. There is no evidence that digitalis prolongs survival of coronary heart disease

patients [5], major limitations in the use of cardiac glycosides are low margin of safety, and inability to retard the process which cause the heart to fail and intoxication are well documented [6]. Catecholamine use is limited by its, insufficient differentiation between positive inotropic and chronotropic actions, potential arrhythmogenic properties, tachyphylaxis due to receptor down regulation and causes a severe oxidative stress in the myocardium through free radical formation [7]. There are so many popular herbs used in traditional practises to cure cardiovascular problems. The greatest disadvantages of present drugs are toxic in nature and costly to buy. So herbal medicine which has less toxic effects and cheap to buy are encouraging. There are many works in progress in this direction. *Ocimum tenuiflorum* is grown traditionally in all houses of India. In telugu language it is known as Krishnatulasi. It has many chemical constituents and popularly used as anti-inflammatory, anticancer, antiageing, Antihyperlipoproteinaemic, Antimutagenic, Antiophidic, Antioxidant, Antiprosthetic, Anthelmintic, Antiatherosclerotic, Anticholinesterase, Antimelanomic, Anticonvulsant agent. *Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. Various strains of *A. niger* are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organisation. *A. niger* fermentation is "generally regarded as safe" (GRAS) by the United States Food and Drug Administration. The present study was undertaken to investigate positive inotropic and chronotropic effects of aqueous extract of fungi infected leaves of *Ocimum tenuiflorum* on isolated frog heart.

MATERIALS AND METHODS

The selected plant species of *Ocimum tenuiflorum* was collected from the premises of Vignan Pharmacy College, Vadlamudi, ChebroluMandalam, Guntur district, Andhra Pradesh, India in august 2009. The basic plant material of *Ocimum tenuiflorum* leaf used for the investigation was identified and it was authenticated by Mr. M.Raghu Ram, MSc.,Ph.D., Department of Botany, AcharyaNagarjuna University, Guntur.

Infection of leaves

Wash the leaves of the live species of *Ocimum tenuiflorum* with distilled water. Surface sterilized with 60% Isopropyl alcohol. Dip the sterilized leaves one by one into 1% solution of *Aspergillus niger*. Keep it for seven days to get complete infection of leaves and take this leaves for further study.

Preparation Of Aqueous Extract Of Uninfected & Infected Leaves Of *Ocimum tenuiflorum*

The shade dried coarsely powdered leaves of *Ocimum tenuiflorum*(50gm) was extracted using water as solvent by continuous hot extraction process using soxhlet apparatus. The extraction was continued till the completion. After completion of extraction the extract was concentrated under reduced pressure. The extract was stored in an air light container in a refrigerator below 10°C. The above process is done separately for both uninfected and infected plant species of *Ocimum tenuiflorum*.

Preliminary Phytochemical Screening

The Aqueous extracts of leaves of both uninfected and infected plant species of *Ocimum tenuiflorum* was subjected to preliminary Phyto Chemical screening for the presence or absence of active Phyto Chemical constituents by following methods.

Isolation of frog heart

Pith the frog and pin to the board. Give amid line incision on the abdomen to remove the pectoral girdle and expose heart. Carefully remove the pericardium and put a few drops of frog ringer over the heart and trace the inferior venecava, put the thread around it and give a small cut in order to insert the venous canula which is in turn connected to a perfusion bottle containing frog ringer. Insert the canula in the vein and tie the thread to assure the canula in place. Give a small cut in one of the aorta for the perfusate to come out. Adjust a proper venous pressure of 2-4cm by altering the height of the perfusion bottle the effective venous pressure is height in centimetres from level of venous canula and ringer level in the perfusion bottle. Start the perfusion by opening screw clamp attached. Pass a thin pin hook through the tip of the ventricles and with a thread attached it to the lever. Record the normal contraction of the heart on the smoked drum [8].

Evaluation of ionotropic and chronotropic effects:

The procedure followed with selection of standard drugs which are regulating the force of contraction, heart rate and cardiac output by acting on various receptors, such as alpha & beta receptors (adrenaline), ion channels (potassium chloride), L- type calcium channels (calcium chloride), muscarinic & nicotinic receptors (acetyl choline), beta blocker (propranolol), muscarinic & nicotinic receptor blocker (atropine) were preferred to compare with test drug in optimum concentration.

Table no 1: Preliminary PhytoChemical screening results

Sl.No	Plant	Test/Reagent	Aqueous extract of normal leaves of <i>Ocimum tenuiflorum</i>	Aqueous extract of fungus infected leaves of <i>Ocimum tenuiflorum</i>
1.	Steroids	Salkovaski	---	+++
2.	Alkaloids	Dragendroff's test	---	---
		Hager's test	---	---
		Mayer's test	---	---
		Wagner's test	---	---
3.	Saponins	Foam test	---	---
		Haemolysis test	---	---
4.	Fats and oils	Filter paper test	+++	+++
5.	Tannins and phenolic	Ferric chloride test	+++	---
		Lead acetate test	+++	---
		Plot. Dichromate	+++	---
		Bromine water	+++	---
6.	Flavonoids	Shinoda test	+++	+++
		Lead acetate test	+++	+++
7.	Carbohydrates	Molisch test	+++	+++
		Fethling's test	+++	+++
		Barfoed's test	+++	+++
8.	Proteins	Millon's test	+++	---
		Xanthoproteic test	+++	---
9.	Amino acid test	Ninhydrine test	---	+++

+++ indicates present ; --- indicates absent

RESULTS AND DISCUSSION

Comparative Preliminary Screening of Normal and Infected Leaves of Aqueous Extract of *Ocimum tenuiflorum*

After successful extraction of aqueous extract of normal leaves and *Aspergillusniger* infected leaves preliminary various phytochemical screening include tests were performed. Here this screening shows the presence of carbohydrates, flavanoids, fats and oils in both normal and infected aqueous extracts. Further it shows that in the normal uninfected aqueous extract tannins and proteins are present and steroids, alkaloids, saponins, amino acids are absent. Where as in the infected leaves aqueous extract shows the presence of steroid, amino acids and absence of saponins, alkaloids, proteins and tannins.

Comparative positive Inotropic and Chronotropic effects of normal versus infected leaves of aqueous extracts of *Ocimum tenuiflorum*

Primarily aqueous extract of normal leaves of *Ocimum tenuiflorum* was been compared with universal standard drugs such as adrenaline, calcium chloride, potassium chloride, acetylcholine, propranolol, atropine. Adrenaline and calcium chloride are used for C.V.S stimulant action. Acetylcholine and potassium chloride are used for C.V.S depressant action. Propranolol and atropine are used as blockers. From this primary conclusion, the non-infected leaves were not matched with any universal standard drugs and it is then compared with digitoxin and found to have similar actions. Further this study has been undergone with infected leaf extracts and compared with universal standards. It is found that infected leaves having similar actions like adrenaline. Infected shows positive ionotropic and positive Chronotropic effects.

Table no 2: Table showing action of various drugs on Frog heart

Sl.No.	Materials	Dose	Cardiac Output	Heart Rate
1.	Normal	0.2 ml	18.0 ml	44
2.	Acetylcholine	0.2 ml	12.0 ml	28
3.	Adrenaline	0.2 ml	21.6 ml	51
4.	Calcium Chloride	0.2 ml	22.2 ml	21
5.	Digitoxin	0.2 ml	20.4 ml	23
6.	Potassium Chloride	0.2 ml	10.5 ml	19
7.	Test	0.2 ml	10.0 ml	20

Table no: 3 Table showing comparisons b/w Uninfected test drug and Infected test drug

Sl.No.	Materials	Dose	Cardiac Output	Heart Rate
1.	Uninfected test drug	0.2 ml	9.5 ml	20
2.	Infected test drug	0.2 ml	19.8 ml	50

CONCLUSION

Aqueous extract of infected leaves shows significant positive ionotropic and chronotropic activity. This may be because of biotransformation taken place after the infection of healthy and live plant species of *Ocimum tenuiflorum* which is indicated by the presence of aminoacids and a steroidal compound. This study concludes after infection of normal leaves shows the presence of steroids and amino acids might be reason for activity. Further study suggest isolating the type of steroid present after bio transformation of phytochemical to be identified with the help of modern analytical procedure and bring out single compound for the treatment of specific disease.

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