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INVESTIGATE ON THE LEAVES OF *CINCHONA OFFICINALIS* L – IT'S DISTINCT *IN VIVO* REVERSAL OF TACHYCARDIA INDUCED BY SYNTHETIC CATHINONES ON MICROCRUSTACEAN *DAPHNIA MAGNA* ARRHYTHMIC HEART.

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ABSTRACT

Objective: To screen the *invivo* cardio protective potential of alkaloid enriched fraction of the leaves *Cinchona officinalis L* Family Rubiaceae using the model organism *Daphnia magna* along with acute toxicity assessment. **Method:** The cardioprotective effect of ethanolic extract (EE) of leaves *C.officinalis in vivo* syntheticcathinone induced cardiac arrhythmia on the myogenic heart of *D.magna* (water flea) innovative model system for studying chronotropic and inotropic effects and its effectiveness comparable to currently available therapeutic agent verapamil (1.5µM).Video microscopy and digital image processing were used to study the pharmacological effects on the heart. Initialy, the leaves extracted with ethanol and separate the alkaloidal enriched fraction, acute toxicity assessment were determined. HPTLC profile of EE *C.officinalis* showed the presence of the presence of Rutin (2mg/g), Quinine (1.5mg/g), Gallic acid (1.12mg/g), and Quercetin (2.4mg/g). **Results:** Normal heart beat of the *D.magna* at 20±2°C was found to be 215 beats/min. Arrhythmia was induced by synthetic cathinone (2µM) in the bathing medium. The EE of the leaves of *C.officinalis* (0.5, 1.0, 1.5µg/ml) prevented the synthetic cathinone induced arrhythmia in dose dependent manner. Assessment of acute toxicity showed LC50 of EE *C.officinalis* 1.5µg/ml. *C.officinalis* is economically potential species**. Conclusion:** EE of the leaves of *C.officinalis* may be therapeutically effective agent on arrhythmic heart without toxicity. We also assumed that the cardio protection may be due do the presence of quinine, rutin, quercetin. We recommend further investigations in animal model and clinical trials to confirm this potential therapeutic effect.

KEYWORDS: *Cinchona officinalis*, Rubiaceae, cardiac protection, *Daphnia magna*, Alkaloid.

INTRODUCTION

Cinchona officinalis is one traditional medicinal plantbelonging to the family Rubiaceae. Its extracts are the principle ingredient in Indian Ayurvedic formulations to cure various fever types including malaria.[9]

C.officinalis Linn (CROWN or LOXA BARK) is known by other names Quinine, Quinine bark, Peruvian bark (English), Sinkona, Kunayanah (Sanskrit), Cinchona, Kunain (Hindi), Cinkona (Tamil).[12]

A genus of evergreen shrubs or trees, distributed in tropical America and grown in India, Indonesia, Srilanka and more recently in Africa.At present cinchona is cultivated in the states of west Bengal and Tamil Nadu (Anamalai, Naduvattam, Shevroys, Pulney and parts of Tirunelveli).[2,12] The plant growing up to a height of 10-12 meters with a diameter ranging from 15-30 cm.[12]It is 6-15cm in height with rough brown bark, having pale yellow markings, inside and dull white ones outside, 7-15cm long. Ovate-lanceolate leaves with reddish greenpetioles, red flowers in coryliform cymes, oblong 12-20mmlong capsules and elliptic winged seeds. It thrives well at higher elevations up to 2,600m in South India.[2]

Cinchona distributed worldwide in the second part of the 19th century. Around 1880, Srilanka had become a major producer of cinchona bark, albeit of low quality. By 1895it had been superseded by the Dutch East Indies (Indonesia) as the main producer, mainly because the better quality ofthe bark (*C.officinalis*). The crop was introduced into west and East Africa and central Africa in the 1930s. In Asia, cinchona cultivation is still important in Indonesia and India.The centre of variety lies along the Andes Mountains of Bolivia, Peru, Ecuador, Colombia and Venezuela. The humid eastern slopes at 800-3000-3700m attitude.[15] Decoction of leaves from *C.officinalis* is found effective against amebiasis.[16]It was reported that leaves contain quercetin, kaempferol and avicularin, in fresh leaves of *C.officinalis* found 0.0035% of alkaloid; 0.0015% of which was quinine.[4] Previous report, the Quinine is an effective and convenient antiarrhythmic drug for the suppression of ventricular arrhythmias in humans.[13]

The present study investigate the cardio protective effect of the alkaloid of the leaves of *C.officinalis* (Quinine) using the model organism *Daphnia magna*. The purpose of this study is to determine if *D. magna* heart rate behaves similarly to the heart rate of humans, transparent, size and this organ can be easily observed by optical methodologies.[10] Therefore, the water flea model is potentially useful for the evaluation of toxicity of candidate compounds in therapeutic drug screening for arrhythmia.

MATERIALS AND METHODS

Spirulina, Elendt- Bias (M4) medium, *D.magna*,spring water, glass tube with rubber teats, synthetic cathinone, Verapamil, ethanolic extract, CAMAG TLC Scanner 3, video microscopy CCTV and photomicrograph.

COLLECTION AND AUTHENTICATION OF LEAVES OF *C.OFFICINALIS*

Leaves of the plant ***C.officinalis*** selected for our study was collected from **Valparaitaluk, Coimbatore District**, Tamilnadu, India during the month of July 2016 and wasauthenticated by **Dr.Stephen,** Department of Botany, American college, Madurai and **Dr.Sasikala,** Director (Retd) of Siddha Central Research Institute, Arumbakkam, Chennai.

ETHANOL EXTRACTION OF *C.OFFICINALIS* LEAF AND ITS SEPARATION OF ALKALOIDAL ENRICHED FRACTION

Leaf parts were cut into very small quatrates and boiled in alcohol containing ½% of HCL (20cc.concentration HCL on the Liter) for an hour. This took place on the water bath in Erlenmeyer bottles, stoppered by a cork into which a long glass tube, serving as a cooler, was put the alcohol was afterwards poured into porcelain dishes, placed on the water bath and the whole evaporated until nearly dry. Afterwards the dishes were filled up with water and evaporated again until nearly dry also, so as to be sure of the total escape of the filtrate collected in a separatory. After adding caustic potash until alkaline solution it is shaken with chloroform, the chloroform collected in a watch glass, put on the water bath and all chloroform evaporated. The residue is dissolved in water bath, containing ½% of HCL (20cc. concentration HCL to the Liter.) By strong rubbing the resinous substances, sticking to the watch glass are mixed with this solution, the whole filtered and the filtrate used for the alkaloid tests.[4]

We obtained HPTLC profile of ethanolic extract of alkaloid enriched fraction using CAMAG TLC Scanner 3.The following parameters used winCATS Planar Chromatography Manager

software, Detection- 254nm, Stationary phase- HPTLC plates silica gel 60 F 254, Sample preparation- 100mg per ml of sample was prepared in ethanol solvent, Sample solution- 5µL, Standard solution- 5µL, Drying device- Oven, Temperature- 60°C, Volume- 10.0ml, Scanning speed- 20mm/s, Time- 5minutes, Wavelength- 254.

CULTURE OF *D.MAGNA*

D.magna obtained from the local aquarium in Madurai, Tamilnadu. It was identified and authenticated by Prof (Major) P.Chandrasekaran, Principal, ManonmaniamSundaranar University Constituent Model College, Vilathikulam, Nagalapuram 628 904, ThoothukudiDt, Tamil Nadu. (Formerly Faculty of PG and Research, Dept of Zoology and Biotechnology, Vivekananda College, Thiruvedakam West 625 217, Madurai,Tamilnadu) *D.magna*was cultured by using Elendt- Bias (M4) medium and maintained photoperiod ±12hr. spirulina used as a feed in spring water. Aerated for 48hr to obtain O2 concentration not less than 4mg/ml. experiment was carried out at 20°C±2°C and away from the sunlight.[6,8]

ASSESMENT OF ACUTE TOXICITY OF ALKALOID ENRICHED FRACTION OF *C.OFFICINALIS* LEAVES ON*DAPHNIA MAGNA*

24 hr old *Daphnids* selected for this study. Since neonates may be more sensitive than elder one. Moreover more specificity, simplicity and do not reproduce. *Daphnids*in spring water culture transferred to depression cavity (n=20). No food feed throughout the study. Administrate different concentrations of extract (0.5, 1, 1.5,2, 2.5µg/ml) temperature 20°C±2°C maintained. Observe the mortality rate and immobility after 24 hr. LC50 was calculated by using probit analysis method.[1,3,7]

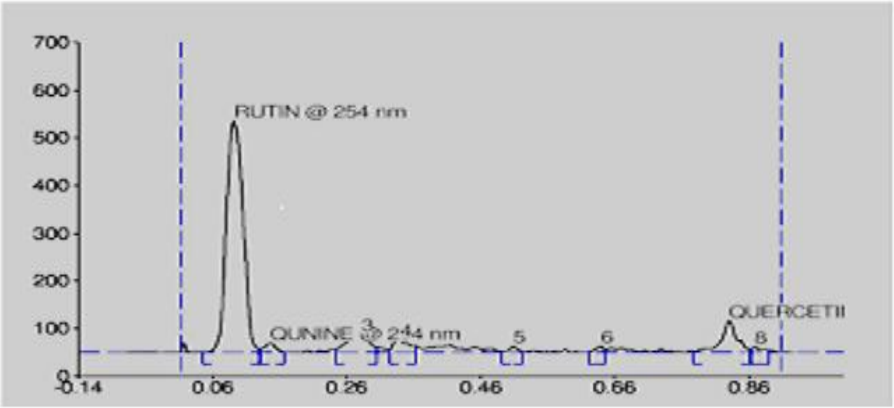
EVALUATION OF THE EFFECT OF ALKALOID ENRICHED FRACTION OF *C.OFFICINALISON* SYNTHETIC CATHINONE INDUCED ARRHYTHMIC HEART OF *D. MAGNA*

Place the *Daphnia* in the depression cavity along with drops of water using glass tube with rubber teats. Divided *Daphnia* into three groups (n=10).*D.magna* incubated 30 seconds in three different solutions. Control, synthetic cathinone (2Mm) induced, test drug treated (0.5, 1, 1.5µg/ml), standard drug Verapamil treated (1.5µM) on the heart of *D.magna*. Negative control of *D.magna* treated with water. Heart rate and rhythm were observed under low power microscope with video microscopy CCTV and photomicrograph. Image processing: The image processor used to allow real time operations (i.e 25 frames/s as input source which was essential for analyzing fast heart movements as the implementation of algorithms

possible, if the operation is divided into sequentially executable parts. The data were analysed by one way ANOVA.[5, 10, 14]

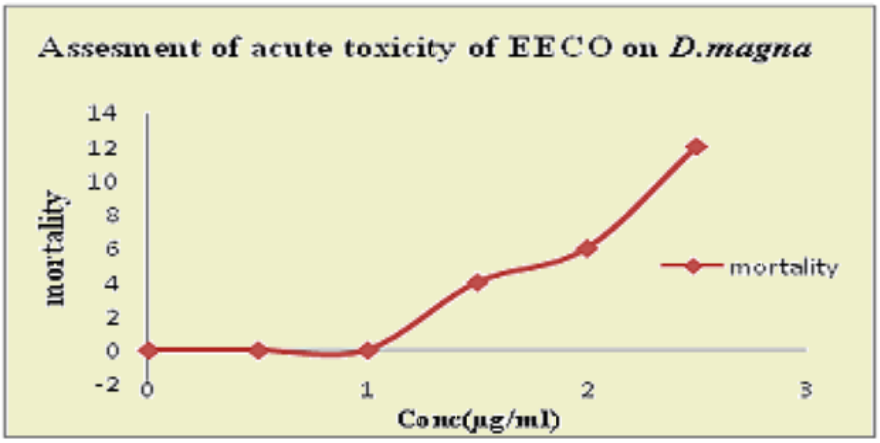
RESULT AND DISCUSSION

- From the chemical test to report the presence of alkaloid in ethanolic extract of *C.officinalis* leaves.
- HPTLC profile of the ethanolic extract of alkaloid showed the presence of rutin, quinine, galic acid and quercetin. ("Fig.1")



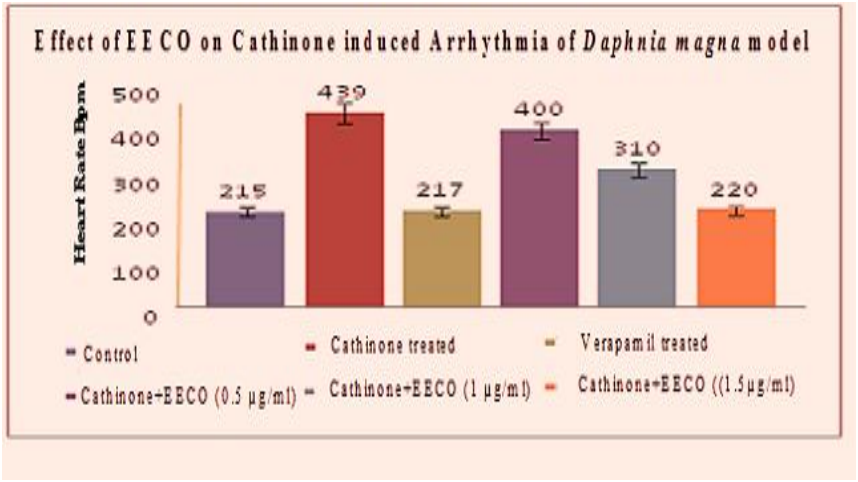
"Fig. 1: "HPTLC profile of the ethanolic extract of the leaves of *Cinchona officinalis L*.

- From that report of acute toxicity assessment, LC50 of ethanolic extract of *C.officinalis* of the leaves was found to be 1.5µg/ml denotes it's safe and non-toxic to *Daphnia*. **("Fig. 2")**



"Fig. 2:" Assesment of acute toxicity of ethanolic extract of *C.officinalis* on *D.magna*.

- The heartbeat of control, synthetic cathinone induced, and ethanolic extract of *C.officinalis* leaves 0.5, 1, 1.5, 2, 2.5µg/ml treated, standared drug Verapamil 1.5µM were found to be 215, 439 and 400, 310, 220, 217 bpm respectively. (**"Fig. 3")**



"Fig. 3: "Heart rate of control and different concentration of EE of *C.officinalis* treated on synthetic cathinone induced arrhythmic heart of *D. magna*.

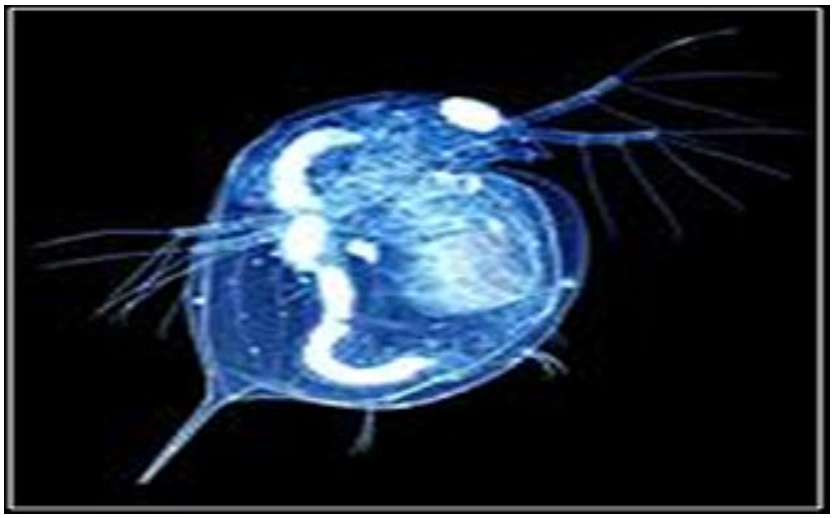


Image of D.magna (water flea).

CONCLUSION

This study covers on the widely available a member of the family Rubiaceae is known botanically as *Cinchona officinalis* L. commonly called as Cinchona. Research on development of herbal products from this plant is required to be initiated immediately for exploring the unique potential of this crop which would also minimize the menacing wastage especially the leaves. It is traditionally known to be useful for the treatment of wide panel of diseases like malaria, analgesic in common cold, cough, influenza, amoebic dysentery,

cardiac depressant, particularly in auricular fibrillation, cardiac arrhythmias, management of irregularity of the heart, mild central nervous system depressants, local anaesthetics, hypotensive, analgesic, apsychic depressant, cytotoxic, sclerosing agent, haemorrhoids, ophthalmology and leave is used for amebicide.[2] It was reported that Quinine is an effective and convenient antiarrhythmic drug for the suppression of ventricular arrhythmias in humans.[13] Its physicochemical parameters were studied. We extracted alkaloid enriched fraction from the leaves of C. officinalis using ethanol. The HPTLC profile of EE C. officinalis was obtained showed the presence of quinine, rutin, and quercetin. We have investigated the effect of EE *C.officinalis* leaves on synthetic cathinone induced *D.magna* on arrhythmic heart. The results were encouraging that it is comparable to that of the standard drug verapamil. Previous report and HPTLC profile showed the presence of quinine. Moderate dose of quinine in useful in cardiac depressant, particularly auricular fibrillation and its used for treating cardiac arrhythmia.[2] Hence the antiarrhythmic potential of alkaloid rich fraction may be due to the presence of quinine and may also be due to the presence of flavonoids rutin, quercetin.

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