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ANTIARRHYTHMIC EFFECT OF THE LEAF VOLATILE OIL OF *ELETTARIA CARDAMOMUM MATON*. ON THE HEART OF *Daphnia magna*

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ABSTRACT

Objective: To prescreen the *in vivo* cardioprotective activity of the leaves of *Elettaria cardamomum.* Maton Family Zingeberaceae using the model organism *Daphnia magna* along with preliminary acute toxicity assessment. **Method:** Thecardioprotective effect of the volatile oil of the leaves of *E.cardamomumin vivo* on the lactose induced arrhythmic heart of the cladocerans *D. magna* (Water flea) a novel model system for studying effects of agonists and toxins on cell signalling and ion channels in situwas evaluated. Initially GCMS of the isolated VO, acute toxicity assessment were determined. **Results**: GC-MS profile of the VO showed the presence of 4-terpineol, 1:8 cineol, α-terpinolene, α-Tujene, α-pinene, sabinene, linalool, α-terpineol, p-cymene, α-terpinene, endbornyl acetate. Normal mean heart beat of the *D.magna*at 20±2°C was found to be 180.1± 1.0 beats/min. Arrhythmia was induced by lactose (50mM) in the bathing medium. The VO of the leaves of *E.cardamomum*(0.5, 1.0, 1.5, 2.0µl/ml) prevented the lactose induced arrhythmia in dose dependent manner. Previous assessment of toxicity showed LC_{50} 5µl/ml. **Conclusion:** *E.cardamomum*. (cardamom) has long been recognized and economically is of appreciable importance as a spice. This study indicates that the VO of the leaves of *E.cardamomum* possesses potential cardio protective activity on the lactose induced arrhythmia of the *Daphnia* heart without any toxicity and mortality. It is assumed that this may be due to the presence of terpenes in the VO.Further investigation requires confirming this activity.

KEYWORDS: *Elettariacardamomum*, Zingeberaceae, Cardioprotective action, *Daphnia magna*, Volatile oil.

INTRODUCTION

Elettaria cardamomum Family: Zingiberaceae is a spice and widely used in traditional medicine. These genera received a great level of scientific interest as they contain medicinally important secondary metabolites possessing useful biological activities.

E.cardamomum is used in folk medicines of South-East Asia, Indonesia, India and many parts of the world. It provides advantages as a profitable multipurpose crop.

The *E.cardamomum* is a perennial herb is known by other names cardamom (Eng.), ChotiIlayachi (Hindi), Chotaelaich (Beng.), Elam (Tamil), Elachi, velloda (Guj & Mar) & Elam, Chittelam (Malayalam).[1], which can grow to heights of 12 feet. It is a native of moist evergreen forests of Western Ghats of Southern India from where it spread to some other tropical countries such as Sri Lanka, Tanzania and a few Central American Countries. It is growing regions of South India are located at elevations ranging from 800- 1300 m above mean sea level with annual rainfall of 1500- 7000mm and temperature range of 10-35°.[2]

The fruits and leaves used in the treatment of cardiac disorder, gastrointestional disorder, indigestion and flatulence and also treatment of diarrhea, nausea, bronchitis and strengthening nervous systemand also used for influenza, infection, asthma, cataract etc.[3] Volatile oil of the leaves of *E.cardamomum*used as high class perfumery and as a flavouring agent in beverages,syrups, baking product, ice cream and pharmaceuticals.[4] It was reported that the leaves contain volatile oil, alkaloids, tannins and lipids. It was reported that the leaves contain 129.6± 6.9 mg/g flavonoid and vitamin C 19.22 ±1.1mg/g.[5,6,14] The present study investigate the cardiac effect of the VO of the leaves of *E.cardamomum*(containing terpenes) using the model organism *Daphnia magna*. The small fresh water crustacean *D.magna* (0.2-3mm) was used in this experiment because of their transparent carapace, which allows for increased visibility of the internal organs and makes monitoring the heart rate of the individual easier.[7] Of all sequenced genomes belonging to the

animal group composed of insects and crustaceans, *Daphnia* share more genes with humans.[8] It exhibits a short life span, rapid maturation and reproduction. The heart of the water flea, *D.magna*, regulated by cholinergic neurons and may be useful as a model for the effect of drugs on cardiovascular function and unusual among crustaceans in that they possess myogenic hearts. Testing the effects of the drugs is simplified in *D.magna* as the fleas are responsive to pharmacological agents added to the water in which they swim. The introduction of these pharmacological agents to water fleas may induce activity directly on the cardiac muscle.[9]

MATERIALS AND METHODS

Lactose, Elendt and Bios medium, spirulina, VO. All chemicals used are Sd fine chemicals. GC-MS JEOL GC MATE II, Laboscope model Microscope with Photomicrograph & CCTV.

Collection and authentication of the leaves of *E.cardamomum*

Leaves of the plant *E.cardamomum*. selected for our study was collected from Hailey buria **estate, Idduki District**, Kerala, India during the month of July 2015 and was authenticated by **Dr. Stephen,** Department of Botany, American college, Madurai and S. Sasikala, Director (Retd) of Siddha Central Institute, Arumbakkam, Chennai. Voucher Specimen was deposited in our laboratory (PCG 290).

Isolation of Volatile Oil (VO) From The Leaves of *E.cardamomum*

Weighed quantity of fresh leaves was subjected to hydrodistillation using Clevenger apparatus used for the determination of VO in which Clevenger oil arm fitted with condenser through which cooled water was circulated to prevent low volatiles from evaporation. The oil sample was dried over anhydrous sodium sulphate and kept in scaled glass bottles and stored in refrigerator.

We obtained GC-MS profile of the Isolated VO using JEOL GC MATE II.

The following parameters used Front temp-220 ° c, Column-HP 5 Ms, Carrier gas-high pure helium, Flow rate-1ml / min, Oven temp-50 to 250 @10°c, Ion chamber temp-250°c, GC interface temp-250°c, Mass analyser-Quadrupole with double focusing mass analyser, Detector-Photon multiplier tube, Scam-50 to 600 amu 70ev, Electron impact ionization

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, Manonmaniam Sundaranar University Constituent Model College, Vilathikulam, Nagalapuram 628 904, Thoothukudi Dt, 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 O_2 concentration not less than 4mg/ml. experiment was carried out at 20°C±2°C and away from the sunlight.[10]

Assesment Of Acute Toxicity Of VO Of *E.cardamomum*Leaves On *D.magna*:

48 hr exposure of *D.magna*to different concentrations (1, 2, 3, 4, 5, 6 µl/ml) of VO was observed. One day old *daphnids*were selected for this study, since neonates may be more susceptible to toxic substance than elder one. Moreover more specificity, simplicity including easily handled in lab & less expensive. Temperature 20°C ±2°C is maintained. No food feed throughout the study. Test substance was added directly to the water at various concentrations. Mortality rate was observed after 24 hr and LC_{50} was determined.[11,12,13]

In vivo Cardioprotective activity of the VO of the leaf of *E.cardamomum* on *D.magna*

The heart rate of control & treated groups (Lactose and VO 0.5, 1.0, 1.5 and 2.0µl/ml) were monitored by transferring *D.magna*to depression slide slightly coated with petroleum jelly.[4] Heart beats were observed under light microscope and recorded by using Nikon coolpix camera. It was counted by image processing technique which allowed real time operations i.e. 25frames/sec.[14,15]

RESULT

1. The yield of isolated volatile oil from the leaves was 1.3%.
2. GC-MS profile of the VO showed the presence of 4-terpineol, 1:8 cineol, α-terpinolene, α-Tujene, α-pinene, sabinene, linalool, α-terpineol, p-cymene, α-terpinene, endbornylacetate.“Fig. 1”.

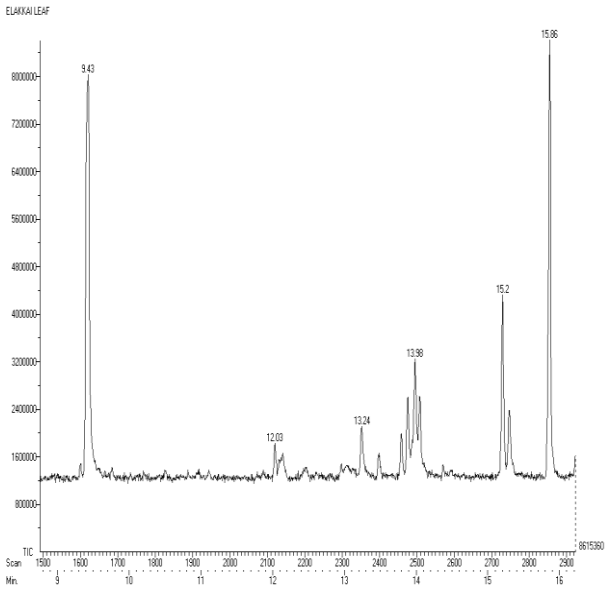

Figure-1: GC MS profile of the VO from the leaves of *E.cardamomum*

3. From the result of toxicity assessment of VO it is clearly observed that it is safe and non toxic to *D.magna*. No significant mortality was observed up to 6µl/ml. LC_{50} 5µl/ml"Fig.2".

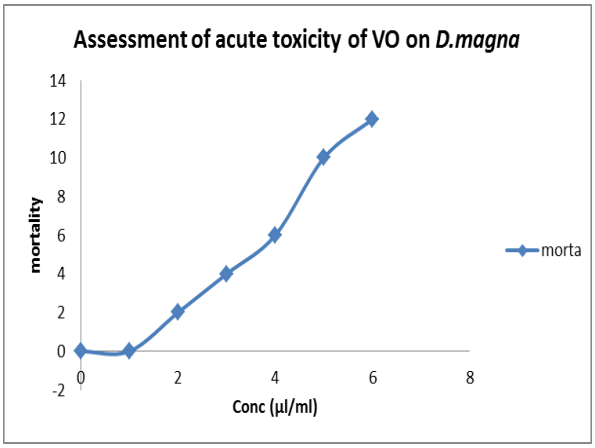

Figure-2 Assessment of Acute Toxicity of VO On *D.magna*

4. The heartbeat of control, lactose induced, VO 0.5, 1.0, 1.5, 2.0µl/ml treated, std drug metoprolol 20µg/ml & 25µg/ml were found to be 180.1± 1.0, 78.6± 1.30, 134.8± 0.79, 161.9± 0.62, 177.7± 0.67 and 179.9± 0.37 and 162.2± 0.89, 181.7± 0.35 bpm respectively. "Fig. 3".

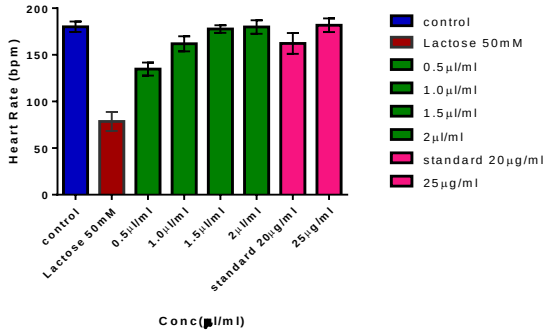

Figure-3: Heart rate of control and different concentration of VO treated on the lactose induced arrhythmic heart of *D.magna.*

Image of *D.magna* (water flea)

DISCUSSION

The leaves of *E.cardamomum* really do not have any match as a cheap natural and easily available plant. It is traditionally known to be useful for the treatment of wide panel of diseases like cancer and as analgesic, antiulcerogenic, antiasthmatic, gut modulatory, blood pressure lowering, diuretic, sedative, enhancement of fibrinolysis, antioxidant, antibacterial, antifungal, antiviral, carminative, gastro protective, immunomodulatory etc.[16] It is used for influenza, infection, asthma, bronchitis, cardiac disorder, diarrhea, nausea, cataracts and strengthening of nervous system etc.[4] It was reported that cardamom leaves has anti diabetic, hypocholesterolemic effect, antimicrobial etc.[17] We isolated the volatile oil from the leaves (1.3%). Its physicochemical parameters.were studied. The GC-MS profile of the isolated volatile oil was obtained. Presence of 4-terpineol, 1:8 cineol, α-terpinolene, α-Tujene, α-pinene, sabinene, linalool, α-terpineol, p-cymene, α-terpinene, endbornyl acetate. The variation in the constitution when compared with the previous report[4] may be due to the change in the environmental conditions. Based on the above facts we have investigated the effect of VO on the lactose induced arrthymia of *D.magna* heart. The results clearly showed the dose dependent protective effect on lactose induced arrthymia of the heart of *D.magna* by VO "Fig.3". It is assumed that this cardioprotective effect may be due to the polyphenolic content and terpenes. Assessment of acute toxicity study reveals its safety and non-toxic nature. So it is concluded that volatile of the leaves of *E.cardamomum* possesses cardio protective effect without toxicity. Further investigation on animal model and clinical trials are required.

Conflict of interest statement

We do not have any conflict of interest.

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