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A new pharmacological insight of cardio protective effect of the leaves of *Cucurbita maxima*Duch. ex Lam (family Cucurbitaceae)on the crustacean *Daphnia magna*- A synoptic review

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ABSTRACT

This study aims to evaluate the effect of the *in vivo* cardio protective activity of the chloroform extract of *Cucurbita maxima*leaves (CECML) *in vivo* on the lactose induced arrhythmic heart of the cladocerans*Daphnia magna*(water flea), a novel model system for studying effects of agonist and toxins on cell signalling and ion channel *in situ*. Initially performed various phytochemical screening, acute toxicity assessment, Electron dispersive X-ray Spector photo meter(EDS) for the presence of various trace elements, determination of flavonoid content, total phenolic content, identification of the presence of cucurbitacins by TLC. Preliminary phytochemical screening of appropriate solvent extract of the leaves showed the presence of proteins, aminoacids, flavonoids, terpenoids, saponin, carbohydrates, tannins, phyto sterol, glycoside and alkaloids. Volatile oil and fixed oil were found to be absent. The presence of cucurbitacin was confirmed by TLC. Total Flavonoid content of CECML 21.6 mg/g (as quercetin). Total phenolic content 18.2 mg/g (as gallic acid).Trace elements analysis by EDS showed the presence Ca, K, P and Mg. Acute toxicity assessment using benchtop bioassay BSLA (Brine Shrimp Lethality Assay) showed LC_{50} 4.65 µg/mL in 24 hours. The heart beat of control, lactose induced CECML (10, 20, 30, 40 µg/ml) treated were found to be 193.2±1.5, 93±0.89, 136.2±1.12, 157.6±1.1, 178.8±0.9, 189.1±0.68 respectively. Standard drug metoprolol showed 180.3±2.5, 191.2±0.75. The result was dose dependent and statistically significant ($p<0.001$). It was conclude that the study indicates that the CECML possess potential cardio protective activity on the lactose induced arrhythmiaof the *D.magna*heart without any toxicity and mortality. It is assume that this may be due to the presence of cucurbitacin, polyphenol content and trace element like Ca, K and Mg. Further investigation required to confirm the activity.

Keywords: *Cucurbita maxima, Cucurbitaceae, Cucurbitacin, Daphnia magna, anti arrhythmic.*

1. INTRODUCTION

C All plant parts are used as well known home remedies for throughout the world, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the world drug market. In this study we selected a widely available plant *Cucurbita maxima*(Cucurbitaceae). It is popularly known as Pumpkin, in English and parankikai, sakkaraipoosani in Tamil.It is monoecious, trailing herb, Annual plant, cylindrical stem 10meter length strongly branched, latral branches are

longer then vein, tendrils are mostly branched, climbing by latral, 2-5 branched tendrils. Stems rounded soft long running softly pubescence. The leaves are reniform, simple, not lobed, softly hairy, occasionally with white plotches, 5-8-13 cm in size, green colour, finely toothed margin, deeply cordated, 3-veined from the base and petiole 5-20 cm long. It was reported that fresh leaves contains β-carotene 743.9±35.0 µg/gm dry weight, lutein-1534.448µg/gm dry weight, Neoxanthine-327.813.7µg/gm dry weight Vialoxanthine-818.528µg/gm dry weight [1], protein-18.6%, crude fibre-9.46%, lipid-4.46%, carbohydrate-51.3%, moisture-7.21% and ash-8.95%. tannin-0.220, phylate-10.3, phenolic-0.15, phytin phosphorus-2.9, oxalate-1.42mg/100gm dry weight and saponions-0.85, flovanoid-0.51% Minerals percentage Na-19.5, K-32.4, Ca-15.4, Mg-11.5, Zn-17.7, Iron-5.22 lead- <0.001, Cd<0.001, Mn-5.6, Cu-0.37, P-65.8, Na/K-0.602, Ca/Mg-1.34, K/Na-1.66,Ca/P-0.234mg/100gm of the leaf dry weight and [K(Ca/Mg)-2.41meq ratio] [2]. *C.maxima* leafs contain 569mg/100gm of methionine and it is good source of Vitamin A, C,E and B and iron. Steam distillation of leaves does not give essential oil [3]. Leaves are used in nervous disorder, cancer, helminthiasis, cooling effectin the body, gastrointestinal problem, joint pain, cold, piles, dysentery, oedema, skin disorder, leprosy, rheumatoid arthritis, chicken pox [4], anaemia, malaria [5], anti-HIV [6], diabetic, inflammation, immunomodulatory, antibacterial [8], hypertension [17], burns, scalds, inflammation, abscesses, boils, migraine, neuralgia, haemoptysis [9], diuretic, refrigerants, prostate,problem, ascarisis [10], aerial parts are used for hypoglycaemic [11], antioxidant [12], hepatoprotectivity [13], antidiabetic [14], anti cancer [7, 15], caridoprotectivity [16-20]. In short, there is good level of traditional and experimental evidences to support various claims and advantages of this widely available plant. As mentioned earlier several reports have been published on the effects of the plant extract and chemical constituents on different biological activities *in vitro* and *in vivo*.

There have been a plethora of reports on the role of cucurbitacin for their cytotoxic, hepatoprotective, cardiovascular and anti diabetic effects[21]. Cucurbitacina terpenoid possess immense pharmacological potential and may prove to be lead molecule for future research. We planned investigation to explore its effect on heart 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 [22] Of all sequenced genomes belonging to the animal group composed of insects and crustaceans, Daphnia share more genes with humans [23]. 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 [24].

2. Materials and Methods

Lactose, Elendt and Bios medium, spirulina, double distilled water. All chemicals used are SD fine chemicals. For the determination of trace element by Hitachi Scanning electron Microscope 3000 H model and research binocular microscope (laboscope) with eye piece camera linked to monitor.

2.1. Collection and authentication of the leaves of *C.maxima.*

The leaves of the healthy plant *C.maxima* selected for our study was collected from Keelvani, Erode Dist, Tamilnadu, India during the month of July 2017 and was authenticated by Dr.Stephen, Department of Botany, American college, Madurai.

2.2. Preparation of extract

The shadow dried leaves was powdered sieved in a No.60 meshand macerated with chloroform. The filtrate evaporated under vacuum. The residue obtained (CECML) was stored in the refrigerator until further use.

2.3. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedures [25-27].

2.4. Estimation of flavonoid content

The flavonoid content of plant extract was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm [28-30].

2.5. Estimation of total phenolic content

The total phenolic content of the CECM was determined by FolinCio-calteau reagent. This reagent consists of phosphotungstate and phosphomolybdate mixture which is reduced to mixture of blue molybdenum and tungsten oxides while phenolic content of the extract was oxidized. The intensity of colour is proportional to the amount of phenolic content of the extract and which was measured at 765nm. The total phenolic content in CECML was expressed as milligrams of gallic acid equivalent (GAE) per gm of extract [31-32].

2.6. Determination of trace elements

Percentage of various trace elements like Ca, Mg, K, Na etc was determined by Energy dispersive X-ray spectrometer (EDS) built in scanning electron microscope (SEM) Back scattered electron images in the SEM display compositional contrast yet results from different atomic number of the elements and there distribution [33-35]. Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with I sq. cm glass slide and kept in carbon adhesive sheet. Samples were coated with gold to a thickness of 100 AO using hitachi vacuum evaporator. Coated sample were analysed ina Scanning electron Microscope

2.7. TLC profile of the CECML

CECML was applied as a band on aluminium sheet pre coated with slica gel 60 GF 254. The plates were developed in the mobile phase Toluene : Ethyl acetate (25: 75) to a distance of 10 cm. Vanillin sulphuric acid was used as a spraing agent. Spots absorbed under UV 360nm [36].

2.8. Toxicity assessment of CECML

Toxicity assessment of CECML 48 hr exposure of *D.magna* to different concentrations (1, 2, 3, 4, 5, 6 µg/L) of CECML 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 handle in lab & less expensive. Temperature 20°C ±2°C is maintained. No food feed through 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 [37].

2.9. Culture of *Daphnia magna*

D.magna obtained from the local aquarium in Madurai, Tamilnadu, India. It was identified & authenticated by Prof (Major) P.Chandrasekaran, Principal, ManonmaniamSundaranar University Constituent Model College, Vilathikulam, Nagalapuram 628 904, ThoothukudiDt,Tamil Nadu, India. (Formerly Faculty of PG and Research, Dept of Zoology and Biotechnology, Vivekananda College, Thiruvedakam West 625 217, Madurai, Tamilnadu, India. *D.magna*were cultured by using Elendt-Bias(M4) medium[38] (figure 1) 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 at 20°C±2°C.*In vivo*cardioprotective activity of the CECML on *D. magna*: The heart rate of control & treated groups (Lactose and CECML 10, 20, 30, 40 µg/ml), metoprolol (20, 25 µg/ml)was used as standard drug were monitored by transferring *D.magna* to depression slide slightly coated with petroleum jelly. Observed under light microscope, heart beats were noted and recorded by using Nickon high pixel camera. It was counted by image processing technique which allowed (25 frames/sec) real time operations [22-24]. Data expressed as ± SEM and graphically presented. Statistical significance was calculated by one way Anova.

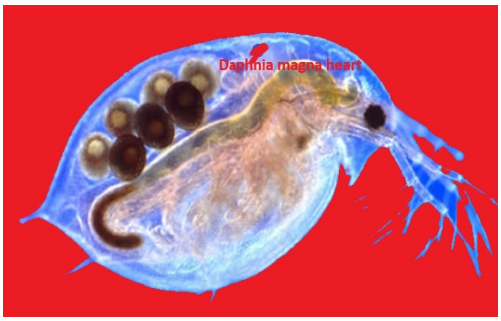

Figure - 1: Image of Daphnia magna [Water flea]

3. Results

Pale green viscus CECML (19.5%w/w) was obtained. Preliminary phytochemical screening of appropriate solvent extract of the leaves showed the presence of alkaloids, steroids, tannins, proteins and aminoacids, flavonoids, terpenoids, saponin, glycosides, carbohydrates and absence of volatile oil, mucilage, fixed oil. Flavonoid content of CECML in terms of quercetin was found to be 21.6 mg/g. Total phenolic content of CECML in terms of gallicacid was found to be 18.2 mg/g.Estimation of the elements by EDS showed the following mg weight percentage O- 46.72, Mg- 0.76, P- 0.12, S- 0.07, Cl- 0.22, K- 1.50, Ca- 2.82, C- 40.40, N- 4.11, Al- 0.22, Si- 3.06 and atomic percentage O- 42.65, Mg- 0.46, P- 0.06, S- 0.03, Cl- 0.09, K- 0.56, Ca- 1.03, C- 49.12, N- 4.29, Al- 0.12, Si- 1.59.The Co TLC of CECML showed 3 spots of R_f value 0.03, 0.36, 0.82 respectively (the $R_f$value of cucurbitacin was 0.36 (correlated with

reported value) [36]. The presence of cucurbitacin in the CECML was confirmed by Co TLC.

Acute toxicity study was performed by benchtop bioassay BSLA (Brine Shrimp Lethality Assay) LC_{50} for them was found to be 4.65µg/ml and non-toxic.

The heart beat of control, lactose induced CECML (10,20,30,40 µg/ml) treated were found to be 193.2±1.5, 93±0.89, 136.2±1.12, 157.6±1.1, 178.8±0.9, 189.1±0.68 respectively. Standard drug metoprolol showed 180.3±2.5, 191.2±0.75 (figure 2). The result was statistically significant ($p<0.001$).

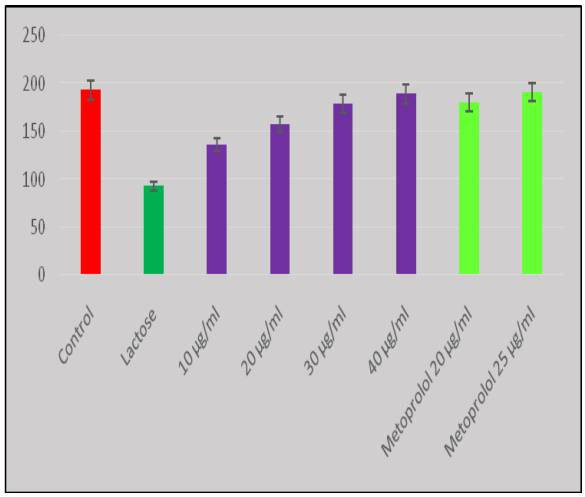

Figure-2: Heart rate of control and different concentration of CECML, standard treated on the lactose induced arrhythmic heart of *D. magna*

4. Discussion

As reported earlier several reports have been published on the effects of the plant extract and chemical constituents on different biological activities *in vitro* and *in vivo*.Preliminary phytochemical screening of the leaves showed the presence of biologically active constituents like flavonoids, terpenoids, steroids, tannins etc. Remarkable quantity of flavonoids and total phenolic content, various trace elements were observed. Cucurbitacins which are structurally diverse triterpenes, are found in many cucurbitaceous plants possesses immense pharmacological potential including cardio vascular effect [21]. Cucurbitacin contain a basic 19-(10-9β)-abeo-10α-lanost-5-ene ring skeleton. The difference of terpenes cucurbitacins from steroidal nucleus lies in the fact that in basis structure of cucurbitacin a methyl group is located at C-9 rather than C-10. The presence of cucurbitacin was identified by TLC.Assessment of acute toxicity study reveals its safety and non-toxic nature. Based on the above facts we have investigated the effect of CECML on the lactose induced arrhythmia of *D.magna* heart. The results clearly showed the dose dependent protective effect on lactose induced arrhythmia of the heart of *D.magna* by CECML. It is assumed that this cardioprotective effect may be due to the phenolic content, cucurbitacin and the influence of calcium, potassium, magnesium content and antioxidant activity. It can be assumed that research focused on this unattended medicinal herb from the nature can prove to be of immense significant in generating scientifically validated data with regard to their efficacy and possible role in various diseases.

5. Conclusion

So it is concluded that leaves of *C.maxima* 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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