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ANTI-ARRHYTHMIC ACTIVITY OF THE FRUIT OF *Benincasa hispida* (Thunb.) Cogn. ON THE HEART OF *Daphnia carinata*

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ABSTRACT

Background: Plants have an important role as a source of useful compounds to mankind. *Benincasa hispida* (Thunb.) Cogn. belongs to Cucurbitaceae family is known as Neer Poosanikai in Tamil Nadu.

Aim and objective: This study aims to evaluate anti-arrhythmic activity from ethanolic extract of the *Benincasa hispida* fruit (EEBHF) in lactose induced arrhythmic heart of the *Daphnia carinata* (water flea) along with preliminary acute toxicity assessment.

Methods: EEBHF evaluated for *in-vitro* antioxidant by DPPH method and *in-vivo* lactose induced arrhythmic activity using *Daphnia carinata*.

Results: The results for *in-vitro* antioxidant using various concentration of EEBHF (50, 100, 150, 200, 250 µg/ml) were found to be 51.61 ± 0.09, 62.93 ± 0.22, 73.44 ± 0.04, 81.89 ± 0.09, 92.47 ± 0.03.The results for *in vivo* antiarrhythmic activity using *D. carinata* was as follows, heartbeat of control, disease control and lactose induced EEBHF (20, 40, 60, 80 µg/ml) treated were found to be 192 ± 1.84, 94 ± 1.26, 135 ± 2.04, 155 ± 1.84, 179 ± 2.40, 188 ± 2 (bpm) respectively. Standard drug metoprolol (25 µg/ml) showed 190 ± 1.26. The results were statistically significant ($p<0.001$).

Discussion and Conclusion: It was concluded that the study indicates that the EEBHF possess potential cardio protective activity on the lactose induced arrhythmia of the *D. carinata* heart without any toxicity and mortality. It was assumed that this cardioprotective effects may be due to the presence of secondary metabolites and cucurbitacin and the influence of calcium, potassium, magnesium content present in the fruit.

Keywords: *Benincasa hispida*, anti-arrhythmic activity, Cardioprotective, Antioxidant, *Daphnia carinata*

INTRODUCTION

Medicinal plants have been utilized to cure human ailments for thousands of years **[1]**. For primary healthcare, 80 % of people in some Asian and African countries utilise traditional herbal medicines whereas in many developed nations, between 70-80% of people uses complementary and alternative medicine (CAMs), which mostly consist of herbal products **[2]**. India possesses world's most extensive traditions of plant-based medicine. In India's rural areas, there are about 25,000 powerful plant-based medicines available with traditional medical applications. Plants are the essential medical resources for early-stage drug development, particularly those with ethnopharmacological applications **[3]**. Traditionally, natural compounds served as the primary source of many drugs **[4-5]**.

Benincasa hispida, also called as winter melon, ash gourd, wax gourd, white guard, winter gourd, gourd melon, white pumpkin, tallow gourd. It is a member of gourd family (Cucurbitaceae) **[10].** *Benincasa hispida* is a widely grown trailing or climbing plant that is grown as a vegetable in India's plains and on hillsides as high as upto 1,200 meters **[6]**. This well-liked vegetable crop is particularly accessible to Asian people for both culinary and therapeutic uses. Most likely, it was grown in India and other warm nations, as well as in Japan and Java. The plant was used medicinally to manage a range of conditions, including diabetes mellitus, respiratory conditions, heart diseases, gastrointestinal problems, and urinary infections. These fruits were also used to treat a variety of blood diseases, dyspepsia, schizophrenia and other psychological disorders, jaundice, menstrual disorders, diuretic, insanity, laxative, aphrodisiac, fever insanity, epilepsy, cardiotonic, and urinary calculi **[7]**. *Benincasa hispida* has anti-cancer, antihelmintic, gastroprotective, anti-inflammatory, antidiarrhoeal, antidiabetic, antimicrobial, antiobesity, anti-convulsant, antioxidant, antipyretic, antidepressant and analgesic activity **[8]**. The fruit *Benincasa hispida* contains lupeol, α-sitosterol and their acetates, cucurbitin, triacontanol, adenin, trigonelline, and histidine. The waxy layer of fruit contains pentacyclic triterpene and isomultiflorenol acetate **[9]**.

The present study investigates the cardiac effect of the ethanol extract of *Benincasa hispida* fruit (containing polyphenols including flavonoids and triterpenoids) using model organism *Daphnia carinata*. The small fresh water crustacean *D. carinata* 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.

The heart of the water flea, *Daphnia* 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 the possess myogenic hearts. Testing the effects of the drugs is simplified in *Daphnia*, 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 **[10-13]**.

BRADYCARDIA

Bradycardia is a commonly observed arrhythmia and a frequent occasion for cardiac consultation. Defined as a heart rate of less than 50–60 bpm, bradycardia can be observed as a normal phenomenon in young athletic individuals, and in patients as part of normal aging or disease. Pathology that produces bradycardia may occur within the sinus node, atrioventricular (AV) nodal tissue, and the specialized His-Purkinje conduction system **[14]**.

MATERIALS AND METHODS

Lactose and light microscope were used from laboratory of Madras Medical College, Chennai. Metoprolol was purchased from local pharmacy in Chennai. Depression slide, petroleum jelly and plastic pipette were purchased from Lab chemicals, Chennai.

Collection and Authentication of plant

The fruit of *Benincasa hispida* (Thunb.) Cogn. was collected from Villivakkam local market, Chennai, Tamil Nadu, India on the month of October 2023. The plant material was identified and authenticated (Certificate No. 703-28122309) by Dr. KN Sunil Kumar Research Officer / Sci-II and HOD, Department of Pharmacognosy, Siddha Central Research Institute, Government of India, Arumbakkam, Chennai- 6000106.

Figure 1: *Benincasa hispida* (Thunb.) Cogn. fruit

Preparation of extract

The cuticle of the fruit was peeled. The fruit was cut and freed from seeds and endocarp. Fresh pulp of fruits of *Benincasa hispida* was cut into small pieces and mased well using an electric juicer. The ground pulp was macerated using ethanol for 7 days. Then the mother liquor was separated from

the marc by using a muslin cloth and allowed to evaporate using rotary vacuum evaporator **[15]**. Semisolid brownish colored extract thus obtained was stored in a refrigerator. The yield of the extract was 3.5 % w/w.

***In vitro* antioxidant activity**

DPPH radical scavenging assay

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay. The hydrogen atom donating ability of the plant extracts was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in ethanol at different concentrations (50–250 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity = {(A0-A1)/ A0} × 100

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then the % of inhibition was plotted against concentration, and the experiment was repeated three times at each concentration **[16]**.

Collection and authentication of *Daphnia carinata*

Daphnia carinata was obtained from the local aquarium in Kolathur, Chennai, Tamil Nadu, India. It was identified and authenticated by Dr. P. C. Sathiya Narayanan, Assistant Professor, Research Department of Zoology, Pachaiyappa's College, Chennai-6000 030.

Acute toxicity study

Acute toxicity study was performed as per OECD guidelines 202. For the acute toxicity study, neonates with less 24 hours old were selected, since neonates may be more susceptible to toxic substance than the elder ones. The neonates were more specificity, simple and easy to handle in the lab. No feed was given throughout the study. Temperature is maintained at 20°C +2°C. The different concentrations of 1, 2, 3, 4, 5 and 6 mg/l extracts of the drug extract was added directly to the 100 ml of water. *Daphnia carinata* is exposed to the extracts in six different concentrations for 48 hrs. Mortality rate was observed after 24 hours **[13, 17]**.

***In vivo* antiarrhythmic activity**

Daphnia carinata of 10 days old were used and separately placed in beakers containing water as control. Each *Daphnia* was chosen randomly and removed from a

beaker of fresh water with a plastic pipette and transferred individually onto a depression slide slightly coated with petroleum jelly. Any water remaining on the slide was entirely absorbed with a cotton. Then *Daphnia* was first induced for arrhythmias using lactose (200 mM). Subsequently, *Daphnia* were allowed to acclimatize to the environment for few minutes, and then the heart rate was recorded for one minute. Metoprolol (25 µg/ml) was used as a standard drug. Heart rate of control, standard and treated groups (lactose and ethanolic extract of fruit of *Benincasa hispida* 20, 40, 60, 80 µg/ml) were monitored by transferring individual *Daphnia* to the depression slide for each four concentration of test drug. The heart rate of control, standard and test drug treated groups were observed under light microscope **[12, 13, 18]**.

RESULTS

Free radical scavenging activity of fruit of *Benincasa hispida* was determined using DPPH assay. Results for various concentration of ethanolic extract of fruit of *Benincasa hispida* (50, 100, 150, 200, 250 µg/ml) are given in following **Table 1**.

Table 1: DPPH radical scavenging activity of ethanolic extract of *Benincasa hispida* fruit

Concentration µg/ml	Ascorbic acid	Ethanolic extract
50	46.24 ± 0.12	51.61 ± 0.09
100	54.51 ± 0.65	62.93 ± 0.22
150	65.29 ± 0.46	73.44 ± 0.04
200	72.65 ± 0.03	81.89 ± 0.09
250	80.12 ± 0.04	92.47 ± 0.03

Values are expressed as mean ± SEM, n = 3 in each group

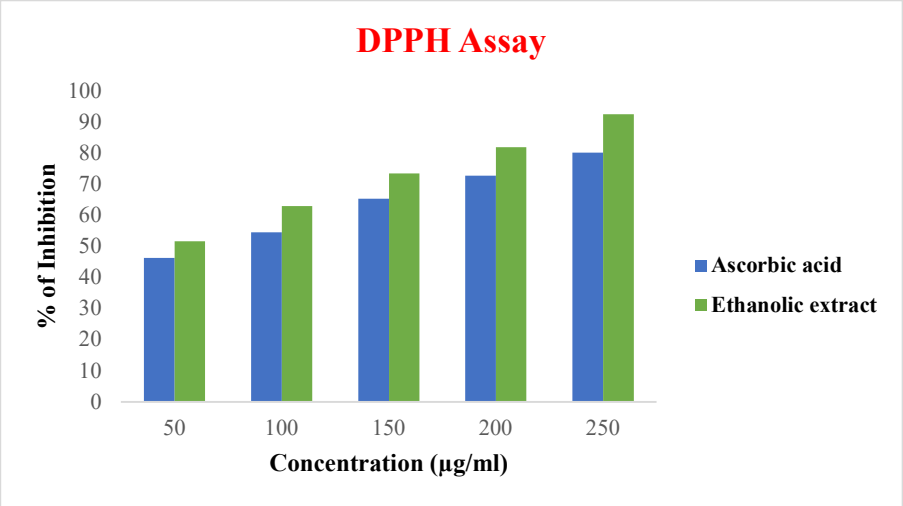

Figure 2: Comparative effect of ethanolic extract of *Benincasa hispida* fruit and ascorbic acid on DPPH assay

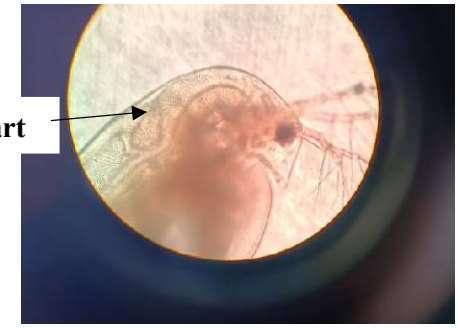

Figure 3: Heart of *Daphnia carinata* under light microscope

Table 2: Mean heart beat (bpm) of *Daphnia carinata*

Groups	Heart beat (bpm)
Control	192 ± 1.84
Lactose (200 mM)	94 ± 1.26####
Metoprolol (25 µg/ml)	190 ± 1.26****
Test drug (TD_1) (20 µg/ml)	135 ± 2.04****
Test drug (TD_2) (40 µg/ml)	155 ± 1.84****
Test drug (TD_3) (60 µg/ml)	179 ± 2.40****
Test drug (TD_4) (80 µg/ml)	188 ± 2****

Values are expressed as mean ± SEM, n = 6 in each group
####p< 0.0001 Lactose, when compared with control. ****p< 0.0001 Metoprolol, TD 1, TD 2, TD 3, TD 4, when compared with Lactose

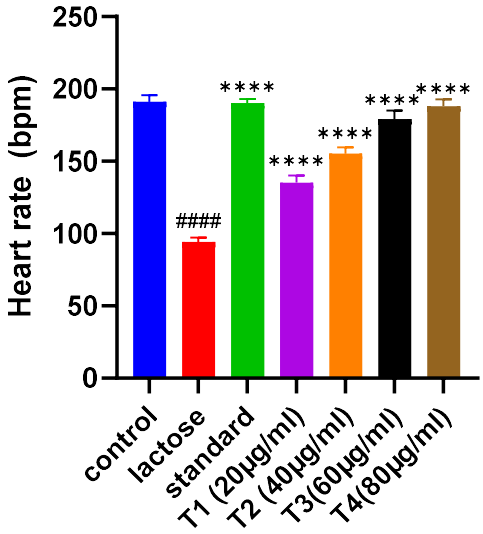

Figure 4: Heart rate of control and different concentration of ethanol extract of fruit of *Benincasa hispida* treated on the lactose induced arrhythmic heart of *D. carinata*

DISCUSSION

Both atrial fibrillation and heart failure, a condition with increased arrhythmic risk, are associated with excess amount of reactive oxygen species (ROS). Ethanolic extract of fruit of *Benincasa hispida* possess potential antioxidant activity compared to ascorbic acid, they will minimize or prevent deleterious effects of the ROS which exhibit influence in many cardiovascular diseases. The results showed that the increase in treatment concentration had positive response against cardiac arrhythmia induced myogenic heart of

Daphnia carinata which behaves similarly to the heart rate of humans. The ethanolic extract of fruit of *Benincasa hispida* was found to possess significant antiarrhythmic activity in lactose induced arrhythmia in *Daphnia carinata.* It is assumed that this cardioprotective effect may be due to the presence of secondary metabolites and cucurbitacin and the influence of calcium, potassium, magnesium content and antioxidant activity of the fruit of *Benincasa hispida.*

CONCLUSION

The fruit was collected from Villivakkam local market, Chennai. It was identified and authenticated. The extract was prepared by maceration method by using ethanol as a solvent. It is concluded that fruit of *Benincasa hispida* possesses cardio protective effect without toxicity. Ethanolic extract of fruit of *Benincasa hispida* possess potential antioxidant activity compared with ascorbic acid. Thus, it can be used to treat the diseases caused by free radicals and oxidative stress. The results of acute toxicity study clearly revealed that no significant mortality was observed upto 6 mg/l of the ethanolic extract of fruit of *Benincasa hispida* (Thunb.) Cogn. The assessment of acute toxicity study revealed that it was safe and non-toxic in nature. The heartbeat of *D. carinata* of various groups viz, control, disease control and lactose induced ethanolic extract of fruit of *Benincasa hispida* (20, 40, 60, 80 µg/ml) treated were found to be 192 ± 1.84, 94 ± 1.26, 135 ± 2.04, 155 ± 1.84, 179 ± 2.40, 188 ± 2 (bpm) respectively. Standard drug metoprolol (25 µg/ml) showed heartbeat of 190 ± 1.26. The results of the extracts treated groups were statistically significant ($p<0.001$) with the standard drug metoprolol treated group. It is anticipated to see an increase in demand as a result of its growing appeal as a health food and as an ingredient in various foods and drink items. *Benincasa hispida* fruit demand is also expected to be driven by the growing interest in natural and traditional treatments for many illness. The current study findings confirmed the traditional use of *Benincasa hispida* in the treatment of heart diseases. Further investigation on animal model and clinical trials were to be carried out.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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