ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

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Human Journals
Research Article
October 2015 Vol.:4, Issue:3

Cardiac Depressant and Cytotoxic Activities of Hydro Methanoloic Extract of *Bacopa monnieri L.*

ISSN 2349-7203
IJPPR
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Submission: 4 October 2015
Accepted: 9 October 2015
Published: 25 October 2015

Keywords: *Bacopa monnieri*, cardiac depressant, MCF, MIT assays, cytotoxic activity

ABSTRACT

Bacopa monnieri is a well known herb for its use in enhancing memory and in treatment of epileptic disorders ever since from ancient times. While effects of hydro-methanolic extracts of *Bacopa monnieri* on isolated heart has not been reported yet, so in this present study we aim to investigate effect of 75% hydro-methanolic extract of *Bacopa monnieri* on isolated frog heart and cytotoxic activity by using Human MCF-7 cell line. The extract produced significant results by dramatically decreasing the heart rate in a tachycardia induced heart. It shows decrease in the heart rate with an increase in the concentration of dose, showing a negative inotropic and chronotropic effects at 50 µg/ml and 75 µg/ml. Where at 25 µg/ml it showed a significant decrease in the rate of contraction but no change in the heart rate. 1 µg/ml adrenaline was used to induce the tachycardia. The *in-vitro* cytotoxic study was carried out by following the determination of cell viability by MTT assay on MCF-7 cell line. It produced a significant result with an IC50 median value of 370±0.0 µg/ml. The current study reveals that the hydro-methanolic extract of *Bacopa monnieri* has a significant cardiac depressant activity and also the cytotoxic activity in a dose dependent manner.

1. INTRODUCTION

Almost more than 50% of the drugs today are extracted from the plants [1-3]. This use of plants as medications and to treat various diseases was started 60000 years ago and more recently, a 5000 year-old Sumerianclay slab was discovered verifying the utilization of medicinal plants for the preparation of drugs [1]. *Bacopa monnieri* is a traditional herb which has been mentioned in ancient ayurveda for the use of mental disorders, improving memory conditions and energizing the nervous system. The major chemical constituents are dammarane type triterpenoids, saponins known as bacosides with jujubogenin or pseudo jujubogenin moieties as aglycone units. It also contains saponins, cucurbitacin, D-mannitol, hersaponin etc. The major constituent responsible for memory enhancing was found to be Bacosides A [4-8].

In the developing countries cardiac disease seems to be one of the most common causes for the death. In India it is estimated that it accounts for nearly one third of total deaths in the near future. Among these cardiac diseases, arrhythmia is one of the case which leads to cardiac arrest or cardiac failure. Although many Anti arrhythmic drugs like procainamide, amiodarone, sotalol gives significant results but possess severe side effects like chest pain and fainting. Herbal Medicine showing a scope of treatment with far lesser side effects when compared to the synthetic drugs.

Another major life threatening disease is Cancer, which is responsible for over 8.2 million deaths all over the world in 2012 [10]. As the cancer cells are showing significant resistant to chemotherapeutic drugs and therefore development of new therapeutic drugs for cancer is clinically important work. As per our examination *Bacopa monnieri* shows a significant cytotoxic effect on the MCF-7 cell line.

2. MATERIALS AND METHODS

2.1 Plant Material

Bacopa monnieri L. family Scrophulariaceae was obtained from wet lands of Koringa Forests, Kakinada, Andhra Pradesh. The plant species was identified and authenticated by Botanical survey of India, Deccan Hyderabad regional centre [Ref. No: BSI/DRC/2012-13/Tech./1736].

2.2 Preparation of Extract

The whole plant was washed with distilled water and shade dried. Then 500 g of plant was minced and extracted with 75% Methanol with occasional stirring at room temperature (25-28^{o}C) for 10 days. Thus obtained extract was concentrated to the desired level and yield 3 to 4 grams was stored in refrigerator for further use.

2.3 Experimental animals

Frogs of *Rana tigrina* species weighing 200-250 g were collected from the animal house Koringa College of pharmacy were used for the study and were maintained as per the CPCSEA guidelines.

Instruments Used

Sherington rotating drum, Sterling's heart lever, Symes venous cannulae

2.4 Drugs and Solutions Used

The marketed Adrenaline (Vasocon from Neon Laboratories) was obtained from the local market, Frog Ringer Solution.

2.5 Evaluation of Cardiovascular effect

Isolated frog heart perfusion technique (IFHP) was carried out to evaluate the effect of *Bacopa monnieri* extract. The frog was pithed and pinned to the frog board. An incision was given on the abdomen and pectoral girdle was removed in order to expose the heart. The inferior vena cava was traced, a thread was tied around it and a small cut was given in order to insert the venous cannula. The cannula was inserted into the vein and a thread was tied to assure that the cannula was in place which in turn was connected to a reservoir of ringer solution. The heart was isolated and attached to the stand with the moderate ringer flow. A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread attached to the hook; the other end was tied to free limb of the Sterling's lever. The basal cardiac response was recorded on a smoked kymograph drum. Then tachycardia was produced using adrenaline and then different concentrations of 25, 50, 75 and 100 µg/ml of test samples were administered and responses

were recorded. The frog heart was washed with the ringer solution after every administration of drug and brought back to normal state [10,11].

Table 1: Composition of ringer solution

S.No	Ingredients	Quantity
1	Sodium chloride (NaCl)	6.5 gm
2	Potassium chloride (KCl)	0.14 gm
3	Calcium chloride ($CaCl_2$)	0.03 gm
4	Glucose ($C_6H_{12}O_6$)	2.0 gm
5	Sodium bicarbonate ($NaHCo_3$)	0.2 gm
6	Distilled water	1000 ml

Table 2: Cytotoxic activity of chemicals

Chemical Name	Manufacturer Name
3-(4,5-dimethylthiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT)	Sigma Aldrich Co, St Louis, USA
Fetal Bovine serum (FBS)	
Phosphate Buffered Saline (PBS)	
Dulbecco's Modified Eagle's Medium (DMEM)	
Trypsin	
EDTA	Hi-Media Laboratories Ltd., Mumbai
Glucose	
Antibiotics	E.Merck Ltd., Mumbai, India.
Dimethyl Sulfoxide (DMSO)	
Propanol	

2.6 Cell lines and culture Medium

MCF-7 (Human breast adenocarcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS).

The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.7 Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Principle

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue colour product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well micro titre plates, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in micro titre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^{o}C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilise the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated

using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

$$\textbf{\% growth inhibition} = 1 - \frac{MeanODofindividualtestgroup}{MeanODofControlGroup} \textbf{ X 100}$$

3. RESULTS AND DISCUSSION

3.1 Cardiac depressant activity

Table 3: Cardiac depressant activity

BM Extract Concentration	Normal Ringer solution		Adrenaline 1mg/ml		*Bacopa monnieri*	
	HFC (cm)	Heart rate beats/min	HFC (cm)	Heart rate beats/min	HFC (cm)	Heart rate beats/min
25 µg/ml	0.9	54	---	---	0.6	53
50 µg/ml	1.1	52	1.4	73	1.0	56
75 µg/ml	0.8	56	1.2	77	0.7	51
100 µg/ml	1.0	53	---	---	0.8	45 (3 mins) Cardiac arrest

--- : without administration; HFC= Height of Force of Contraction

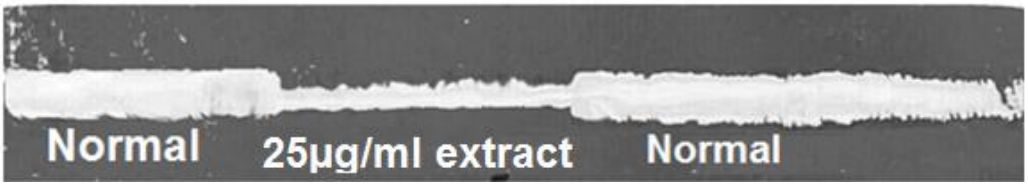

Fig.1: Effect of 25 µg/ml extract on isolated frog heart

At 25 µg/ml concentration of the drug, the force of contraction was decreased by 0.3 mm towards normal but the heart rate was found to be nearly at normal rate.

Fig. 2: Effect of 50 µg/ml extract on isolated frog heart

When concentration of the drug was increased to 50 µg/ml, there was a decrease in the heart rate but the force of contraction was same as that of for the adrenaline. The decrease in the heart rate was nearly by 17 beats/ min.

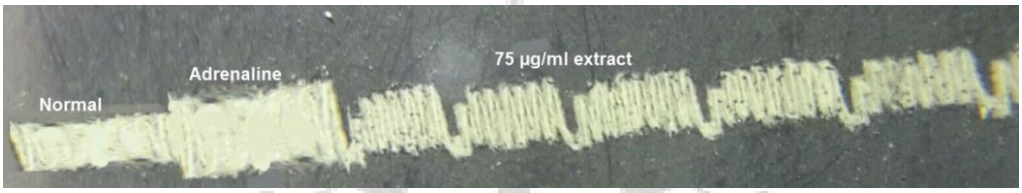

Fig.3: Effect of 75 µg/ml extract on isolated frog heart

There was a sudden fall in the heart beat at regular intervals along with the decrease in the force of contraction at 75 µg/ml concentration. The duration of drug action at this concentration was extremely high i.e. for approximately 8-9 minutes from the time of administration, even after the washing with high flow of ringer solution. This may be due to its lipophilic property towards the receptors of myocardium.

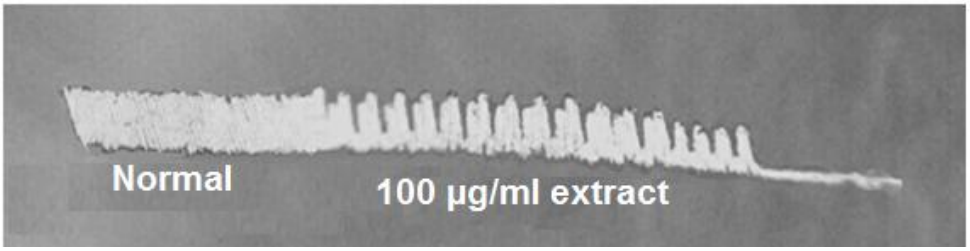

Fig. 4: Effect of 100 µg/ml extract on isolated frog heart

At 100 µg/ml concentration the force of contraction was reduced along with the heart rate. There was a notable decrease in the force of contraction at regular short intervals of the heart rate. After a span of 3 minutes from the administration of the drug, cardiac arrest was observed.

The hydro-methanolic extract of whole plant of *Bacopa monnieri L.* showed the cardiac depressant action on the isolated frog heart. The heart rate and the force of contraction gradually decreased as the concentration of the drug was increased. It showed a Negative Inotropic and Chronotropic effects.

3.2 Cytotoxic Activity

Through the MTT method, the median cytotoxic concentration (IC_{50}) on MCF & cell line was established from hydro-methanolic extract of whole plant of *Bacopa monnieri*. There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (22.24%, 26.40%, 28.61%, 72.02% and 74.10% for the concentrations 62.5, 125, 250, 500, 1000 µg/ml respectively) against the cell line MCF-7 cell line. In the Table the median value of IC_{50} observed for MCF-7 cell was 370±0.00.

Table 4: Cytotoxic activity

S. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
1	***Bacopa monnieri*** **Hydro-methanolic extract**	1000	74.10±0.2	370.00±0.00
		500	72.02±0.3	
		250	28.61±0.1	
		125	26.40±0.3	
		62.5	22.24±0.4	

Note: In case of cardiac depressant activity, this experiment is solely conducted in order to identify the activity and hence not compared with any standard drug.

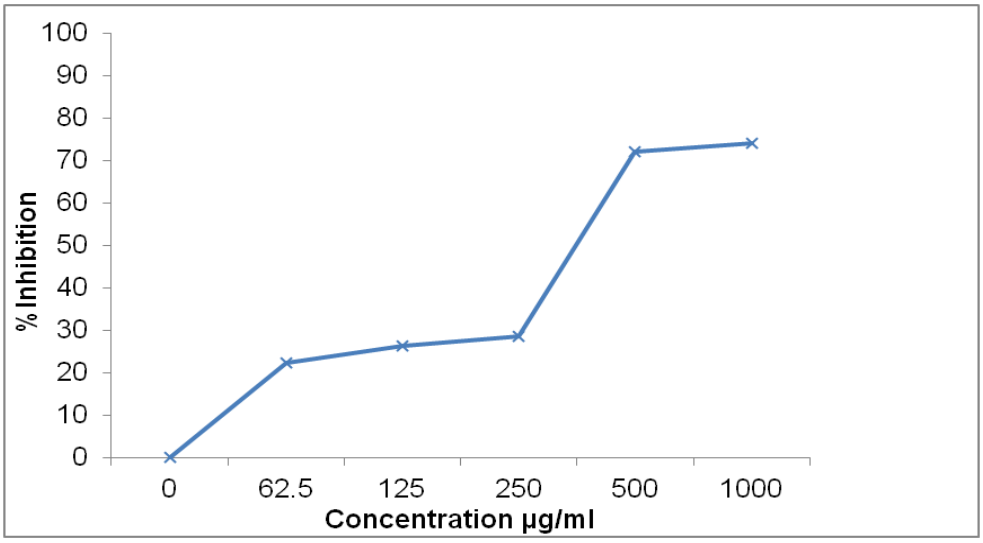

Fig. 5: Graphical representation of cytotoxic effect

CONCLUSION

The *Bacopa monnieri* which is a herbal medicine widely used for the memory enhancing and mental disorders, through this study it conclude that it also has a cardiac depressant property with a Negative Inotropic and Negative Chronotropic effect which may be used as anti- arrhythmic drug for ventricular tachyarrhythmia. At the same time it shows a significant cytotoxic property which may be used as a chemotherapeutic agent in the treatment of cancer. *Bacopa monnieri* shows a lot of research scope for not only the effects on nervous systems but also on the heart and cancer cells. It would be interesting in the isolating the compounds responsible for the respective effects and possible mechanism of action. It promises a lot of scope for future research.

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