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EFFECT OF BACOSIDE EXTRACT ON H_2O_2 STRESSED LYMPHOCYTES

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ABSTRACT

Organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components. Antioxidant property of Bacopa monniera is rendered by bacoside against H_2O_2 stressed lymphocytes which was extracted by Soxhlet apparatus and analyzed by TLC method. Hydrogen peroxide was found to be lethal for cells (23.80 %viability). While bacoside showed significant cell viability (75 % viability) when treated against hydrogen peroxide stressed cells. In this regard there is a great scope for further investigation of Bacopa monniera against certain drugs producing free radicals having negative side effects on cell.

KEYWORDS: Antioxidant, H_2O_2, lymphocytes, bacoside, cell viability, free radicals.

INTRODUCTION

Living organisms require oxygen for their existence but sometimes it is highly reactive molecule that can damage the cells by producing highly reactive oxygen species. Antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell[1]. The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O2−)[2]. The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms[3,4], while damage to proteins causes enzyme inhibition, denaturation and protein degradation[5]. Today's modern lifestyle encourages quick meals such as fries, burgers, pizzas, pastries, ice-creams, chocolate bars etc. They not only lack antioxidants but are also loaded with oxidants. Free radicals are the major cause of over a hundred human diseases. The process of ageing is also hastened by the onslaught of oxidants in the body. Oxidants are normally produced during healthy cellular metabolism, wherein 98 per cent of the oxygen consumed by a cell is converted to water. The remaining 1 to 2 per cent of the unutilized oxygen is free to escape as free radicals. Several medicinal plants are well known for their antioxidant properties. *Bacopa monniera* (Brahmi) commonly grows in marshy areas throughout India, it has been reported that the plant is used in traditional Ayurvedic treatment for epilepsy, asthma [6] ,improvement of memory[7] and intellectual activity[8] .The sulfhydryl and polyphenol components of *Bacopa monniera* extract have also been shown to impact the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipoxygenase activity and reducing divalent metals[9] . As the Lymphocytes play an integral role in the body's defenses; it is crucial to maintaine their repair against certain stress conditions. In the present study, effect of extracted bacoside from *Bacopa monniera* was checked against stressed lymphocytes to reduce cytotoxicity caused due to hydrogen peroxide administration.

MATERIALS AND METHODS

Extraction of plant material[10]

The fresh leaves of *Bacopa monniera* were collected from Town Hall Garden, Kolhapur, Maharashtra in the month of June 2011. The plant was identified at the department of botany, Shivaji University, Kolhapur, Maharashtra, India. The freshly harvested herb *Bacopa monniera* was

thoroughly washed with tap water followed by distilled water, shade dried, crushed in a disintegrator to obtain ground powder and stored in air tight bottles. 44 gram of shade dried powder of plant was filled in the thimble and extracted with hexane solvent in Sohxlet extractor. The hexane extract was removed from the soxhlet apparatus when the solution became transparent and was dried at room temperature. The dried herb was extracted with acetone for a time period 4 to 8 hours to obtain an acetone extract containing unwanted colour and non-bacoside constituents and dried the herb. The dried herb was extracted with methanol to obtain a methanol extract containing bacosides. The methanol extract was concentrated under vacuum evaporator. This concentrated methanol extract was added gradually to acetone with stirring to effect preferential precipitation of bacosides. The bacosides were filtered in a Wattman filter paper to obtain a bacoside residue. This bacoside residue was dissolved in distilled water to obtain an aqueous solution.

% yield of bacoside was calculated by formula,

% yield = Weight of crude extract / Weight of dried plant x 100

Thin layer Chromatography (TLC) of Bacopa *monniera* extract

The extract or the isolated bacoside dissolved in methanol and spotted over silica gel G plates. The plates were eluted in ethyl acetate-pyridine-water (4: 1: 1). Then the TLC plates were sprayed with trichloroacetic acid (25 %) and chloroform. Distance traveled by sample and by solvent was measured. The RF (Retension factor) value calculated by the formula,

RF value= distance traveled by sample / distance traveled by solvent

Standard RF value for bacoside A= 0.43

Culture of lymphocyte cells

The lymphocyte cell line was obtained from the whole blood in four culture bottles. The culture of lymphocytes was propagated in RPMI 1640 media (RPMI 1640 media 1.64 gm + Foetal bovine serum 11 ml + Antibiotic Pen Strep 3 ml + Phytoheamagglutinin 2 µl .Each for 100 ml of Lymphocyte media.). After propagation of these components in autoclaved distilled water, this media was filtered with help of syringe filter (0.22 microns) .Then 5 ml of media was added in each tube. Two drops of fresh blood were added in these tubes aseptically. The tubes were maintained in humidified atmosphere of 5% CO_2 at 37 ^{0} C until confluent in CO_2 incubator for 72 hours. The lymphocyte cell line was obtained from the whole blood in four culture bottles and named as,

Tube A (Normal): Lymphocytes, Tube B (Extract Toxicity): Lymphocytes + Extract, Tube C (Toxicity): Lymphocytes + hydrogen peroxide and Tube D (Treatment Effect): Lymphocytes + Hydrogen Peroxide +Extract.

Determination of sub lethal concentration of H_2O_2

H_2O_2 was diluted to 0.5 % concentration in phosphate buffer saline. To determine the sub lethal concentration, H_2O_2 was taken in quantities of 0.5, 0.05, 0.1, 0.15, 0.20 and 0.25 (In ml). 0.1 ml of lymphocyte culture was added in each tube and total volume was made upto 0.6 ml by adding media. After doing additions, each set was incubated for 20 hrs in CO_2 incubator and then cell count was taken for each set by using haemocytometer and sub lethal concentration of H_2O_2 was determined.

Extract treatment

After 24 hours, treatment of 5 % autoclaved stock of bacoside extract (5 gm of bacoside extract + 100 ml phosphate buffer saline buffer), filtered stock of hydrogen peroxide (0.5 µl of Hydrogen peroxide in 100 ml phosphate buffer saline) and bacoside extract with hydrogen peroxide filtered stock solution (0.5 gm of bacoside extract + 10 ml H_2O_2 stock solution) was checked on lymphocytes.

Cell Counting using Haemocytometer

The concentration of a cell suspension was determined by placing the cells in a Haemocytometer under a microscope. The cell number within a defined area of known depth (i.e., within a defined volume) were counted, and the cell concentration was derived from the count. Cell viability was checked by using Giemsa staining for live cell count and Trypan blue staining for dead cells.

RESULTS

% yield of bacoside

% yield = Weight of crude extract / Weight of dried plant x 100

Where weight of crude extract- 4.2 gm

Weight of dried plant material-44 gm

% yield of bacoside = 4.2/44 ×100 = 9.54 %

Thin layer chromatography of bacoside

RF value= distance traveled by sample/distance traveled by solvent

Distance travelled by sample=7.2cm

Distance travelled by solvent=16.5 cm

Thus, Calculated RF value = 7.2 / 16.5= 0.436 which is approximately equals to standard RF value of bacoside is 0.43, Therefore presence of bacoside was confirmed.

Sub lethal concentration of H_2O_2

From the cell count, it is clear that minimum cell growth was found in tube added with 50µl H_2O_2 and above this concentration no cell growth was observed ,therefore 50 µl concentration is sub lethal concentration of H_2O_2 for lymphocyte.

Cell viability count

Antioxidant activity of bacoside was checked against H_2O_2 stressed lymphocytes. When hydrogen peroxide was added to the lymphocyte culture, there was tremendous decrease in the viable cell count even at the low concentration of H_2O_2 (Table 5).

Table no.5 Cell Viability Count

	Live cells Per ml Average	Dead Cells Per ml Average	Percentage Viability %
Tube A (Lymphocytes)	13.5×10^6	0.75×10^6	94.73
Tube B (Lymphocytes+H_2O_2)	2.5×10^6	8×10^6	23.80
Tube C (Lymphocytes+ Extract)	10.75×10^6	1.5×10^6	87.75
Tube D (Lymphocytes+H_2O_2+Extract)	7.5×10^6	2.5×10^6	75

Tube D containing bacoside showed 7.5×10^6 live cells per ml and 75 % viability of lymphocytes. While, H_2O_2 exhibited 2.5×10^6 /ml and 8×10^6 /ml live and dead cells respectively. Percentage viability of H_2O_2 treated lymphocyte was found to 23.80, which is very less as compared to the normal lymphocytes in tube A and only bacoside treated lymphocytes of tube C. From the Percentage Viability, it is clear that hydrogen peroxide is lethal for cells and when the bacoside extract added to hydrogen peroxide stressed cell; it reduced the effect of free radicals on cells.

DISCUSSION

Brahmi, has been used in the Ayurvedic system of medicine for centuries[11,12]. Extracted bacoside is found to be useful in many cell recoveries. The present study reveals the antioxidant effect of bacoside on stressed lymphocytes. There are several reports showing curative effect of *Bacopa monniera* against various diseases. Janani and group studied the hepatoprotective activity of bacoside A against *N*-nitrosodiethylamine-induced liver toxicity in adult rats [(13)]. Anbarasi and group studied Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats [(14)] , in which administration of Bacoside A improved the antioxidant status. These results are of resemblance with present work. Higher

epithelialization of the excision wound by Bacoside-A is noticed by Sharath and group [15]. The damage caused to cells by H_2O_2 was due to formation of free radicals in media which results in oxidative stress on lymphocytes. The results of the present investigations exhibit that highly purified bacoside extract can reduce cytotoxicity caused due to hydrogen peroxide administration, where there is death of lymphocytes. Overall, the results of this study support the use of *Bacopa monniera* in traditional Indian medicine and show that extracted bacoside can be used as an easily accessible source of a natural antioxidant and can be of assistance in some ageing problems.

CONCLUSION

The bacoside was successfully extracted by using soxhlet apparatus. The presence of bacoside in bacopa extract was analysed by TLC. From cytotoxic analysis, it is clear that the treatment with bacopa extract and hydrogen peroxide on lymphocytes resulted in significant changes in cell viability. The treatment of hydrogen peroxide on lymphocytes caused sudden increase in cell death. Cells subjected to hydrogen peroxide showed less viability as compared to cells subjected to bacopa extract. So we can conclude that the extract of *Bacopa monniera* can reduce the stressed effect of hydrogen peroxide and thus probably of other oxidants and have minimal damage to cell viability.

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