EXPLORING THE PROTECTIVE ROLE OF WATER EXTRACT OF SPIRULINA PLATENSIS ON GEMCITABINE-INDUCED LIPID PEROXIDATION USING COMMON LABORATORY Markers

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ABSTRACT

The present study was designed with an aim to explore the protective role of water extract of Spirulina platensis on gemcitabine-induced lipid peroxidation. The work was carried out in vitro and goat liver was used as model lipid source. Two common laboratory markers such as malondialdehyde and reduced glutathione were used for the model. The results showed that gemcitabine has the ability to induce lipid peroxidation to a significant extent and it was also found that water extract of the Spirulina platensis has the ability to suppress the gemcitabine-induced toxicity.

Keywords: Gemcitabine, Spirulina platensis, lipid peroxidation, malondialdehyde, reduced glutathione

INTRODUCTION

Gemcitabine is a pyrimidine nucleoside produrg. Gemcitabine has wide application in cancer therapy particularly breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer and bladder cancer. But along with its use the compound also produces several side effects such as pale skin, easy bruising or bleeding, numbness or tingly feeling, weakness, nausea, vomiting, upset stomach, diarrhea, constipation, headache, skin rash, drowsiness, hair loss etc.1.

Spirulina is an algae and it contain 60-70% protein by weight and it contain a high amount of vitamins like vitamin B12, β-carotene (provitamin A), and minerals, especially iron2. It has direct effect on reactive oxygen species. It also contains an important enzyme superoxide dismutase (SOD) (1700 units / gm of dry mass) that acts indirectly by slowing down the rate of oxygen radical generating reactions3.

Lipid peroxidation is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically4,5. The cytotoxic end products of lipid peroxidation are mainly aldehydes as exemplified by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc.6. It was reported that resveratrol and capsuin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model7. In another study Gemcitabine showed improved efficacy when used along with vitamin E8.

In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation9,10 the present work has been carried out in vitro to evaluate the antiperoxidative potential of water extract of Spirulina platensis on gemcitabine-induced lipid peroxidation.

MATERIALS AND METHODS

Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi. Sigma chemicals Co. St. Louis, MO, USA. 1,1,3,3, tetraethoxypropane and reduced glutathione were from Sigma chemicals Co. St. Louis, MO, USA. 5, 5’ dithiobis(2-nitrobenzoic acid) was from SRL Pvt. LTD., Mumbai. Spirulina was obtained from INDO LEENA, Biotech private ltd., Spirulina Farm, Namakkal, Tamil Nadu. Pure sample of gemcitabine used in present study was obtained from Parchem, New Rochelle, New York, USA. All other reagents were of analytical grade. Goat liver was used as the lipid source.

Preparation of water extract of Spirulina platensis

Attempt was made to determine the maximum concentration of the algae in water extract. For this purpose, first 2.5 g of spirulina powder was weighed accurately and taken in a beaker. Then 200 ml of water was added to it. The mixture was heated cautiously in a steam bath until the volume was reduced to 50 ml. The hot solution was filtered at a suction pump using single filter paper. After that the filtrate was again filtered at a suction pump using double filter paper. Then the filtrate was transferred in a 50 ml volumetric flask and the volume was made up to the mark with double distilled water. The concentration of the solution was determined as follows: At first a clean petridish was weighed accurately. Then 1 ml of the extracted solution was placed on it. Then the solution was heated on a steam bath to remove the water and last traces of water were removed by drying in hot air oven. It was then kept in a desiccator to cool to room temperature. The weight of the petridish along with the solid material was weighed. Then further 1 ml of the extract was added and the same procedure was done. In this way a total of 5 ml of extract was added to petridish and water was evaporated. Finally the weight of the petridish and solid material was taken. The amount of solid present in 5 ml extract was calculated by difference from the empty weight of petridish. The concentration of the water extract determined in this way was 0.92% w/v. The same procedure was followed with 4g, 5g, 6g, 7g of spirulina powder and the concentrations were 1.4%, 1.7%, 2.0%, 2.4% respectively.
1.7%, 1.7% w/v respectively. It was found that the maximum extractable concentration of the algae using 200 ml of water would be 1.7% w/v. The \( \lambda_{\text{max}} \) of the water-extracted solution was found at 259 nm.

### Preparation of tissue homogenate

Goat liver was collected from Silchar Municipal Corporation approved outlet. Goat liver was selected because of its easy availability and close similarity with human liver in its lipid profile\(^{31} \). Goat liver perfused with normal saline through hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were then transferred in a sterile vessel containing phosphate buffer (pH 7.4) solution. After draining the buffer solution as completely as possible, the liver was immediately grinded to make a tissue homogenate (1 g/ml) using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts, which were then treated differently as mentioned below.

One portion of the homogenate was kept as control (C) while a second portion was treated with the gemcitabine (D) at a concentration of 1.2 mg/g tissue homogenate. The third portion was treated with both gemcitabine at a concentration 1.2 mg/g tissue homogenate and water extract of *Spirulina platensis* at a concentration of 0.1666 mg/g tissue homogenate (DA) and the fourth portion was treated only with water extract of *Spirulina platensis* at a concentration of 0.1666 mg/g tissue homogenate (A). After gemcitabine and/or water extract of *Spirulina platensis* treatment, the liver tissue homogenate samples were shaken for two hours and the malondialdehyde and reduced glutathione content of various portions were determined.

### Estimation of malondialdehyde (MDA) level from tissue homogenate

The extent of lipid peroxidation was measured in terms of malondialdehyde (MDA) content using thiobarbituric acid (TBA) method\(^2\). The estimation was done at two hours of incubation and repeated in three animal sets. In each case three samples of 1 ml of incubation mixture were treated with 1 ml of 5% (w/v) TCA in 1 ml EDTA centrifuged at 2000 g for 10 minutes. After that 1 ml of the filtrate was mixed with 5 ml of 0.1M phosphate buffer (pH 8.0) and 0.4 ml of 5, 5'-dithiobis(2-nitrobenzoic acid in 0.01% in phosphate buffer pH 8.0) (DTNB) was added to it. The absorbances of the solutions were measured at 412 nm against blank (prepared from 6.0 ml of phosphate buffer and 0.4 ml of DTNB) (0.01% in phosphate buffer). The concentrations of reduced glutathione were determined from standard curve, which was constructed as follows. Different aliquots from standard reduced glutathione solution were taken in 10.00 ml volumetric flask. To each solution 0.04 ml of DTNB solution was added and volume was adjusted up to the mark with phosphate buffer. The absorbances of each solution were noted at 412 nm against a blank containing 9.60 ml phosphate buffer and 0.04 ml DTNB solution. By plotting absorbances against concentrations a straight line passing through the origin was obtained. The best-fit equation is \( A=0.001536 M - 0.00695 \), where \( M= \) nanomoles of GSH, \( A= \) absorbance, \( r = 0.995, \) SEE= 0.0067, \( F=1638.83 \) (df=1,8).

### Estimation of reduced glutathione (GSH) level from tissue homogenate

The extent of lipid peroxidation was measured in terms of reduced glutathione level by Ellmann’s method\(^3\). The estimation was done at two hours of incubation and repeated in three animal sets. In each case three samples of 1 ml of incubation mixture were treated with 1 ml of 5% (w/v) TCA in 1 ml EDTA centrifuged at 2000 g for 10 minutes. After that 1 ml of the filtrate was mixed with 5 ml of 0.1M phosphate buffer (pH 8.0) and 0.4 ml of 5, 5'-dithiobis(2-nitrobenzoic acid in 0.01% in phosphate buffer pH 8.0) (DTNB) was added to it. The absorbances of the solutions were measured at 412 nm against blank (prepared from 6.0 ml of phosphate buffer and 0.4 ml of DTNB) (0.01% in phosphate buffer). The concentrations of reduced glutathione were determined from standard curve, which was constructed as follows. Different aliquots from standard reduced glutathione solution were taken in 10.00 ml volumetric flask. To each solution 0.04 ml of DTNB solution was added and volume was adjusted up to the mark with phosphate buffer. The absorbances of each solution were noted at 412 nm against a blank containing 9.60 ml phosphate buffer and 0.04 ml DTNB solution. By plotting absorbances against concentrations a straight line passing through the origin was obtained. The best-fit equation is \( A=0.001536 M - 0.00695 \), where \( M= \) nanomoles of GSH, \( A= \) absorbance, \( r = 0.995, \) \( \text{SEE}=0.0067, \) \( F=1638.83 \) (df=1,8).

### STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure\(^{4,10} \) were also performed on the percent changes data of various groups such as gemcitabine-treated (D), gemcitabine and water extract of *Spirulina platensis* (DA) and only water extract of *Spirulina platensis* -treated (A) with respect to control group of corresponding time.

### RESULTS & DISCUSSION

The percent changes in MDA and GSH content of different samples at two hours of incubation were calculated with respect to the control of the corresponding time of incubation and was considered as indicator of the extent of lipid peroxidation.

From Table 1 it was evident that tissue homogenates treated with gemcitabine showed an increase in MDA (9.80 %) content in samples with respect to control at two hours of incubation to a significant extent. The observations suggest that gemcitabine could significantly induce the lipid peroxidation process. MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism\(^1\). But the MDA (18.36 %) content were significantly reduced in comparison to gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with water extract of *Spirulina platensis*. Again the tissue homogenates were treated only with the water extract of *Spirulina platensis* then the MDA (-16.00%) level were reduced in comparison to gemcitabine treated group. This decrease may be due to the free radical scavenging property of the water extract of *Spirulina platensis*. So the decrease in MDA content of samples, when treated with gemcitabine and water extract of *Spirulina platensis* implies the free radical scavenging property of water extract of *Spirulina platensis*.
In a particular group, differences (F2) are insignificant which shows that there is no statistical difference in animals in a particular group (Tables 1 & 2). The Table 1 also indicates that for MDA content, gemcitabine-treated group is statistically different from gemcitabine and water extract of Spirulina platensis-treated and only water extract of Spirulina platensis -treated groups. But there is no difference between gemcitabine and water extract of Spirulina platensis-treated and only water extract of Spirulina platensis -treated groups. However for GSH level (Table 2), all three groups i.e. gemcitabine –treated, gemcitabine and water extract of Spirulina platensis-treated and only water extract of Spirulina platensis -treated groups are statistically significantly different from each other.

CONCLUSION

The findings of the work showed the lipid peroxidation induction potential of gemcitabine, which may be related to its toxic potential. The results also suggest the antiperoxidative effects of water extract of Spirulina platensis and demonstrate its potential to reduce gemcitabine induced toxic effects.

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