Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

EXPLORING THE PROETECTIVE ROLE OF WATER EXTRACT OF *SPIRULINA PLATENSIS* ON GEMCITABINE-INDUCED LIPID PEROXIDATION USING COMMON LABORATORY MARKERS

Sarbani Dey Ray *

Department of Pharmaceutical Sciences, Assam University, Silchar 788011, India

*Corresponding Author Email: sarbanideyray09@gmail.com

DOI: 10.7897/2277-4572.07383

Received on: 26/05/18 Revised on: 15/06/18 Accepted on: 17/06/18

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 falls under the class of antimetabolites. Chemically it is a pyrimidine nucleoside prodrug. 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 B_{12}, β-carotene (provitamin A), and minerals, especially iron[2]. 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 reactions[2].

Lipid peroxidation is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically[3-5]. 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 capsaicin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model[7]. In another study Gemcitabine showed improved efficacy when used along with vitamin E[8].

In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation[9-10] 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. 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 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%,

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 λ_{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[11]. 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 1.2mg/g tissue homogenate. The third portion was treated with both gemcitabine at a concentration 1.2mg/g tissue homogenate and water extract of *Spirulina platensis* at a concentration of 0.1666 mg / g 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 thiobarbuturic acid (TBA) method[12]. The estimation was done at two hours of incubation and repeated in three animal sets. In each case three samples of 2.5 ml of incubation mixture were treated with 2.5 ml of 10% (w/v) trichloroacetic acid (TCA) and centrifuged at room temperature at 3000 rpm for 30 minutes to precipitate protein. Then 2.5 ml of the supernatant was treated with 5 ml of 0.002 (M) TBA solutions and then volume was made up to 10 ml with distilled water. The mixture was heated on a boiling water bath for 30 minutes. Then tubes were cooled to a room temperature and the absorbance was measured at 530 nm against a TBA blank (prepared from 5 ml of TBA solution and 5 ml of distilled water). The concentrations of MDA were determined from standard curve, which was constructed as follows. Different aliquots from standard 1, 1, 3, 3-tetrahydroxypropane (TEP) solution were taken in graduated stoppered test tubes and volume of each solution was made up to 5 ml. To each solution, 5 ml of TBA solution was added and the mixture was heated in a steam bath for 30 minutes. The solutions were cooled to a room temperature and their absorbances were measured at 530 nm against TBA as blank. By plotting absorbances against concentrations a straight line passing through the origin of grid was obtained. The best-fit equation is A=0.006776 M + 0.003467, where M= nanomoles of MDA, A= absorbance, r = 0.996, SEE= 0.0037, F=1068.76 (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 Ellman's method[13]. 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 mM 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).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure[14-15] 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[16]. 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*.

Table 1: Effect of Water extract of *Spirulina platensis* on gemcitabine induced lipid peroxidation: Changes in MDA profile

Hours of incubation	Animal sets	% Changes in MDA content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
2	AL1	13.04[a]	-18.13[b]	-9.27[a]	F1=52.90 [df=(2,4)]
	AL2	13.13[a]	-16.71[b]	-19.20[b]	F2=3.02[df=(2, 4)]
	AL3	3.24[a]	-20.25[a]	-19.54[a]	Pooled variance $(S^2)^*$ =13.84
	AV.	9.80	-18.36	-16.00	Critical difference,(p=0.05)[#]
	(± S.E.)	(±3.28)	(±1.03)	(±3.36)	LSD =4.04
					Ranked means[**] (D) (DA, A)

% Changes with respect to controls of corresponding hours are shown: a>99%; b=97.5-99%; c=95-97.5%; d=90-95%; e=80-90%; f=70-80%; g=60-70%; h<60%; Theoretical values F: p=0.05 level F1=6.94 [df=(2,4)], F2=6.94 [df=(2, 4)]. F1 and F2 corresponding to variance ratio between groups and within groups respectively. D, DA, A indicate gemcitabine-treated, gemcitabine and *Spirulina platensis*-treated, *Spirulina platensis*-treated respectively. AV.= Averages of three animal sets; S.E.= Standard Error (df=2); df= degree of freedom; * Error mean square, # Critical difference according to least significant procedure [15] ** Two means not included within same parenthesis are statistically significantly different at p=0.05 level

Table 2: Effect of Water extract of *Spirulina platensis* on gemcitabine induced lipid peroxidation: Changes in GSH profile

Hours of incubation	Animal sets	% Changes in GSH content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
2	AL1	-17.88[a]	8.52[a]	3.34[a]	F1=250.89 [df=(2,4)]
	AL2	-16.50[b]	10.66[a]	6.86[a]	F2=6.63[df=(2, 4)]
	AL3	-16.49[a]	14.29[b]	10.99[a]	Pooled variance $(S^2)^*$ =2.75
	AV.	-16.96	11.16	7.06	Critical difference,(p=0.05)[#]
	(± S.E.)	(±0.46)	(±1.68)	(±2.21)	LSD =3.12
					Ranked means[**] (D) (DA) (A)

% Changes with respect to controls of corresponding hours are shown: a>99%; b=97.5-99%; c=95-97.5%; d=90-95%; e=80-90%; f=70-80%; g=60-70%; h<60%; Theoretical values F: p=0.05 level F1=6.94 [df=(2,4)], F2=6.94 [df=(2, 4)]. F1 and F2 corresponding to variance ratio between groups and within groups respectively. D, DA, A indicate gemcitabine-treated, gemcitabine and *Spirulina platensis*-treated, *Spirulina platensis*-treated respectively. AV.= Averages of three animal sets; S.E.= Standard Error (df=2); df= degree of freedom; * Error mean square, # Critical difference according to least significant procedure [15]

** Two means not included within same parenthesis are statistically significantly different at p=0.05 level

It was also evident from Table 2 that tissue homogenates treated with gemcitabine showed a decrease in GSH (-16.96%) content in samples with respect to control to a significant extent. The observations suggest that gemcitabine could significantly induce the lipid peroxidation process. Glutathione is a small protein composed of three amino acid, such as cysteine,glutamic acid and glycine[17]. It is an important antioxidant and plays a very important role in the defence mechanism for tissue against the reactive oxygen species[18]. But the GSH content was significantly increased (11.16%) in comparison to control and gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with water extract of *Spirulina platensis*. Again the tissue homogenates was treated only with water extract of *Spirulina platensis* then the GSH level was increased (7.06%) in comparison to the control and the gemcitabine treated group. This increase may be explained by the free radical scavenging property of the water extract of *Spirulina platensis*.

To compare means of more than two samples, multiple comparison analysis along with analysis of variance was performed on the percent changes data with respect to control of corresponding hours. It is seen that there is significant differences among various groups (F1) such as gemcitabine-treated, gemcitabine and water extract of *Spirulina platensis* -treated and only water extract of *Spirulina platensis* -treated. But within 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.

REFERENCES

1. Zhang XW, Ma YX, Sun Y, Cao YB, Li Q, Xu CA. Gemcitabine in Combination with a Second Cytotoxic Agent in the First-Line Treatment of Locally Advanced or Metastatic Pancreatic Cancer: a Systematic Review and Meta-Analysis. Target Oncol, 2017; 12: 309-21.
2. Belay A. The potential application of spirulina (arthrospira) as a nutritional and therapeutic supplement in health management. JANA 2002; 5: 26-48

3. Gutteridge, JMC, Halliwell B. Free radicals and antioxidants in the year 2000. A historical look to the future. Ann N Y Aca Sci 2000; 899: 136-47.
4. Stohs SJ. The role of free radicals in toxicity and disease. J Basic Clin Physiol Pharmacol 1995; 6: 205-28.
5. Romero RJ, BoschMorell F, Romero MJ, Jareno EJ, Romero B, Marin N, Roma J. Lipid peroxidation products and antioxidants in human disease. Environ Health Perspect 1998; 106: 1229-34.
6. Esterbauer H, Zollner H, Schauer RJ. Hydroalkenals: Cytotoxic products of lipid peroxidation. Biochem Mol Biol 1998; 1: 311-19.
7. Vendrely V, Peuchant E, Buscail E, Moranvillier I, Rousseau B, Bedel A, Brillac A, de Verneuil H, Moreau-Gaudry F, Dabernat S. Resveratrol and capsaicin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model. Cancer Lett 2017; 390: 390-91
8. Abu-Fayyad A, Nazzal S. Gemcitabine-vitamin E conjugates: Synthesis, characterization, entrapment into nanoemulsions, and in-vitro deamination and antitumor activity. Int J Pharm 2017; 528: 463-70.
9. Ray S, Dey Ray S. Paclitaxel-induced lipid peroxidation: Role of water extract of *Spirulina platensis*. J Pharm Sci Innovation 2016; 5: 38-41.
10. Ray S. Exploring the protective role of water extract of *Spirulina platensis* on docetaxel-induced lipid peroxidation using malondialdehyde as model marker. J Pharm Sci Innovation 2015; 4: 65-7
11. Hilditch TP, Williams PN. The Chemical Constituents of Fats. London: Chapman & Hall; 1964.
12. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-58.
13. George E L. Tissue Sulfhydryl Groups, Arch Biochem Biophys 1959; 82: 70-7.
14. Snedecor GW, Cochran WG. Statistical Methods. New Delhi: Oxford & IBH Publishing Co Pvt Ltd; 1967.
15. Bolton S. Statistics. In: Gennaro AR, editor. Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 124-58.
16. Yahya MD, Pinnsa JL, Meinke GC, Lung CC. Antibodies against malondialdehyde (MDA) in MLR / lpr / lpr Mice: Evidence for an autoimmune mechanism involving lipid peroxidation. J Autoimmun 1996; 9: 3-9.
17. Benet L Z, Schewartz J B. In Goodman & Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York: Mc Graw- Hill; 1996. p. 707-16.
18. Wilkinson G R. In Goodman & Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York: Mc Graw- Hill; 2001. p. 10-20.

How to cite this article:
Sarbani Dey Ray. Exploring the protective role of water extract of *Spirulina platensis* on gemcitabine-induced lipid peroxidation using common laboratory markers. J Pharm Sci Innov. 2018;7(3):66-69.
http://dx.doi.org/10.7897/2277-4572.07383

Source of support: Nil, Conflict of interest: None Declared