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Research Article

PACLITAXEL INDUCED LIPID PEROXIDATION: ROLE OF WATER EXTRACT OF *SPIRULINA PLATENSIS*

Supratim Ray *, Sarbani Dey Ray
Department of Pharmaceutical Sciences, Assam University, Silchar, India
*Corresponding Author Email: supratimray_in@yahoo.co.in

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ABSTRACT

The present study showed the free radical scavenging activity of water extract of *Spirulina platensis* on paclitaxel-induced lipid peroxidation. This *in vitro* work was carried out with goat liver as lipid source using malondialdehyde and 4-hydroxy-2-nonenal as model markers. The findings suggest that paclitaxel could 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 paclitaxel-induced toxicity.

Keywords: Paclitaxel, *Spirulina platensis*, lipid peroxidation, malondialdehyde, 4-hydroxy-2-nonenal

INTRODUCTION

Lipid peroxidation is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically[1-3]. The cytotoxic end products of lipid peroxidation are mainly aldehydes as exemplified by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc[4]. Paclitaxel is one of the popular drugs in breast cancers in women of developed and developing countries. However, the drug produces several side effects due to production of free radicals in the body[5]. It is reported that paclitaxel in combination with antioxidant reduces the drug induced lipid peroxidation [6].
Medicinal plants are used as antioxidant and their use has been increased tremendously through out the world[7]. Spirulina is 60-70% protein by weight and contain a rich source of vitamins especially vitamin B_{12}, β-carotene (provitamin A), and minerals, especially iron[8]. Spirulina 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[8].
In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation[9-11] the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of water extract of *Spirulina platensis* on paclitaxel-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 was from Sigma Chemicals Co. St. Louis, MO, USA. 2, 4-Dinitrophenylhydrazine (DNPH) was procured from SD Fine Chem. Ltd., Mumbai. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Aurora, Ohio. Spirulina was obtained from INDO LEENA, Biotech private ltd., Spirulina Farm, Namakkal, Tamil Nadu. Pure sample of paclitaxel used in present study was provided by United Biotech (P) Ltd., New Delhi, India. 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[12]. 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 paclitaxel (D) at a concentration of 0.143µM/g tissue homogenate. The third portion was treated with both paclitaxel at a concentration 0.143µM/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 paclitaxel and /or water extract of *Spirulina platensis* treatment, the liver tissue homogenate samples were shaken for five hours and the malondialdehyde and 4-hydroxy-2-nonenal 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[13]. The estimation was done at 5 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) using Shimadju UV-1700 double beam spectrophotometer. 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=0.007086M, where M= nanomoles of MDA, A= absorbance, r = 0.995, SEE= 0.006.

Estimation of 4-hydroxy-2-nonenal (4-HNE) level in tissue homogenate

The estimation was done at 5 hours of incubation and repeated in three animal sets. In each case three samples of 2 ml of incubation mixture was treated with 1.5 ml of 10% (w/v) TCA solution then centrifuged at 3000 rpm for 30 min. 2 ml of the filtrate was treated with 1 ml of 2, 4-dinitrophenyl hydrazine (DNPH) (100 mg / 100 ml of 0.5 M HCl) and kept for 1 hour at room temperature. After that, the samples were extracted with hexane, and the extract was evaporated to dryness under argon at 40^0 C. After cooling to a room temperature, 2 ml of methanol was added to each sample and the absorbance was measured at 350 nm against methanol as blank [14] using Shimadju UV-1700 double beam spectrophotometer. The values were determined from the standard curve. The standard calibration curve was drawn based on the following procedure. A series of dilutions of 4-HNE in different concentrations of solvent (phosphate buffer) were prepared. From each solution 2 ml of sample pipette out and transferred into stoppered glass tube. 1 ml of DNPH solution was added to all the samples and kept at room temperature for 1 hour. Each sample was extracted with 2 ml of hexane for three times. All extracts were collected in stoppered test tubes. After that extract was evaporated to dryness under argon at 40^0C and the residue was reconstituted in 1 ml of methanol. The absorbance was measured at 350 nm using the 0 µM standard as blank. The best-fit equation is: Nanomoles of 4-HNE = $(A_{350}$ - 0.005603185) / 0.003262215, where A_{350} = absorbance at 350nm, r = 0.999, SEM = 0.007

Statistical Analysis

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure[15-16] were also performed on the percent changes data of various groups such as paclitaxel-treated (D), paclitaxel 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 4-HNE content of different samples at 5 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 Figure 1 it was evident that tissue homogenates treated with paclitaxel showed an increase in MDA (31.06 %) content in samples with respect to control at 5 hours of incubation to a significant extent. The observations suggest that paclitaxel 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[17]. But the MDA (5.28 %) content was significantly reduced in comparison to paclitaxel-treated group when tissue homogenates were treated with paclitaxel 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 (3.22%) level were reduced in comparison to paclitaxel 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 paclitaxel and water extract of *Spirulina platensis* implies the free radical scavenging property of water extract of *Spirulina platensis*.

It was also evident from Figure 2 that tissue homogenates treated with paclitaxel showed an increase in 4-HNE (16.28%) content in samples with respect to control to a significant extent. The observations suggest that paclitaxel could significantly induce the lipid peroxidation process. 4-HNE is formed due to lipid peroxidation occurring during episodes of oxidant stress, readily forms adducts with cellular proteins; these adducts can be assessed as a marker of oxidant stress in the form of lipid peroxidation [18]. Lipid peroxidation leads to the generation of a variety of cytotoxic products. Moreover, it causes disruption of membrane structure and change in fluidity [19]. But the 4-HNE content was significantly reduced (-10.26%) in comparison to control and paclitaxel-treated group when tissue homogenates were treated with paclitaxel in combination with water extract of *Spirulina platensis*. 4-HNE as well as related aldehydes display strong cytotoxicity [20-21] and their effective removal could play an important role in a broad defense system of the liver *in vivo* against damaging effects of lipid peroxidation [22].

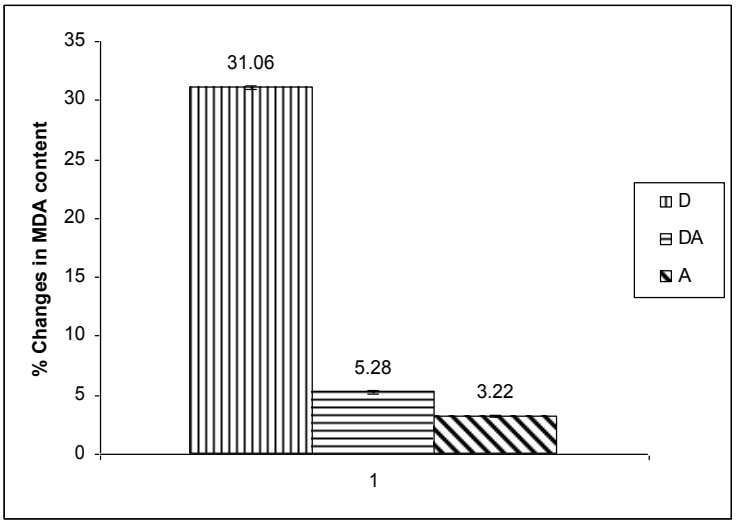

Figure 1: Effects of water extract of *Spirulina platensis* on paclitaxel-induced changes in MDA content (n=3); D, DA & A indicate only paclitaxel-treated, paclitaxel & water extract of *Spirulina platensis* -treated and only water extract of *Spirulina platensis* –treated samples

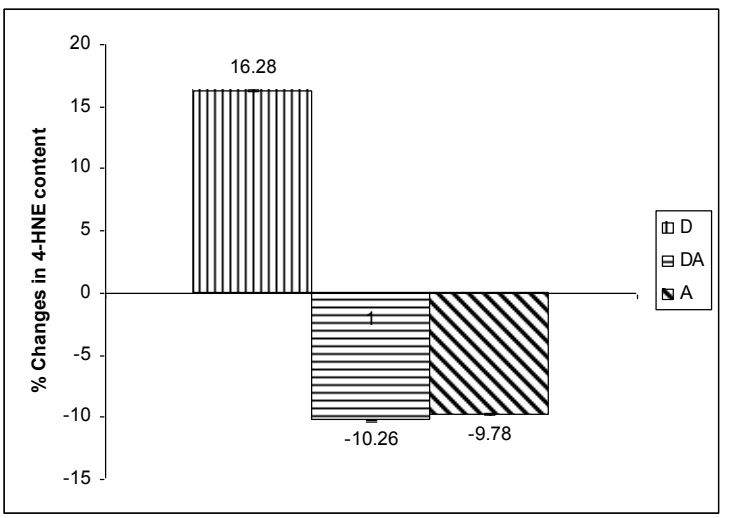

Figure 2: Effects of water extract of *Spirulina platensis* on paclitaxel-induced changes in 4-HNE content (n=3); D, DA & A indicate only paclitaxel-treated, paclitaxel & water extract of *Spirulina platensis* -treated and only water extract of *Spirulina platensis* –treated samples

Table 1: ANOVA & Multiple comparison for changes of MDA and 4-HNE content

Name of the antioxidant	Marker of lipid peroxidation	Analysis of variance and multiple comparison
Water extract of *Spirulina platensis*	MDA	F1=21823.71[df=(2,4)], F2=0.32[df=(2,4)], Pooled variance $(S^2)^*$=0.033, Critical difference $(p=0.05)^{\#}$ LSD=0.342 Ranked means** (D) (DA) (A)
	4-HNE	F1=99127.2 [df=(2,4)], F2=0.27[df=(2,4)], Pooled variance $(S^2)^*$=0.007, Critical difference $(p=0.05)^{\#}$ LSD=0.16 Ranked means** (D) (DA) (A)

Theoretical values of 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 only paclitaxel-treated, paclitaxel & water extract of *Spirulina platensis* -treated and only water extract of *Spirulina platensis* –treated samples * Error mean square, # Critical difference according to least significant procedure (LSD)
**Two means not included within same parenthesis are statistically significantly different at p=0.05 level.

Again the tissue homogenates were treated only with water extract of *Spirulina platensis* then the 4-HNE level was reduced (-9.78%) in comparison to the control and the paclitaxel treated group. This decrease 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 paclitaxel-treated, paclitaxel 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). The Tables also indicate that the level of MDA / 4-HNE in all three groups i.e. paclitaxel – treated, paclitaxel 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 paclitaxel, 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 paclitaxel induced toxic effects.

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