



## EXPLORING THE PROTECTIVE ROLE OF WATER EXTRACT OF *SPIRULINA PLATENSIS* ON DOCETAXEL-INDUCED LIPID PEROXIDATION USING MALONDIALDEHYDE AS MODEL MARKER

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### ABSTRACT

This *in vitro* study was designed with an aim to evaluate free radical scavenging activity of water extract of *Spirulina platensis* on docetaxel-induced lipid peroxidation using malondialdehyde as model marker. In this study goat liver has been used as liver source. The results suggest that docetaxel 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 docetaxel-induced toxicity.

**Keywords:** Docetaxel, *Spirulina platensis*, lipid peroxidation, malondialdehyde

### INTRODUCTION

Docetaxel is a semi synthetic derivative of paclitaxel which is obtained from the rare Pacific yew tree *Taxus brevifolia*<sup>1</sup>. It is primarily used for the treatment of breast, ovarian and non-small cell lung cancer. As docetaxel is a cell cycle specific agent, it is cytotoxic to all dividing cells in the body<sup>2</sup> and produces several toxic side effects due to damage of normal cell like hair follicles, bone marrow and other germ cells. It was reported that docetaxel has the capability of inducing lipid oxidation and membrane damage in human hepatoma cells<sup>3</sup>. Lipid peroxidation leads to generation of peroxides and hydroperoxide that can decompose to yield a wide range of cytotoxic end products most of which are aldehydes as exemplified by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc<sup>4</sup>. It is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically<sup>5-7</sup>. The latter form is associated mostly with cellular damage as a result of oxidative stress<sup>8</sup>. Free radicals are constantly being generated in the body through various mechanisms and also being removed by endogenous antioxidant defense mechanism that acts by scavenging free radicals, decomposing peroxides and/or binding with pro-oxidant metal ion. Free radical mediated oxidative stress results usually from deficient natural antioxidant defense. In case of reduced or impaired defense mechanism and excess generation of free radicals that are not counter balanced by endogenous antioxidant defense exogenously administered antioxidants have been proven useful to overcome oxidative damage<sup>9</sup>. Use of medicinal plants as antioxidant has been increased tremendously through out the world<sup>10</sup>. *Spirulina* is 60-70 % protein by weight and contain a rich source of vitamins especially vitamin B<sub>12</sub>,  $\beta$ -carotene (provitamin A) and minerals, especially iron<sup>11</sup>. It was found that spirulina potentiate the immune system leading to suppression of cancer development and viral infection<sup>12</sup>. It also contains phycocyanin (7 % dry weight basis) and polysaccharides, both of them have antioxidant properties. *Spirulina* has direct effect on reactive oxygen species. It also contains an important enzyme superoxide dismutase (SOD) (1700 units/g of dry mass) that acts indirectly by slowing down the rate of oxygen radical generating reactions<sup>11</sup>. In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation<sup>13-15</sup> the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of water extract of *Spirulina platensis* on docetaxel-induced lipid peroxidation.

### MATERIALS AND METHODS

Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India. 1,1,3,3, tetraethoxypropane was from Sigma chemicals Co. St. Louis, MO, USA. *Spirulina* was obtained from INDO LEENA, Biotech private ltd., *Spirulina* Farm, Namakkal, Tamil Nadu, India. Pure sample of docetaxel used in present study was provided by Fresenius Kabi, Kalyani, 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 4 g, 5 g, 6 g, 7 g 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  $\lambda_{max}$  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<sup>16</sup>. 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 docetaxel (D) at a concentration of 0.143 μM/g tissue homogenate. The third portion was treated with both docetaxel at a concentration 0.143 μM/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 docetaxel and /or water extract of *Spirulina platensis* treatment, the liver tissue homogenate samples were shaken for five hours and the malondialdehyde content of various portions were determined. Then the samples were stored at 10-12°C for 24 hours for next determinations.

**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<sup>17</sup>. The estimation was done at 2 hours of incubation and repeated in five 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

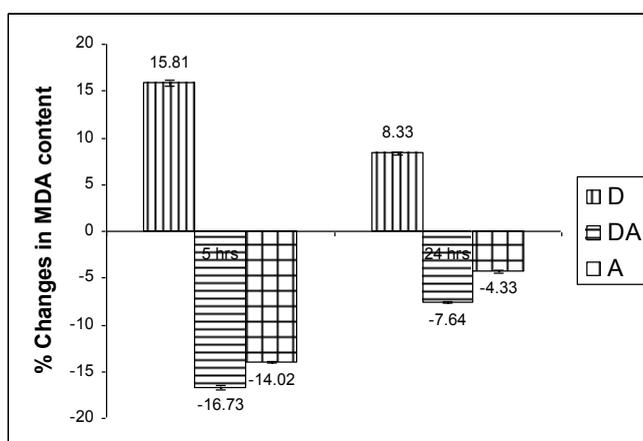
then volume was made up to 10 ml with distilled water. The mixture was heated on a boiling water bath for 30 minutes. Then tubes were cooled to a room temperature and the absorbance was measured at 530 nm against a TBA blank (prepared from 5 ml of TBA solution and 5 ml of distilled water) using Shimadzu 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  $A = 0.007086 M$ , where M = nanomoles of MDA, A = absorbance,  $r = 0.995$ ,  $SEE = 0.006$ .

**Statistical Analysis**

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure<sup>18-19</sup> were also performed on the percent changes data of various groups such as docetaxel-treated (D), docetaxel 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 AND DISCUSSION**

The percent changes in MDA content of different samples at 5 and 24 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 docetaxel showed an increase in MDA (15.81 and 8.33 %) content in samples with respect to control of 5 and 24 hours of incubation to a significant extent. The observations suggest that docetaxel could significantly induce the lipid peroxidation process.



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

**Table 1: ANOVA and Multiple Comparisons for changes of MDA content**

Name of the antioxidant	Time of incubation (h)	Analysis of variance and multiple comparison
Water extract of <i>Spirulina platensis</i>	5	F1 = 3238.36[df = (2, 4)], F2 = 0.13[df = (2, 4)], Pooled variance (S <sup>2</sup> ) = 0.3, Critical difference (p = 0.05) <sup>#</sup> LSD = 1.03 Ranked means** (D) (DA) (A)
	24	F1 = 2628.28 [df = (2, 4)], F2 = 1.15[df = (2, 4)], Pooled variance (S <sup>2</sup> ) = 0.08, Critical difference (p = 0.05) <sup>#</sup> LSD = 0.53 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 and A indicate only docetaxel-treated, docetaxel and 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

MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism<sup>20</sup>. But the MDA (-16.73 and -7.64 %) content were significantly reduced in comparison to control and docetaxel-treated group when tissue homogenates were treated with docetaxel 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 (-14.02 and -4.33 %) level were reduced in comparison to the control and the docetaxel 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 docetaxel and water extract of *Spirulina platensis* as well as only with water extract of *Spirulina platensis* implies the free radical scavenging property of 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 docetaxel-treated, docetaxel 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 in all three groups i.e. docetaxel –treated, docetaxel 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 data presented in this work demonstrate the lipid peroxidation induction potential of docetaxel, which may be related to its toxic potential. The results also suggest the antiperoxidative effects of water extract of the algae and demonstrate its potential to reduce docetaxel induced toxic effects. The antioxidant effect is attributed due to its various constituents working individually or in synergy. However, further extensive study is required to draw any final conclusion.

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