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

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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\(^2\). 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\(_12\), \(\beta\)-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\(^9\).

In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation\(^{10-11}\) 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 \(\lambda_{max}\) 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\(^12\). Goat liver perfused with normal saline through hepatic
The extent of lipid peroxidation was measured in terms of
Estimation of malondialdehyde (MDA) level from tissue
absorbance, r = 0.995, SEE = 0.006.

The observations suggest that paclitaxel could significantly
induce the lipid peroxidation process. 4

It was also evident from Figure 2 that tissue homogenates
treated with paclitaxel in combination with 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 significantly induce the lipid peroxidation process.

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 a significant 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. 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. 14 Lipid peroxidation leads to the generation of a variety of cytotoxic products. Moreover, it causes disruption of membrane structure and change in fluidity. 15 But the 4-HNE content was significantly reduced (10.26%) in comparison to control 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.

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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.

![Graph showing changes in MDA content](image1)

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.

![Graph showing changes in 4-HNE content](image2)

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

<table>
<thead>
<tr>
<th>Name of the antioxidant</th>
<th>Marker of lipid peroxidation</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract of <em>Spirulina platensis</em></td>
<td>MDA</td>
<td>$F_{1}=21823.71$ [df=(2, 4)], $F_{2}=0.32$ [df=(2, 4)], Pooled variance $(S') = 0.033$, Critical difference $(p=0.05)$ LSD $= 0.342$ Ranked means** (D) (DA) (A)</td>
</tr>
<tr>
<td></td>
<td>4-HNE</td>
<td>$F_{1}=99127.2$ [df=(2, 4)], $F_{2}=0.27$ [df=(2, 4)], Pooled variance $(S') = 0.007$, Critical difference $(p=0.05)$ LSD $= 0.16$ Ranked means** (D) (DA) (A)</td>
</tr>
</tbody>
</table>

Theoretical values of F: $p=0.05$ level $F_{1}=6.94$ [df=(2, 4)], $F_{2}=6.94$ [df=(2, 4)] $F_{1}$ and $F_{2}$ 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.

**REFERENCES**


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