Polish Pharmaceutical Society

Free radical production and lipid peroxidation are potentially important mediators for pathophysiology of many diseases (1). Lipid peroxidation is the introduction of a functional group containing two catenated oxygen atoms O-O, into unsaturated fatty acids in a free radical chain reaction (2). 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. (3). Free radicals are highly reactive molecules with odd number of electrons. They 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 prooxidant metal ion (4). 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 counterbalanced by endogenous antioxidant defense, exogenously administered antioxidants have been proven useful to overcome oxidative damage (4).

Ascorbic acid has versatile medicinal properties. It has been now established that besides its own physiological effects it has potential antioxidant property. Ascorbic acid has been reported to have protective role against cadmium induced thyroid dysfunction due to its antioxidant action (5). *Spirulina plantensis* (blue green algae) has diverse biological activities. Due to high content of highly valuable proteins, indispensable amino acids, vitamins, β-carotene and other pigments, mineral substances, indispensable fatty acids and polysaccharides, spirulina has been found suitable for use as bioactive additive (6). 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 (7). It was found that besides antioxidant effects spirulina has versatile properties like immunomodulation effects (8), anticaner effects (9), effects on hyperlipidemia (10, 11) and diminishing toxicities from heavy metal and other compounds (12). In view of the above findings it would be of interest to obtain more information regarding antiperoxidative potential of ascorbic acid and spirulina.

**Abstract:** Considering drug-induced lipid peroxidation as a possible mediator of drug-induced toxicity and exploiting the free radical scavenging action of antioxidants, the present study was designed to evaluate the protective effects of ascorbic acid (AA) and water extract of *Spirulina plantensis* (SP) to minimize 5-fluorouracil (5-FU)-induced lipid peroxidation. The study has been performed *in vitro* using goat liver as an experimental model. This evaluation was done by measuring the malondialdehyde (MDA), reduced glutathione (GSH), 4-hydroxy-2-nonenal (4-HNE) and nitric oxide (NO) content of the tissue as markers of lipid peroxidation. The results suggest that ascorbic acid and water extract of *Spirulina plantensis* could suppress the 5-FU-induced lipid peroxidation to a significant extent.

**Keywords:** lipid peroxidation, 5-fluorouracil, ascorbic acid, *Spirulina plantensis*, nitric oxide, malondialdehyde, reduced glutathione, 4-hydroxy-2-nonenal.

---

* Correspondence: e-mail: supratimray_in@yahoo.co.in

---

**IN VITRO EVALUATION OF PROTECTIVE EFFECTS OF ASCORBIC ACID AND WATER EXTRACT OF *SPIRULINA PLANTESIS* (BLUE GREEN ALGAE) ON 5-FLUOROURACIL-INDUCED LIPID PEROXIDATION**

SUPRATIM RAY1*, KUNAL ROY2 and CHANDANA SENGUPTA2

1 Division of Pharmaceutical Chemistry, Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim, 737 136, India, 2 Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, West Bengal, India

---

**Abstract:** Considering drug-induced lipid peroxidation as a possible mediator of drug-induced toxicity and exploiting the free radical scavenging action of antioxidants, the present study was designed to evaluate the protective effects of ascorbic acid (AA) and water extract of *Spirulina plantensis* (SP) to minimize 5-fluorouracil (5-FU)-induced lipid peroxidation. The study has been performed *in vitro* using goat liver as an experimental model. This evaluation was done by measuring the malondialdehyde (MDA), reduced glutathione (GSH), 4-hydroxy-2-nonenal (4-HNE) and nitric oxide (NO) content of the tissue as markers of lipid peroxidation. The results suggest that ascorbic acid and water extract of *Spirulina plantensis* could suppress the 5-FU-induced lipid peroxidation to a significant extent.

**Keywords:** lipid peroxidation, 5-fluorouracil, ascorbic acid, *Spirulina plantensis*, nitric oxide, malondialdehyde, reduced glutathione, 4-hydroxy-2-nonenal.
5-Fluorouracil (5-FU), an anticancer drug, has several toxic side effects besides its antitumor property. It has been reported that 5-FU produces nephrotoxicity on Wistar rats (13). It has also been observed that the drug induces cardiotoxicity (14) because 5-FU treatment causes impairment in the myocardial antioxidant defense system and leads to cardiac peroxidation and it has been postulated that antioxidant therapy might have therapeutic advantage (15). Lipid peroxidation induction capacity of drugs may be related to their toxic potential as exemplified by adriamycin-induced cardiotoxicity, which occurs through free radical mediated process (16). Thus, the evaluation of antioxidants as suppressor of drug induced lipid peroxidation provides a scope of further investigation for their co-administration with drugs to reduce drug-induced toxicities that are possibly mediated by free radical mechanism.

In the ongoing search of the present authors for antioxidants that may reduce drug induced lipid peroxidation (17-27), the present work has been carried out in vitro to evaluate the antioxidant effect of ascorbic acid (AA) and water extract of Spirulina plantesis (SP) on 5-fluorouracil-induced lipid peroxidation.

EXPERIMENTAL

Thiobarbituric acid (TBA), 2,4-dinitrophenylhydrazine (DNPH), sodium nitrite and trichloroacetic acid (TCA) were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi; 5,5'- dithiobis(2-nitrobenzoic acid) was from SRL Pvt. Ltd., Mumbai; sulfanilamide from S.D. Fine Chem. Ltd., Mumbai; N-naphthylethylenediamine dihydrochloride was from Loba Chemie Pvt. Ltd., Mumbai; 1,1,3,3-tetraethoxypropane, reduced glutathione were from Sigma Chemicals Co. St. Louis, MO, USA. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Ohio, USA. All other reagents were of analytical grade.

The study was performed on goat (Capra capra) liver using some common laboratory markers of lipid peroxidation like measurement of the malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), reduced glutathione were from Sigma Chemicals Co. St. Louis, MO, USA. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Ohio, USA. All other reagents were of analytical grade.

The study was performed on goat (Capra capra) liver using some common laboratory markers of lipid peroxidation like measurement of the malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), reduced glutathione were from Sigma Chemicals Co. St. Louis, MO, USA. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Ohio, USA. All other reagents were of analytical grade.

Preparation of water extract of Spirulina plantesis

Spirulina was obtained from INDO LEENA, Biotech Private Ltd., Spirulina Farm, Namakkal, Tamil Nadu. 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 and added to 200 mL of water. The mixture was heated cautiously until the volume was reduced to 50 mL. The hot solution was twice filtered at a suction pump using one and then double filter paper. 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 Petri dish was weighed accurately and 1 mL of the extract was placed on it and heated on steam bath to remove the water. The last traces of water were removed by drying in hot air oven. The Petri dish was cooled in desiccator and weighed. The procedure was repeated fourfold yielding the amount of solid in 5 mL of extract. The concentration of water extract determined in this way was 0.92% w/v. The same procedure was followed with 4 g, 5 g, 6 g , and 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 concentration of the water extract of the algae was 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 Kolkata Municipal Corporation (KMC) approved outlet. 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).

Incubation of tissue homogenate with drug and/or antioxidant

The tissue homogenate was divided into four parts of 50 mL each. The first portion was kept as control (C), while the second portion was treated with drug (D) at a concentration of 0.012 mg/g of tissue homogenate. The third portion was treated with drug and antioxidant (AA / SP) (DA) and the fourth one was treated with antioxidant alone at a concentration of 0.166 mg/g of tissue homogenate. After treatment with drug and/or antioxidant, the different portions of liver homogenate were shaken for 1 h and incubated at ambient temperature for 4 h for further work.
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 (29). The estimation was done at 2 and 4 h 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 min to precipitate protein. Then, 2.5 mL of the supernatant was treated with 5 mL of 0.002 M TBA solution and the volume was made up to 10 mL with distilled water. The mixture was heated on a boiling water bath for 30 min. The tubes were cooled to 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 min. The solutions were cooled to 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 reduced glutathione (GSH) level from tissue homogenate

Reduced glutathione (GSH) was measured acc. to Ellman’s method (30). The estimation was done at 1 and 2 h of incubation and repeated in five 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 min. After that, 1 mL of the filtrate was mixed with 5 mL of 0.1 M phosphate buffer (pH = 8.0) and 0.4 mL of 5,5′-dithiobis(2-nitrobenzoic acid) in 0.01% 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). The concentrations of reduced glutathione were determined from standard curve, which was constructed as follows. Different aliquots of standard reduced glutathione stock solution were taken in 10 mL volumetric flasks. To each solution 0.4 mL of DTNB solution was added and volume was adjusted up to the mark containing 9.6 mL of phosphate buffer (pH = 8.0) and 0.4 mL of DTNB solution. By plotting absorbances against concentration a straight line passing through the origin of grid was obtained. The best-fit equation was A = 0.00151C, where C = nanomoles of reduced glutathione, A = absorbance, r = 0.997, SEE = 0.008.

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

The estimation was done only at 2 h of incubation and it was repeated in 5 animal sets. In each

<table>
<thead>
<tr>
<th>Name of the antioxidant</th>
<th>Hours of incubation</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>2</td>
<td>F1= 41.29 [df = (2,8)], F2 = 5.44 [df = (4,8)], Pooled variance (S²) = 4.79, Critical difference, (p = 0.05), LSD = 4.12, Ranked means” (D) (DA, A)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>F1= 67.92 [df = (2,8)], F2 = 0.26 [df = (4,8)], Pooled variance (S²) = 8.94, Critical difference, (p = 0.05), LSD = 5.63, Ranked means”, (D) (DA, A)</td>
</tr>
<tr>
<td>Water extract of Spirulina platensis</td>
<td>2</td>
<td>F1= 59.33 [df = (2,8)], F2 = 1.153 [df=(4,8)], Pooled variance (S²) = 14.06, Critical difference, (p = 0.05), LSD = 7.06, Ranked means”, (D) (DA, A)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>F1= 27.23 [df = (2,8)], F2 = 0.13 [df = (4,8)], Pooled variance (S²) = 56.90, Critical difference, (p = 0.05), LSD = 14.20, Ranked means”, (D) (DA, A)</td>
</tr>
</tbody>
</table>

Theoretical values of F: p = 0.05 level F1 = 4.46 [df = (2,8)], F2 = 3.84 [df = (4,8)]; p = 0.01 level F1 = 8.65 [df = (2,8)], F2 = 7.01 [df = (4,8)]; F1 and F2 corresponding to variance ratio between groups and within groups, respectively. Error mean square, * Critical difference according to the least significant procedure (34); ** Two means not included within the same parenthesis are statistically significantly different at p = 0.05 level.
case three samples of 2 mL of incubation mixture were treated with 1.5 mL of 10% TCA solution and centrifuged at 3000 rpm for 30 min. Then, 2 mL of the filtrate was treated with 0.5 mL of Griess reagent. After 10 min the absorbances of the solutions were measured at 540 nm against blank (prepared from 5.0 mL of distilled water and 0.5 mL of Griess reagent). The values were calculated using the best-fit equation: Nanomoles of 4-HNE = \left( \frac{A_{540} - 0.005603185}{0.003262215} \right), where A = absorbance at 540 nm, r = 0.999, SEE = 0.007.

### Table 2. ANOVA and multiple comparison for changes of 4-HNE content.

<table>
<thead>
<tr>
<th>Name of the antioxidant</th>
<th>Hours of incubation</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>2</td>
<td>F1 = 34.99 [df = (2,8)], F2 = 4.64 [df = (4,8)], Pooled variance (S^2) = 48.89, Critical difference, (p = 0.05)^*, LSD = 13.16, Ranked means^** (D) (DA, A)</td>
</tr>
<tr>
<td>Water extract of Spirulina plantesis</td>
<td>2</td>
<td>F1 = 50.25 [df = (2,8)], F2 = 1.10 [df = (4,8)], Pooled variance, (S^2) = 41.18, Critical difference, (p = 0.05)^*, LSD = 12.08, Ranked means^** (D) (DA, A)</td>
</tr>
</tbody>
</table>

Theoretical values of F: p = 0.05 level F1 = 4.46 [df = (2,8)], F2 = 3.84 [df = (4,8)]; p = 0.01 level F1 = 8.65 [df = (2,8)], F2 = 7.01 [df = (4,8)]; F1 and F2 corresponding to variance ratio between groups and within groups, respectively. * Error mean square, ^** Critical difference according to the least significant procedure (34); ** Two means not included within the same parenthesis are statistically significantly different at p = 0.05 level.

### Table 3. ANOVA and multiple comparison for changes of GSH content.

<table>
<thead>
<tr>
<th>Name of the antioxidant</th>
<th>Hours of incubation</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>2</td>
<td>F1 = 28.62 [df = (2,8)], F2 = 5.30 [df = (4,8)], Pooled variance (S^2) = 8.49, Critical difference, (p = 0.05)^*, LSD = 5.48, Ranked means^** (D) (DA, A)</td>
</tr>
<tr>
<td>Water extract of Spirulina plantesis</td>
<td>2</td>
<td>F1 = 17.87 [df = (2,8)], F2 = 1.05 [df = (4,8)], Pooled variance (S^2) = 22.71, Critical difference, (p = 0.05)^*, LSD = 8.97, Ranked means^** (D) (DA, A)</td>
</tr>
</tbody>
</table>

Theoretical values of F: p = 0.05 level F1 = 4.46 [df = (2,8)], F2 = 3.84 [df = (4,8)]; p = 0.01 level F1 = 8.65 [df = (2,8)], F2 = 7.01 [df = (4,8)]; F1 and F2 corresponding to variance ratio between groups and within groups, respectively. * Error mean square, ^** Critical difference according to the least significant procedure (34); ** Two means not included within the same parenthesis are statistically significantly different at p = 0.05 level.

The estimation was done at 1 and 2 h of incubation and it was repeated in five animal sets. NO content was determined by reaction with the Griess reagent. This reagent was prepared by mixing equal volumes of sulfanilamide (1% w/v in 3 M HCl) and 0.1% w/v N-naphthylethylenediamine dihydrochloride (32). In each case three samples of 4.0 mL of tissue homogenate were treated with 2.5 mL of 10% TCA solution and centrifuged at 3000 rpm for 30 min. Then 5 mL of the filtrate were treated with 0.5 mL of Griess reagent. After 10 min the absorbances of the solutions were measured at 540 nm against blank (prepared from 5.0 mL of distilled water and 0.5 mL of Griess reagent). The values were calcu-
lulated from standard curve, which was constructed as follows. Different aliquots from standard sodium nitrite solution were taken in 5 mL volumetric flasks. To each solution 0.5 mL of Griess reagent was added and the volume was adjusted up to the mark with phosphate buffer. The absorbances of each solution were noted at 540 nm against a blank containing the buffer and Griess reagent. By plotting absorbance against concentration a straight line passing through the origin was obtained. The best-fit equation is \( A = 0.0108 \times M \), where \( M = \) nanomoles of NO, \( A = \) absorbance, \( r = 0.99581 \), SEE = 0.0064.

The percent changes in MDA, GSH, 4-HNE and NO level of different samples at different hours of incubation were calculated with respect to the control of the corresponding hours of incubation and the changes in MDA/GSH/4-HNE/NO levels were considered as an indicator of the extent of lipid peroxidation. Interpretation of the results was supported by Mann-Whitney U test (33) and also by statistical multiple comparison analysis using the least significant different procedure (34, 35).

RESULTS

The results of the studies on 5-fluorouracil-induced lipid peroxidation and its inhibition with ascorbic acid and water extract of Spirulina plantensis are shown in bar chart along with standard deviation (Figs.1-8). From Figures 1-4, it is evident that incubation of the liver homogenates with 5-FU caused an increase in MDA and 4-HNE content with respect to control to a significant extent after incubation for varying period of time. However, the MDA and 4-HNE content were significantly reduced with respect to drug treated group when the tissue homogenates were treated with 5-FU in combination with antioxidants (AA / SP). The observations suggest that 5-FU could significantly induce the lipid peroxidation process. So, the lipid peroxidation induction capacity of the drug may be related to its toxic potential. It was also found that the antioxidants (AA / SP) could suppress the 5-FU induced lipid peroxidation to a significant extent. It was also noted that when the tissue homogenates were treated with ascorbic acid alone, it showed some increase in MDA content with respect to control (1 h), but when the tissue homogenates were treated with spirulina alone, then there was a decrease in MDA content with respect to the corresponding control.

From Figures 5-8 it is evident that incubation of the liver homogenates with 5-FU caused a decrease in GSH and NO content with respect to control to a significant extent. The decrease in GSH and NO content was associated with an increase in lipid peroxidation. When the tissue homogenates were treated with drug and antioxidants (AA / SP), then the GSH and NO levels increased in comparison to drug treated group of corresponding hours. Again, when the tissue homogenates were treated with antioxidants (AA / SP) alone, the GSH and NO contents were increased in comparison to the control samples. The increase in GSH and NO level suggest the antiperoxidative potential of antioxidants (AA /SP). The Z values obtained from Mann-Whitney test for comparison of different groups (D-DA, DA-A, D-A) (Table 5) indicate that the drug treated groups are significantly different from the groups treated with drug and antioxidant (DA), and antiox-
Table 5. Results of Mann-Whitney U-Test.

<table>
<thead>
<tr>
<th>Name of Antioxidant</th>
<th>Markers Used</th>
<th>Hours of incubation</th>
<th>D-DA</th>
<th>DA-A</th>
<th>D-A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean Ranks</td>
<td>U</td>
<td>Z</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>2</td>
<td>40,15</td>
<td>0.00</td>
<td>2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40,15</td>
<td>0.00</td>
<td>2.611</td>
<td>0.009</td>
</tr>
<tr>
<td>GSH</td>
<td>1</td>
<td>17,38</td>
<td>2.00</td>
<td>-2.193</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16,39</td>
<td>1.00</td>
<td>-2.402</td>
<td>0.016</td>
</tr>
<tr>
<td>4-HNE</td>
<td>2</td>
<td>38,17</td>
<td>2.00</td>
<td>2.193</td>
<td>0.028</td>
</tr>
<tr>
<td>NO</td>
<td>1</td>
<td>15,40</td>
<td>0.00</td>
<td>-2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15,40</td>
<td>0.00</td>
<td>-2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>2</td>
<td>40,15</td>
<td>0.00</td>
<td>2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40,15</td>
<td>0.00</td>
<td>2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>1</td>
<td>15,40</td>
<td>0.00</td>
<td>-2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15,40</td>
<td>0.00</td>
<td>-2.611</td>
<td>0.009</td>
</tr>
<tr>
<td>4-HNE</td>
<td>2</td>
<td>40,15</td>
<td>0.00</td>
<td>2.611</td>
<td>0.009</td>
</tr>
<tr>
<td>NO</td>
<td>1</td>
<td>15,40</td>
<td>0.00</td>
<td>-2.611</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15,40</td>
<td>0.00</td>
<td>-2.611</td>
<td>0.009</td>
</tr>
</tbody>
</table>

D, DA, A indicate drug treated, drug & antioxidant treated and antioxidant treated, respectively.
In vitro evaluation of protective effects of ascorbic acid... 341

In this study ANOVA was used in addition to non-parametric Mann-Whitney test to confirm the results of differences between various groups and also within a particular group. From Tables 1-4, it is seen that there are significant differences among various groups (F1), but within a particular group, differences (F2) were insignificant. The differences among objects within a treatment, is a measure of the variability of the observation. If the F test is significant and more than two treatments are included in the experiments, it may not be obvious immediately, which treatments are different. To solve the problem, multiple comparisons in ANOVA have been done.

DISCUSSION

It has been understood that lipid peroxidation induction capacity of drugs may be related to their toxic potential. This is an analogy to insulin defi-

Figure 1. Effects of ascorbic acid on 5-FU induced lipid peroxidation changes in MDA profile (n = 5).

Figure 2. Effects of ascorbic acid on 5-FU induced lipid peroxidation changes in 4-HNE profile (n = 5).

Figure 3. Effects of water extract of *Spirulina plantesis* on 5-FU induced lipid peroxidation changes in MDA profile (n = 5).

Figure 4. Effects of water extract of *Spirulina plantesis* on 5-FU induced lipid peroxidation changes in 4-HNE profile (n = 5).
crenity diabetes induced by alloxan (36) that are mediated through free radical mechanism. An increase in MDA and 4-HNE levels or a decrease in GSH and NO levels of the drug treated group suggests the occurrence of lipid peroxidation. MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism (37). An increase in the accumulation of MDA in cells can result into cellular degradation, some biochemical changes and even cell death (38). 4-HNE is a major product formed due to lipid peroxidation through the oxidative degradation of arachidonic acid bound to phospholipid (39, 40). It can diffuse within or even escape from the cell and attack targets far from the site of the original free radical event (41, 42). So, the decrease in MDA and 4-HNE content of tissue homogenates when treated with drug and antioxidants implies the free radical scavenging property of antioxidants. The increase in MDA content (1 h) with respect to control when the tissue homogenates were treated with ascorbic acid alone indicates its prooxidant effect. It was postulated that ascorbic acid could reduce Fe³⁺ to Fe²⁺ which promotes generation of hydroxyl radicals and other reactive oxygen species through Fenton’s reaction (43, 44). Many known antioxidants like vitamins (45), estrogen (46, 47), superoxide dismutase (48), and...
In vitro evaluation of protective effects of ascorbic... 343

flavonoids have been reported to act as prooxidants in the presence of transition metals (49-50) or at high concentration (51). Glutathione is a small protein composed of three amino acids, such as cysteine, glutamic acid and glycine (52). It is an important antioxidant and plays a very important role in the defence mechanism for tissue against the reactive oxygen species (53). The depletion of GSH is associated with an increase in lipid peroxidation. The decrease in GSH level may be a consequence of enhanced utilization of this compound by the antioxidant enzymes: glutathione peroxidase and glutathione-S-transferase. NO plays a very important role in host defense (54). Nitric oxide has versatile role in biology because it can be a signaling molecule in vasodilatation (55), a toxin (56), and a prooxidant (57), and a potential antioxidant (58-61). Thus, the increase in GSH and NO content of tissue homogenates when treated with drug and antioxidant (AA / SP) implies the free radical scavenging activity of the antioxidants. It has been found that spirulina reduces the hepatic cytochrome P₄₅₀ content and increases the glutathione-S-transferase activity (62). It has been proposed that NO causes chain termination reactions during lipid peroxidation as observed in low-density lipoprotein oxidation as well as in chemical systems (58-61).

The data presented in this work demonstrate the lipid peroxidation induction potential of 5-fluorouracil, which may be related to its toxic potential. The results also suggest the antiperoxidative effects of ascorbic acid and water extract of *Spirulina plantesis* and demonstrate their potential to reduce 5-fluorouracil induced lipid peroxidation and thus to increase therapeutic index of the drug by the way of reducing toxicity that may be mediated through free radical mechanisms.

**CONCLUSION**

Our findings clearly show that ascorbic acid and water extract of *Spirulina plantesis* are capable of preventing lipid peroxidation induced by 5-fluorouracil. The concept of antioxidant cotherapy may also be exploited during future formulation design to reduce drug-induced toxicity. Moreover, lipid peroxidation induction capacity of a drug may be tested at individual level to determine the extent of risk from the drug in case of a particular individual, in view of variable *in vivo* antioxidant defence and accordingly, the decision about safe use of a drug and necessary co-administration of antioxidants may be taken. However, further extensive study is required to advance such hypothesis.

**Acknowledgments**

The authors wish to thank Dabur Research Foundation, Ghaziabad (U.P.) for providing the free gift sample of 5-fluorouracil.

**REFERENCES**


Received: 22.1.2006