Research Paper

In Vitro Evaluation of Antiperoxidative Potential of Water Extract of *Spirulina platensis* (blue green algae) on Cyclophosphamide-Induced Lipid Peroxidation

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The aim of the study is to evaluate free radical scavenging activity of water extract of *Spirulina platensis* on cyclophosphamide-induced lipid peroxidation using some common laboratory markers. In this study goat liver has been used as liver source. This *in vitro* evaluation was done by measuring the malondialdehyde, 4-hydroxy-2-nonenal, reduced glutathione and nitric oxide content of tissue homogenates. The results suggest that cyclophosphamide could induce lipid peroxidation to a significant extent and it was also found that water extract of the algae has the ability to suppress the drug-induced lipid peroxidation.

The balance between pro-oxidants and antioxidant is very important for survival because its tilt in favour of the former may lead to cellular damage due to oxidative stress[1]. Oxidative stress in cells can be initiated by the addition of Fe^{2+} in the presence of dioxygen. This stress will result in lipid peroxidation and the associated formation of lipid radicals[2-3]. Free radical mediated oxidative stress results usually from deficient natural antioxidant defences. In case of reduced or impaired defence 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[4].

Spirulina platensis, planktonic blue green algae, is gaining increasing attention because of its nutritional and medicinal properties[5]. It contains phycocyamin (7% dry weight basis) and polysaccharides, both of them having antioxidant properties. They have a direct effect on reactive oxygen species. It also contains an important enzyme superoxide dismutase (1700 units/g) that acts indirectly by slowing down the rate of oxygen radical generating reactions[6]. It was found that beside antioxidant effects, spirulina had versatile properties like immunomodulatory effect[7], anticancer effect[8] and antiviral effects[9-10]. In view of the above findings and widespread use of spirulina, it would be of interest to obtain more information regarding antiperoxidative potential of spirulina.

Cyclophosphamide, an anticancer drug, is an inactive cytostatic, which is metabilised into active metabolites mainly in the liver. During bioactivation, reactive oxygen species (ROS) are also formed, which can modify the components of both healthy and neoplastic cells leading to decreased antioxidative capacity[11]. It has been reported that cyclophosphamide produces genotoxicity and oxidative stress in mice[12]. It has also been observed that the drug induces testicular gametogenetic and steroidogenic disorder in rats[13]. Cyclophosphamide causes lung toxicity in animals and humans. The mechanism of pulmonary damage caused by the drug is not fully understood but probably by direct toxicity to pulmonary tissue or indirect toxicity through activation of pulmonary inflammatory cells[14].

Lipid peroxidation inducing capacity of drugs may be related to their toxic potential as exemplified by adriamycin-induced cardiotoxicity, which occurs through free radical mediated process[15]. So the evaluation of

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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 by authors for antioxidants that may reduce drug-induced lipid peroxidation[16-27], the present work has been carried out *in vitro* to evaluate the antioxidant effect of water extract of *Spirulina platensis* on cyclophosphamide-induced lipid peroxidation.

MATERIALS AND METHODS

The study had been performed on the goat (*capra capra*) liver using some common laboratory markers of lipid peroxidation like malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), reduced glutathione (GSH) and nitric oxide (NO) content of the tissue. The experiments were performed in accordance with the recommendation of the Institutional Animal Ethics Committee.

Preparation of water extract of *Spirulina platensis:*

Spirulina was obtained from Indo Leena, Biotech Private Ltd., Spirulina Farm, Namakkal, Tamil Nadu. Attempt had been made to determine the maximum concentration of the algae in water extract. For this purpose a mixture of spirulina powder (2.5 g) and water (200 ml) was heated cautiously and the volume was reduced to 50 ml. The hot solution was filtered at pump and the filtrate was transferred to a 50 ml volumetric flask and the volume was made up to the mark with double distilled water and the concentration of the solution was determined. The same procedure was followed with 4, 5, 6 and 7 g of spirulina powder.

Preparation of tissue homogenate:

Goat liver was collected in a sterile vessel containing phosphate buffer (pH 7.4) solution. After draining the buffer solution as completely as possible, the liver was cut into thin pieces and immediately transferred to a homogenizer, a tissue homogenate (1 g/ml) was prepared using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts, which were then treated differently as mentioned below.

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 0.015 mg/g tissue homogenate. The third portion was treated with drug and antioxidant (DA) and the fourth one was treated with antioxidant (A) alone at a concentration of 0.167 mg/g tissue homogenate. After treatment with drug and/or antioxidant, the liver homogenates were shaken for 2 h and incubated below 20^0 for 24 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 thiobarbuturic acid (TBA) method[28]. The estimation was done at 2, 4, 8 and 24 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% trichloroacetic acid (TCA) and centrifuged at 1200 g for 30 min to precipitate protein. Then 2.5 ml of the filtrate 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 and then 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 ELICO MINI SPEC (SL 171). The values were determined from standard curve.

Estimation of reduced glutathione (GSH) level from tissue homogenate:

Reduced glutathione (GSH) was measured in accordance with Ellman's method[29]. The estimation was done at 2, 4, 8 and 24 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% TCA in 1 mM EDTA and 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) (DTNB, 0.01% in phosphate buffer) was added to it. The absorbances of the solutions were estimated at 412 nm against blank (prepared from 6.0 ml of phosphate buffer and 0.4 ml of DTNB) using the above-mentioned spectrophotometer. The values were determined from standard curve.

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

The estimation was done only at 2 and 24 h of incubation and it was repeated in 5 animal sets. In each case three samples of 2 ml of incubation mixture were treated with 1.5 ml of 10% TCA solution and centrifuged at 1200g for

30 min. Then 2 ml of the filtrate was treated with 1 ml of 2,4-dinitrophenylhydrazine (DNPH, 1 g/l in 0.5 M HCl) and kept for 1 h at room temperature. After that the samples were extracted with hexane and the extract was evaporated at 40°. After cooling to room temperature, 2 ml of methanol was added to each sample and the absorbance was measured at 350 nm against methanol as blank[30]. The values were determined from the standard curve.

Estimation of nitric oxide (NO) level from tissue homogenate:

The estimation was done at 2, 4 and 24 h of incubation and it was repeated in five animal sets. NO content was determined by reaction with Griess reagent[31]. In each case three samples of 4 ml of tissue homogenate were treated with 2.5 ml of 10% TCA solution and centrifuged at 1200 g for 30 min. Then 5 ml of the filtrate was treated with 0.5 ml Griess reagent. After 10 min the absorbances of the solutions were measured at 540 nm against blank (prepared from 5 ml of distilled water and 0.5 ml of Griess reagent). The values were calculated from standard curve.

Statistical analysis:

Analysis of variance (ANOVA) and multiple comparisons[32,33] was done based on percent changes data with respect to control of corresponding hours to check statistical significance of the results. In multiple comparison there are two possible sources of error: the random error associated with the replicate measurements and the other due to animal variations. The variations may be calculated and their effects estimated by a statistical method known as the analysis of variance (ANOVA), where the square of the standard deviation s^2 is called the variance V. Thus, $F= s_1^2/s_2^2$ where $s_1^2>s_2^2$, and may be written as $F= V_1/V_2$ where $V_1>V_2$. In our study degree of freedom (df) between samples is (2,8) and that between animals is (4,8). ANOVA is done to compare the means of more than two treatment groups.

If the F test (F= mean square between regimens/mean square within regimens) is significant and more than two treatments are included in the experiment, it may not be obvious immediately which treatments are different. Various multiple-comparison procedures have been proposed to solve this problem. The general procedure of multiple-comparison is to list the ranked means from lowest to highest (or the reverse) and the means that are not statistically significantly different from each other are placed in a same parenthesis. The procedure is carried out by calculating a 5% allowance, which is defined as the critical difference between means which allows one to reject the null hypothesis and accept the alternative hypothesis for any two sample means at p= 0.05.

We have used least significant difference procedure in multiple-comparison analysis. Least significant difference procedure is the least conservative procedure, and this assures that the probability that any one comparison is judged to be significant by chance alone is 5%. However, the probability of one or more comparisons being judged significant would be greater than 5%. Any two means included in the same parenthesis do not differ significantly at p= 0.05. Any two means not included in same parenthesis are statistically significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of the studies on cyclophosphamide-induced lipid peroxidation and its inhibition with water extract of *Spirulina platensis* are listed in Tables 1-4. The MDA, GSH, 4-HNE and NO content of different samples at different hours of incubation were calculated in nM/g wet weight of tissue with corresponding standard errors. The goat liver was selected because of its easy availability and close similarity to the human liver in its lipid profile[34]. For the water extract prepared from 2.5 g of spirulina powder, the concentration was found 0.92% w/v and for those with 4, 5, 6 and 7 g of spirulina powder, the concentrations were 1.4%, 1.7%, 1.7% and 1.7% respectively. It was found that the maximum concentration of the algae present in water extract was 1.7% w/v. The λ_{max} of the water-extracted solution was found at 259 nm.

Incubation of tissue homogenates with cyclophsphamide resulted an increase in MDA content with respect to corresponding control (Table 1). This observation suggests lipid peroxidation induction potential of the drug. Lipid peroxidation leads to the generation of variety of cytotoxic products. Moreover it causes disruption of membrane structure and change in fluidity[35]. Increase in the accumulation of MDA in cells can result into cellular degradation, some biochemical and functional changes and even cell death[37]. It was further found that MDA content was significantly reduced when tissue homogenates were treated with both cyclophosphamide and water extract of *Spirulina platensis*. This implies the free radical scavenging property of the water extract of the algae. When tissue homogenates were treated only with the extract, there was also depletion of MDA level.

TABLE 1: EFFECTS OF WATER EXTRACT OF *SPIRULINA PLATENSIS* ON CYCLOPHOSPHAMIDE INDUCED LIPIDPEROXIDATION: CHANGES IN MDA PROFILE

Hours of incubation	Animal sets	MDA content (Mean±SE) in nM/g wet weight of tissue				Analysis of variance and multiple comparison of percent changes in MDA content with respect to control of corresponding hours
		C	D	DA	A	
2	AL1	6.44 (±0.08)	8.09 (±0.092)	7.28 (±0.083)	6.12 (±0.046)	F1=9.06 [df=(2,8)]
	AL2	3.26 (±0.05)	3.44 (±0.080)	3.08 (±0.093)	2.92 (±0.05)	F2=2.78 [df=(4,8)]
	AL3	4.78 (±0.046)	5.04 (±0.046)	4.59 (±0.046)	5.04 (±0.05)	Pooled variance, $(S^2)^*$ = 39.58,
	AL4	3.78 (±0.139)	3.93 (±0.05)	3.54 (±0.05)	3.46 (±0.05)	Critical difference, (p=0.05)#
	AL5	4.06 (±0.083)	4.72 (±0.046)	3.95 (±0.080)	3.86 (±0.046)	LSD =11.84,Ranked
	AV (±SE)	4.46 (±0.55)	5.04 (±0.81)	4.49 (±0.74)	4.28 (±0.57)	means**(D) (DA, A)
4	AL1	6.08 (±0.046)	7.20 (±0.123)	6.19 (±0.046)	5.68 (±0.046)	F1=15.24[df=(2,8)]
	AL2	2.43 (±0.00)	2.65 (±0.080)	2.18 (±0.046)	2.05 (±0.046)	F2=1.76[df=(4,8)]
	AL3	5.59 (±0.00)	6.04 (±0.080)	4.78 (±0.046)	5.83 (±0.093)	Pooled variance, $(S^2)^*$ = 47.71,
	AL4	2.86 (0±0.096)	3.56 (±0.080)	2.69 (±0.046)	2.65 (±0.080)	Critical difference, (p=0.05)#
	AL5	8.73 (±0.046)	9.07 (±0.122)	7.17 (±0.00)	8.07 (±0.083)	LSD =13.00, Ranked means**
	AV (±SE)	5.14 (±1.15)	5.70 (±1.17)	4.60 (±0.96)	4.86 (±1.31)	(D) (DA, A)
8	AL1	5.51 (±0.123)	6.47 (±0.186)	5.19 (±0.080)	4.87 (±0.123)	F1=42.14 [df=(2,8)]
	AL2	2.29 (±0.046)	2.58 (±0.046)	2.16 (±0.123)	2.07 (±0.046)	F2=1.28[df=(4,8)]
	AL3	6.26 (±0.143)	6.66 (±0.080)	5.90 (±0.186)	5.72 (±0.046)	Pooled variance$(S^2)^*$ = 12.23,
	AL4	3.95 (±0.143)	4.38 (±0.096)	3.89 (±0.080)	3.74 (±0.046)	Critical difference, (p=0.05)#
	AL5	5.17 (±0.046)	5.38 (±0.096)	4.70 (±0.046)	4.74 (±0.139)	LSD =6.58, Ranked means*
	AV(±SE)	4.64 (±0.69)	5.09 (±0.75)	4.37 (±0.64)	4.23 (±0.62)	*(D) (DA, A)
24	AL1	3.84 (±0.083)	4.70 (±0.093)	3.88 (±0.05)	3.33 (±0.083)	F1=21.65 [df=(2,8)]
	AL2	2.89 (±0.05)	3.18 (±0.046)	2.59 (±0.139)	2.56 (±0.046)	F2=1.29[df=(4,8)]
	AL3	5.42 (±0.143)	5.81 (±0.080)	5.57 (±0.046)	5.23 (±0.092)	Pooled variance$(S^2)^*$ =27.54,
	AL4	4.91 (±0.080)	5.27 (±0.046)	4.55 (±0.046)	4.49 (±0.046)	Critical difference, (p=0.05)#
	AL5	4.87 (±0.046)	5.36 (±0.143)	4.46 (±0.083)	4.34 (±0.080)	LSD =9.88, Ranked means**
	AV (±SE)	4.39 (±0.45)	4.86 (±0.45)	4.21 (±0.49)	3.99 (±0.47)	(D) (DA, A)

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 correspond to variance ratio between groups and within groups respectively. C, D, DA, A indicate control, drug treated, drug and antioxidant treated, antioxidant treated respectively. AV.= Averages of five animal sets; S.E.= Standard Error (df=4); df= degree of freedom. * Error mean square, # Critical difference according to least significant procedure[33]. **Two means not included within same parenthesis are statistically significantly different at p=0.05 level

The reduced glutathione (GSH) content of different animal sets and their averages are shown along with statistical analysis in Table 2. Incubation of tissue homogenates with cyclophosphamide decreased the GSH level with respect to corresponding controls. This is due to fast oxidation of GSH induced by cyclophosphamide. The depletion of GSH is associated with 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. GSH is an important antioxidant and plays a very important role in the defence mechanism for tissue against the reactive oxygen species[38]. When the tissue homogenates were treated with both cyclophosphamide and water extract of the algae, the GSH level increased in comparison to drug treated group of corresponding hours. Incubation of tissue homogenates only with extract also enhances the GSH level. These observations suggest that increase in GSH level may be due to antioxidant property of the extract.

The 4-HNE content of different animal sets and their averages are shown along with statistical analysis in Table 3. Incubation of tissue homogenates with drug caused significant increase in 4-HNE content with respect to control. When tissue homogenates were treated with both drug and antioxidant, 4-HNE content was significantly reduced in comparison to drug treated group. 4-HNE as well as related aldehydes display strong cytotoxicity[38,39] and their effective removal could play an important role in a general defence system of the liver *in* vivo against damaging effects of lipid peroxidation[40-42]. Incubation of tissue homogenates only with extract also reduces the 4-HNE levels. This implies that water extract of *Spirulina platensis* inhibits cyclophosphamide-induced lipid peroxidation to a significant extent.

The nitric oxide (NO) content of different animal sets and their averages are shown along with statistical analysis in Table 4. Incubation of tissue homogenates with drug reduced the NO content with respect to corresponding controls. Nitric oxide is a prooxidant[43], potential antioxidant[44-47] and plays a very important role in host defence[48]. It was further found that incubation of tissue homogenates with both drug and water extract

TABLE 2: EFFECTS OF WATER EXTRACT OF *SPIRULINA PLATENSIS* ON CYCLOPHOSPHAMIDE INDUCED LIPIDPEROXIDATION: CHANGES IN GSH PROFILE

Hours of incubation	Animal sets	GSH content (Mean±SE) in nM/g wet weight of tissue				Analysis of variance and multiple comparison of percent changes in GSH content with respect to control of corresponding hours
		C	D	DA	A	
2	AL1	411.92 (±1.38)	377.04 (±0.58)	387.86 (±1.34)	429.14 (±0.76)	F1=12.40 [df=(2,8)]
	AL2	335.09 (±1.01)	317.66 (±0.22)	350.77 (±0.58)	347.24 (±1.72)	F2=1.35[df=(4,8)]
	AL3	196.03 (±1.15)	177.92 (±1.23)	218.32 (±0.22)	211.70 (±0.22)	Pooled variance(S^2)* = 14.47,
	AL4	357.39 (±0.44)	333.99 (±0.22)	359.59 (±1.01)	363.57 (±0.38)	Critical difference, (p=0.05)#
	AL5	344.81 (±0.21)	323.84 (±0.38)	349.67 (±1.01)	351.87 (±0.58)	LSD =7.16, Ranked means**
	AV(±SE)	329.05 (±35.81)	306.09 (±33.68)	333.24 (±29.55)	340.70 (±35.46)	(D) (DA, A)
4	AL1	346.79 (±0.79)	336.86 (±0.58)	372.84 (±1.37)	360.27 (±1.32)	F1=9.44[df=(2,8)]
	AL2	201.32 (±0.66)	164.45 (±0.79)	173.06 (±0.96)	205.73 (±0.79)	F2=4.35[df=(4,8)]
	AL3	75.93 (±0.44)	73.50 (±0.38)	84.76 (±1.01)	86.75 (±0.66)	Pooled variance(S^2)* = 29.60,
	AL4	250.32 (±1.01)	220.52 (±0.76)	272.18 (±0.38)	277.90 (±0.58)	Critical difference, (p=0.05)#
	AL5	237.97 (±0.44)	230.90 (±0.44)	236.20 (±0.44)	240.39 (±1.01)	LSD =10.24, Ranked
	AV(±SE)	222.47 (±43.81)	205.25 (±43.15)	227.81 (±48.23)	234.21 (±49.94)	means**(D) (DA, A)
8	AL1	78.15 (±0.86)	75.05 (±0.88)	89.62 (±0.44)	91.83 (±0.22)	F1=12.33 [df=(2,8)]
	AL2	102.65 (±0.76)	94.04 (±0.38)	116.99 (±0.79)	109.27 (±0.38)	F2=1.16[df=(4,8)]
	AL3	51.87 (±0.58)	48.12 (±0.22)	71.07 (±0.44)	58.72 (±0.22)	Pooled variance(S^2)* = 77.53,
	AL4	141.72 (±0.76)	110.81 (±0.96)	163.58 (±0.76)	166.22 (±1.01)	Critical difference, (p=0.05)#
	AL5	87.85 (±0.58)	83.22 (±0.22)	90.29 (±0.79)	91.17 (±0.44)	LSD =16.57, Ranked means**
	AV (±SE)	92.45 (±14.84)	82.25 (±10.42)	106.31 (±16.08)	103.44 (±17.69)	(D) (DA, A)
24	AL1	87.85 (±0.96)	70.64 (±0.79)	109.71 (±0.22)	101.32 (±1.15)	F1=12.42[df=(2,8)]
	AL2	161.58 (±1.32)	153.86 (±1.23)	206.61 (±2.02)	181.67 (±0.79)	F2=1.64[df=(4,8)]
	AL3	62.91 (±0.38)	54.74 (±0.22)	134.21 (±0.22)	86.97 (±1.23)	Pooled variance (S^2)* = 468.66,
	AL4	83.66 (±0.96)	69.53 (±0.38)	130.68 (±0.79)	134.87 (±0.96)	Critical difference, (p=0.05)#
	AL5	71.96 (±0.58)	65.78 (±0.58)	106.84 (±0.96)	99.99 (±1.01)	LSD =40.76, Ranked means**
	AV(±SE)	93.59 (±17.55)	82.91 (±17.96)	137.61(±18.09)	120.96 (±17.11)	(D) (DA, A)

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 correspond to variance ratio between groups and within groups respectively. C, D, DA, A indicate control, drug treated, drug and antioxidant treated, antioxidant treated respectively. AV.= Averages of five animal sets; S.E.= Standard Error (df=4); df= degree of freedom. *Error mean square, #Critical difference according to least significant procedure[33]. **Two means not included within same parenthesis are statistically significantly different at p=0.05 level

TABLE 3: EFFECTS OF WATER EXTRACT OF *SPIRULINA PLATENSIS* ON CYCLOPHOSPHAMIDE INDUCED LIPIDPEROXIDATION: CHANGES IN 4-HNE PROFILE

Hours of incubation	Animal sets	4-HNE content (Mean±SE) in nM/g wet weight of tissue				Analysis of variance and multiple comparison of percent changes in 4-HNE content with respect to control of corresponding hours
		C	D	DA	A	
2	AL1	50.28 (±0.270)	72.46 (±0.540)	62.19 (±0.270)	39.55 (±0.618)	F1=12.09[df=(2,8)]
	AL2	41.24 (±0.798)	45.43 (±0.808)	38.74 (±0.509)	35.42 (±0.367)	F2=1.36[df=(4,8)]
	AL3	32.25 (±0.176)	41.39 (±0.103)	30.72 (±0.306)	29.78 (±0.467)	Pooled variance(S^2)* = 126.4,
	AL4	42.83 (±0.636)	52.07 (±0.569)	44.61 (±0.206)	40.06 (±0.466)	Critical difference, (p=0.05)#
	AL5	36.34 (±0.270)	40.93 (±0.270)	31.99 (±0.10)	34.04 (±0.203)	LSD =21.16,Ranked means**
	AV (±SE)	40.59 (±3.06)	50.46 (±5.85)	41.65 (±5.71)	35.77 (±1.89)	(D) (DA, A)
24	AL1	44.92 (±0.270)	48.14 (±0.099)	31.89 (±0.267)	39.29 (±0.613)	F1=14.24[df=(2,8)]
	AL2	33.33 (±0.176)	36.28 (±0.444)	31.94 (±0.173)	29.39 (±0.366)	F2=2.13[df=(4,8)]
	AL3	39.71 (±4.44)	45.58 (±0.306)	32.61 (±0.670)	31.53 (±0.409)	Pooled variance(S^2)* = 52.12,
	AL4	45.68 (±0.406)	49.41 (±0.176)	44.15 (±0.103)	42.82 (±0.707)	Critical difference, (p=0.05)#
	AL5	41.85 (±0.270)	44.56 (±0.733)	39.04 (±0.103)	39.50 (±0.270)	LSD =13.59, Ranked means**
	AV (±SE)	41.09 (±2.22)	44.79 (±2.29)	35.93 (±2.45)	36.51 (±2.57)	(D) (DA, A)

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 correspond to variance ratio between groups and within groups respectively. C, D, DA, A indicate control, drug treated, drug and antioxidant treated, antioxidant treated respectively. AV.= Averages of five animal sets; S.E.= Standard Error (df=4); df= degree of freedom. *Error mean square, #Critical difference according to least significant procedure[33]. **Two means not included within same parenthesis are statistically significantly different at p=0.05 level

resulted increase in NO content with respect to drug treated group. Incubation of tissue homogenates only with extract also enhances NO level with respect to corresponding controls. These results suggest that NO could inhibit lipid peroxidation to a significant extent. It has been proposed that NO causes chain termination

TABLE 4: EFFECTS OF WATER EXTRACT OF *SPIRULINA PLATENSIS* ON CYCLOPHOSPHAMIDE INDUCED LIPIDPEROXIDATION: CHANGES IN NO PROFILE

Hours of incubation	Animal sets	NO content (Mean±SE) in nM/g wet weight of tissue				Analysis of variance and multiple comparison of percent changes in NO content with respect to control of corresponding hours
		C	D	DA	A	
2	AL1	3.07 (±0.079)	2.82 (±0.140)	3.45 (±0.052)	3.69 (±0.079)	F1=4.40 [df=(2,8)]
	AL2	1.39 (±0.029)	1.33 (±0.029)	1.97 (±0.052)	1.51 (±0.052)	F2=0.684 [df=(4,8)]
	AL3	1.34 (±0.03)	1.24 (±0.03)	1.44 (±0.03)	1.98 (±0.082)	Pooled variance(S^2)* = 223.93,
	AL4	4.37 (±0.082)	4.13 (±0.0599)	4.65 (±0.03)	4.18 (±0.033)	Critical difference, (p=0.05)#
	AL5	0.895 (±0.03)	0.78 (±0.03)	1.04 (±0.0548)	0.98 (±0.03)	LSD =28.17,Ranked means**
	AV (±SE)	2.21 (±0.65)	2.06 (±0.62)	2.51 (±0.67)	2.47 (±0.62)	(D, DA, A)
4	AL1	2.82 (±0.110)	2.65 (±0.029)	3.09 (±0.110)	3.04 (±0.029)	F1=18.79[df=(2,8)]
	AL2	1.27 (±0.051)	1.10 (±0.029)	1.41 (±0.029)	1.43 (±0.059)	F2=1.94[df=(4,8)]
	AL3	1.11 (±0.055)	0.93 (±0.051)	1.19 (±0.03)	1.21 (±0.03)	Pooled variance(S^2)* =28.37,
	AL4	4.19 (±0.03)	3.74 (±0.059)	3.98 (±0.055)	4.08 (±0.03)	Critical difference, (p=0.05)#
	AL5	1.17 (±0.03)	1.07 (±0.03)	1.19 (±0.03)	1.36 (±0.03)	LSD =10.02,Ranked means**
	AV (±SE)	2.11 (±0.609)	1.898 (±0.56)	2.17 (±0.57)	2.22 (±0.57)	(D) (DA, A)
24	AL1	2.07 (±0.079)	1.49 (±0.033)	3.39 (±0.082)	2.92 (±0.063)	F1=12.15[df=(2,8)]
	AL2	0.81 (±0.052)	0.64 (±0.029)	1.34 (±0.052)	0.93 (±0.082)	F2=1.45[df=(4,8)]
	AL3	1.25 (±0.052)	1.09 (±0.052)	1.59 (±0.082)	1.37 (±0.054)	Pooled variance(S^2)* = 323.72,
	AL4	4.31 (±0.085)	3.63 (±0.03)	4.39 (±0.03)	4.52 (±0.0267)	Critical difference, (p=0.05)#
	AL5	0.86 (±0.054)	0.63 (±0.03)	0.96 (±0.033)	0.98 (±0.03)	LSD =33.87,Ranked means**
	AV (±SE)	1.86 (±0.65)	1.496 (±0.556)	2.33 (±0.662)	2.14 (±0.69)	(D) (DA, A)

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 correspond to variance ratio between groups and within groups respectively. C, D, DA, A indicate control, drug treated, drug and antioxidant treated, antioxidant treated respectively. AV.= Averages of five animal sets; S.E.= Standard Error (df=4); df= degree of freedom. *Error mean square, #Critical difference according to least significant procedure[33]. **Two means not included within same parenthesis are statistically significantly different at p=0.05 level

reactions during lipid peroxidation as observed in low-density lipoprotein oxidation as well as in chemical systems[44-47].

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 and Tables 1-4 shows the results. The ranked mean values indicate that there is significant difference in changes of MDA/GSH/4-HNE/ NO content in drug-treated samples and those in antioxidant-treated samples. The data presented in this work demonstrate the lipid peroxidation induction potential of cyclophosphamide, 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 cyclophosphamide 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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