Research Paper

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A STUDY ON DOCETAXEL-INDUCED LIPID PEROXIDATION USING 4-HYDROXY-2-NONENAL AS MODEL MARKER: PROTECTIVE ROLE OF WATER EXTRACT OF SPIRULINA PLATENSIS

Chemistry

KEYWORDS : docetaxel, lipid peroxidation, 4-hydroxy-2-nonenal, Spirulina platensis

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ABSTRACT

The aim of the study was to investigate the antiperoxidative potential of water extract of Spirulina platensis on docetaxel-induced lipid peroxidation using 4-hydroxy-2-nonenal as model marker. Goat liver was used as a source of liver. The results showed that docetaxel had the capacity to induce lipid peroxidation on goat liver tissue homogenates. The study also revealed the antiperoxidative potential of water extract of Spirulina platensis on docetaxel-induced lipid peroxidation. Interpretation of the results is supported by analysis of variance and also by statistical multiple comparison analysis using least significant different procedure.

INTRODUCTION

In general free radicals are constantly generated in the human body, but they also be removed through enzymatic and non-enzymatic defense mechanisms of body 1. Lipid peroxidation leads to generation of peroxides and hydroperoxides that can decompose to yield a wide range of cytotoxic products, most of which are aldehydes, as exemplified by malondialdehyde, 4-hydroxynonenal etc 2. It was observed that exogenously administered antioxidants have been proven useful to overcome oxidative damage in case of reduced or impaired defense mechanism and excess generation of free radicals that are not counter balanced by endogenous antioxidant defense 3.

Docetaxel is a semi synthetic derivative of paclitaxel which is obtained from the rare Pacific yew tree *Taxus brevifolia* 4. It is primarily used for the treatment of breast, ovarian and non-small cell lung cancer but it possesses several toxic side effects 5.

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 6. It was found that spirulina potentiate the immune system leading to suppression of cancer development and viral infection 7. In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation 8-10 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.

2. EXPERIMENTAL

2.1 Materials

Goat liver was used as the lipid source. Chemicals of analytical grade were used for the present study. 2, 4-Dinitrophenylhydrazine (DNPH) and trichloroacetic acid (TCA) were procured from SD Fine Chem. Ltd., Mumbai and Merck, Mumbai, respectively. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Aurora, Ohio. Sulfanilamide was from SD Fine Chem.Ltd., Mumbai; Spirulina was obtained from INDO LEENA, Biotech private ltd., Spirulina Farm, Namakkal, Tamil Nadu. Pure sample of docetaxel used in present study was provided by Fresenius Kabi, Kalyani, India.

2.2 Methods

2.2.1 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 lmax of the water-extracted solution was found at 259 nm.

2.2.2 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 11. 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 0.143μM/g tissue homogenate. The third portion was treated with both docetaxel 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 docetaxel and /or water extract of *Spirulina platensis* treatment, the liver tissue homogenate samples were shaken for five hours and the reduced glutathione content of various portions were determined. Then the samples were stored at 10-12 ^{0}C for 24 hours for next determinations

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

The estimation was done at 5 and 24 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 12 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

2.3 STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure 13-14 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.

3 RESULTS & DISCUSSION

The percent changes in 4-HNE 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 4-HNE (15.72 & 10.63 %) content in samples with respect to control of 5 and 24 hours of incubation to a significant extent. 4-Hydroxy-2-nonenal (4-HNE), a lipid aldehydes that form 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 15. The increase in 4-HNE content was associated with an increase in lipid peroxidation and suggests that docetaxel could significantly induce the lipid peroxidation process. When tissue homogenates were treated both with docetaxel and water extract of *Spirulina platensis* then the 4-HNE (-13.04 & -8.75%) levels decreased in comparison to docetaxel-treated as well as control group. Tissue homogenates treated only with water extract of *Spirulina platensis* also decrease the 4-HNE (-10.57 & -7.40%) contents in comparison to the control samples. The decrease in 4-HNE level suggests the antiperoxidative potential of water extract of *Spirulina platensis.*

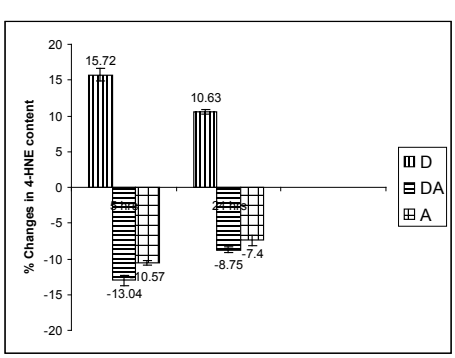

Figure 1: Effects of water extract of Spirulina platensis on docetaxel-induced changes in 4-HNE content (n=3); D, DA & A indicate only docetaxel-treated, docetaxel & water extract of Spirulina platensis -treated and only water extract of Spirulina platensis –treated samples

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 time. 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 4-HNE 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 at 24 hrs of incubation but at 5 hrs of incubation the level of 4-HNE in docetaxel -treated group is only statistically significantly different from the docetaxel and water extract of Spirulina platensis-treated group as well as only water extract of Spirulina platensis -treated group. But there is no statistically significantly difference among the docetaxel and water extract of Spirulina platensis -treated group and only water extract of Spirulina platensis -treated group.

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

Time of incubation (hrs)	Analysis of variance and multiple comparison
5	F1=355.79[df=(2,4)], F2=0.11[df=(2,4)], Pooled variance $(S^2)^*$=2.14, Critical difference (p=0.05)# LSD=2.75 Ranked means** (D) (DA, A)
24	F1=700.61 [df=(2,4)], F2=2.57[df=(2,4)], Pooled variance $(S^2)^*$=0.50, Critical difference (p=0.05)# LSD=1.33 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 docetaxel-treated, docetaxel & 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.

CONCLUSIONS

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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REFERENCE

I. Durak, H. Perk, M. Kavutcu, O. Combolat, O. Akyal, Y. Beduk. Free Radic. Biol. Med. 16, 1994, 825-831. | 2. H. Esterbauer, H. Zollner, R.J. Schauer. Atlas Sci. Biochem. 1, 1998, 311-319. | 3. B. Halliwell. Drugs. 42, 1991, 569-605. | 4. S.J. Clarke, L.P. Rivory. Clin. Pharmacokinet. 36, 1999, 99-114. | 5. H. P. Rang, M.M Dale, J.M. Ritter, P.K. Moore. Pharmacology. 5th ed., Edinburg, Churchill Livingstone, 2003, 666-671. | 6. A. Belay. JANA. 5, 2002, 26-48. | 7. T. Hirahashi, M. Matsumoto, K. Hazeki, Y. Saeki, M. Ui, T. Seya. Inter. Immunopharmacol. 2, 2002, 423- 434. | 8. S. Ray, K. Roy, C. Sengupta. Acta Pol. Pharm. Drug Res. 64, 2007, 335-344. | 9. S. Ray, K. Roy, C. Sengupta. Indian J. Pharm. Sci. 69, 2007, 378-383 | 10. S. Ray, K. Roy, C. Sengupta. Iranian J. Pharm. Sci. 3, 2007, 217-228. | 11. T. P. Hilditch, P.N. Williams. The Chemical Constituents of Fats. London, Chapman & Hall, 1964. | 12. M. Kinter. Free Radicals- A Practical Approach. Oxford, Oxford University Press, 1996, 133-145. | 13. G.W. Snedecor, W.G. Cochran. Statistical Methods. New Delhi, Oxford & IBH Publishing Co Pvt Ltd, 1967. | 14. S. Bolton. Remington: The Science and Practice of Pharmacy Statistics. Philadelphia, Lippincott Williams & Wilkins, 2000, 124-158 | 15. H. Esterbauer, R.J. Schaur, H. Zollner. Free Radic. Biol. Med. 11, 1991, 81-128.