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Exploring Protective Role of Naringin on Paclitaxel-Induced Lipid Peroxidation

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ABSTRACT

Objective: The aim of the present study was to investigate the free radical scavenging activity of naringin on paclitaxel-induced lipid peroxidation. **Methods**: This *in vitro* work was carried out with goat liver as lipid source using malondialdehyde and 4-hydroxy-2-nonenal as model markers. **Results**: The results suggest that paclitaxel could induce lipid peroxidation to a significant extent and it was also found that naringin has the ability to suppress the paclitaxel-induced toxicity. **Conclusion**: The study reveals the antiperoxidative effects of naringin and demonstrates its potential to reduce paclitaxel-induced toxic effects.

1. INTRODUCTION

In the biological system under enzymatic control or non-enzymatically, lipid peroxidation may occur. It is a free radical related process [1-3]. As a result of lipid peroxidation, several cytotoxic products like malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc. are produced [4]. The oxidative stress results from deficient natural antioxidant defense and it is a free radical-mediated process. It has been observed that exogenously administered antioxidants have been proven useful to overcome oxidative damage, in the case of reduced or impaired defense mechanism [5]. Paclitaxel is one of the common drugs used in breast cancers in women. However, the applicability of the drug is reduced due to several side effects due to the production of free radicals in the body [6]. It is reported in earlier studies that paclitaxel in combination with antioxidant reduces the drug-induced lipid peroxidation [7].

A recent study shows that naringin has a protective role against cisplatin-induced oxidative stress [8]. Naringin is a phenolic compound and exhibit antioxidant and antigenotoxic properties [9]. In view of the above findings and the ongoing search for 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 naringin on paclitaxel-induced lipid peroxidation.

2. MATERIALS AND METHODS

Materials

The reagent required for the study such as 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. Naringin was obtained from CDH Pvt. Ltd., New Delhi. A 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.

Goat liver was collected from Silchar Municipal Corporation approved outlet. Due to similarity with human liver in lipid profile, goat liver was selected for the work [12]. The small pieces of

goat liver after drying and cutting with the heavy duty blade were then transferred to a sterile vessel containing phosphate buffer (pH 7.4) solution. Then the livers were immediately grinded to make a tissue homogenate (1 g/ml) using freshly prepared phosphate buffer (pH 7.4) solution. The tissue homogenate was divided into four equal parts, which were then treated differently like; one part of the homogenate was kept as control (C) while a second part was treated with the paclitaxel (D) at a concentration of 0.143µM/g tissue homogenate. The third portion was treated with both paclitaxel at a concentration 0.143µM/g tissue homogenate and naringin at a concentration of 0.1666 mg / g homogenate (DA) and the fourth portion was treated only with naringin at a concentration of 0.1666 mg / g tissue homogenate (A). The liver tissue homogenate samples were shaken for five hours after paclitaxel and /or naringin treatment, and the malondialdehyde and 4-hydroxy-2-nonenal content of various portions were determined.

Methods

Estimation of malondialdehyde (MDA) content

The malondialdehyde (MDA) content was measured using thiobarbituric acid (TBA) method was used as a marker of lipid peroxidation [13]. The estimation was done at 5 hours of incubation and repeated in three animal sets. Three samples of 2.5 ml of incubation mixture in each case were treated with 2.5 ml of 10% (w/v) trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 30 minutes at room temperature to precipitate protein. Then the supernatant (2.5 ml) was treated with 5 ml of 0.002 (M) TBA solutions and then the volume was made up to 10 ml with distilled water. On a boiling water bath, the mixture was heated 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 the standard curve, which was constructed as follows. In graduated stoppered test tubes, different aliquots from standard 1, 1, 3, 3-tetrahydroxypropane (TEP) solution were taken and volume of each solution was made up to 5 ml. Finally, 5 ml of TBA solution was added to each solution 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 the 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 4-hydroxy-2-nonenal (4-HNE) content.

The 4-HNE content was measured at 5 hours of incubation and repeated in three animal sets. Three samples of 2 ml of incubation mixture in each case were treated with 1.5 ml of 10% (w/v) TCA solution. After that, the solutions were centrifuged at 3000 rpm for 30 min. The filtrate (2 ml) was treated with 1 ml of 2, 4-dinitrophenylhydrazine (DNPH) (100 mg / 100 ml of 0.5 M HCl) and kept for 1 hour at room temperature. Finally, the samples were extracted with hexane, and the extract was evaporated to dryness under argon at 40^0 C. After attaining 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 [14] using Shimadzu UV-1700 double beam spectrophotometer. The concentrations were determined from the standard curve. The standard calibration curve was drawn based on the following procedure. Different dilutions of 4-HNE in different concentrations of solvent (phosphate buffer) were prepared. 2 ml of sample was pipetted out from each solution and transferred into the stoppered glass tube. Then 1 ml of DNPH solution was added to all the samples and kept at room temperature for 1 hour. After that, each sample was extracted with 2 ml of hexane for three times. All extracts were collected in stoppered test tubes. Finally, all the 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

Statistical Analysis

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure [15-16] were also performed on the percent changes data of various groups such as paclitaxel-treated (D), paclitaxel, and naringin (DA) and only naringin-treated (A) with respect to control group of the corresponding time.

3. RESULTS AND 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 an indicator of the extent of lipid peroxidation.

From **Figure 1** it was evident that tissue homogenates treated with paclitaxel showed an increase in MDA (8.21 %) 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. MDA is produced in the body as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. Chemically it is a highly reactive three-carbon dialdehyde [17]. But the MDA (-5.56 %) content was significantly reduced in comparison to control and paclitaxel-treated group when tissue homogenates were treated with paclitaxel in combination with naringin. Again the tissue homogenates were treated only with the naringin then the MDA (-4.27%) level was reduced in comparison to control and paclitaxel-treated group. This decrease may be due to the free radical scavenging property of the naringin.

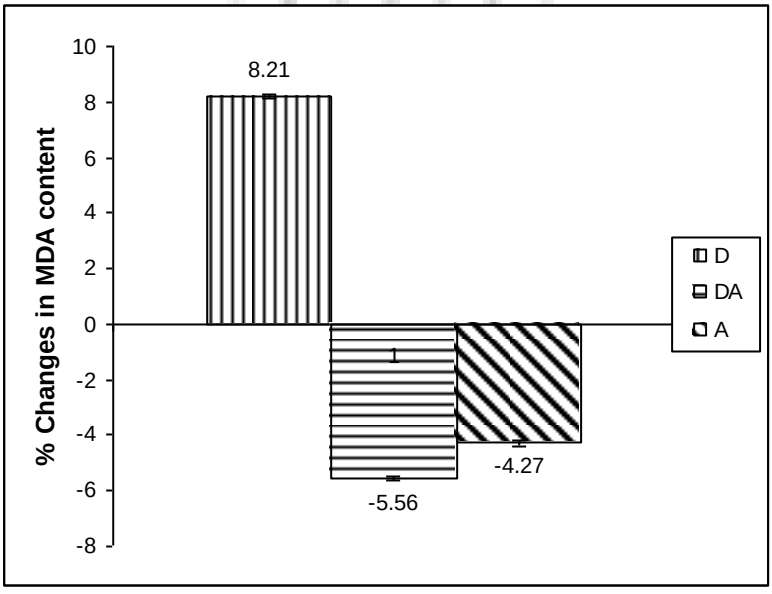

Figure 1: Effects of naringin on paclitaxel-induced changes in MDA content (n=3); D, DA & A indicate only paclitaxel-treated, paclitaxel & naringin-treated and only naringin–treated samples.

So the decrease in MDA content of samples, when treated with paclitaxel and naringin implies the free radical scavenging property of naringin.

It was also evident from **Figure 2** that tissue homogenates treated with paclitaxel showed an increase in 4-HNE (10.19%) content in samples with respect to control to a significant extent. The observations suggest that paclitaxel could significantly induce the lipid peroxidation process. After lipid peroxidation, 4-HNE is formed. It may form due to oxidative stress which is responsible for lipid peroxidation and measurement of 4-HNE may act as a marker of lipid peroxidation [18]. But the 4-HNE content was significantly reduced (-4.24%) in comparison to control and paclitaxel-treated group when tissue homogenates were treated with paclitaxel in combination with naringin. Removal of 4-HNE and related aldehydes by antioxidant could play an important role in a broad defense system of the liver *in vivo* against damaging effects of lipid peroxidation [19].

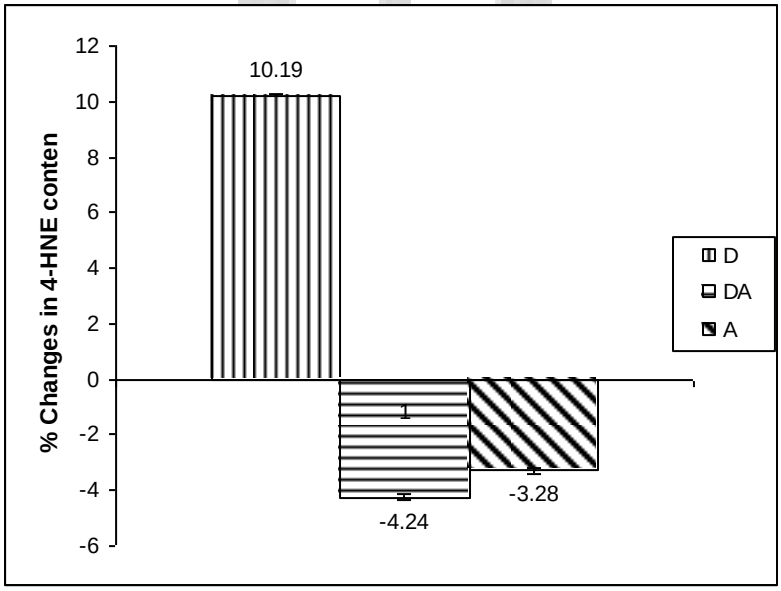

Figure 2: Effects of naringin on paclitaxel-induced changes in 4-HNE content (n=3); D, DA & A indicate only paclitaxel-treated, paclitaxel & naringin-treated and only naringin–treated samples.

Again the tissue homogenates were treated only with naringin then the 4-HNE level was reduced (-3.28%) in comparison to the control and the paclitaxel-treated group. This decrease may be explained by the free radical scavenging property of the naringin.

It is seen that there are significant differences among various groups (F1) such as paclitaxel-treated, paclitaxel and naringin-treated and only naringin-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 naringin-treated and only naringin-treated groups are statistically significantly different from each other.

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

Name of the antioxidant	Marker of lipid peroxidation	Analysis of variance and multiple comparisons
Naringin	MDA	F1=13694.37[df=(2,4)], F2=0.48[df=(2,4)], Pooled variance $(S^2)^*$=0.0126, Critical difference (p=0.05)# LSD=0.211 Ranked means** (D) (DA) (A)
	4-HNE	F1=26765.91 [df=(2,4)], F2=0.732 [df=(2,4)], Pooled variance $(S^2)^*$=0.0073, Critical difference (p=0.05)# LSD=0.161 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 paclitaxel-treated, paclitaxel, & naringin-treated and only naringin–treated samples * Error mean square, # Critical difference according to least significant procedure (LSD) **Two means not included within the same parenthesis are statistically significantly different at p=0.05 level.

CONCLUSION

The results from the work showed that paclitaxel has lipid peroxidation induction capacity. The study also reveals the antiperoxidative effects of naringin and demonstrates its potential to reduce paclitaxel-induced toxic effects.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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