



Research Article

EXPLORING PROTECTIVE ROLE OF MORIN ON PACLITAXEL-INDUCED LIPID PEROXIDATION USING MALONDIALDEHYDE AND 4-HYDROXY-2-NONENAL AS MODEL MARKERS

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ABSTRACT

The present *in vitro* study was designed to explore antiperoxidative potential of morin on paclitaxel-induced lipid peroxidation. Goat liver tissue homogenate was used as source of lipid. Estimation of malondialdehyde and 4-hydroxy-2-nonenal in liver tissue homogenate was used as model markers for paclitaxel-induced lipid peroxidation. The results suggest that paclitaxel could induce lipid peroxidation to a significant extent and it was also found that morin has the ability to suppress the paclitaxel-induced toxicity.

Keywords: Paclitaxel, morin, lipid peroxidation, malondialdehyde, 4-hydroxy-2-nonenal

INTRODUCTION

The polyunsaturated fatty acids of membrane phospholipids are particularly susceptible to peroxidation. Reactive oxygen free radicals are responsible for damage of tissues through lipid peroxidation¹. Free radicals are constantly formed in the human body, but the protection of cellular structures from damage by free radicals can be accomplished through enzymatic and non-enzymatic defense mechanisms². In case of reduced or impaired defense 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 damage³. 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 body⁵. It is reported that paclitaxel in combination with antioxidant reduces the drug induced lipid peroxidation⁴. Dietary antioxidants are gaining increasing importance day by day because of their health protective value, easy availability and biosafety⁵. Morin (3, 5, 7, 2', 4'-pentahydroxyflavone), a member of flavonols, exert antioxidant potential and offer protection against the oxidative stress induced by hydrogen peroxide⁶. Recently study shows that morin could exert a significant chemo preventive effect on colon carcinogenesis induced by 1, 2-dimethylhydrazine⁷. In one study it was found that morin exert beneficial effect on busulfan-induced lipid peroxidation⁸. In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation⁹⁻¹¹ the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of morin 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. Morin was from CDH Pvt. Ltd., New Delhi. 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 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 profile¹². 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 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 morin at a concentration of 0.1666 mg / g homogenate (DA) and the fourth portion was treated only with morin at a concentration of 0.1666 mg / g tissue homogenate (A). After paclitaxel and /or morin, the liver tissue homogenate samples were shaken for five hours and the malondialdehyde and 4-hydroxy-2-nonenal content of various portions were determined.

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) method¹³. The estimation was done at 5 hours of incubation and repeated in three 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 minutes to precipitate protein. Then 2.5 ml of the supernatant was treated with 5 ml of 0.002 (M) TBA solutions and then volume was made up to 10 ml with distilled water. The mixture was heated on a boiling water bath for 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 Shimadju 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 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 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) level in tissue homogenate

The estimation was done at 5 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°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 blank¹⁴ 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°C and the residue was reconstituted in 1 ml of methanol. The absorbance was measured at 350 nm using the 0 μ M 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 procedure¹⁵⁻¹⁶ were also performed on the percent changes data of various groups such as paclitaxel-treated (D), paclitaxel and morin (DA) and only morin-treated (A) with respect to control group of corresponding time.

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

Name of the antioxidant	Marker of lipid peroxidation	Analysis of variance and multiple comparison
Morin	MDA	F1=93927.68[df=(2,4)], F2=0.008[df=(2,4)], Pooled variance (S^2)=0.004, Critical difference (p=0.05) [#] LSD=0.119 Ranked means** (D) (DA) (A)
	4-HNE	F1=72462.8 [df=(2,4)], F2=0.26[df=(2,4)], Pooled variance (S^2)=0.011, Critical difference (p=0.05) [#] LSD=0.197 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 & morin -treated and only morin-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.

RESULTS & DISCUSSION

The percent changes in MDA / 4-HNE content of different samples at five 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 an increase in MDA (22.04 %) content in samples with respect to control to a significant extent. The observations suggest that paclitaxel could significantly induce the lipid peroxidation process. MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism¹⁷. But the MDA contents were significantly reduced (3.47%) in comparison to paclitaxel-treated group when tissue homogenates were treated with paclitaxel in combination with morin. Again the tissue homogenates were treated only with morin then the MDA level were reduced (1.89%) in comparison to the paclitaxel treated group. This decrease may be due to the free radical scavenging property of the antioxidant.

It was also evident from Figure 2 that tissue homogenates treated with paclitaxel showed an increase in 4-HNE (24.22%) 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 be assessed as a marker of oxidant stress in the form of lipid peroxidation¹⁸. But the 4-HNE content was significantly reduced (-5.25%) in comparison to control and paclitaxel-treated group when tissue homogenates were treated with paclitaxel in combination with morin. Again the tissue homogenates were treated only with morin then the 4-HNE level was reduced (-4.16%) in comparison to the control and the paclitaxel treated group. This decrease may be explained by the free radical scavenging property of morin.

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 morin-treated and only morin-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 morin-treated and only morin-treated groups are statistically significantly different from each other.

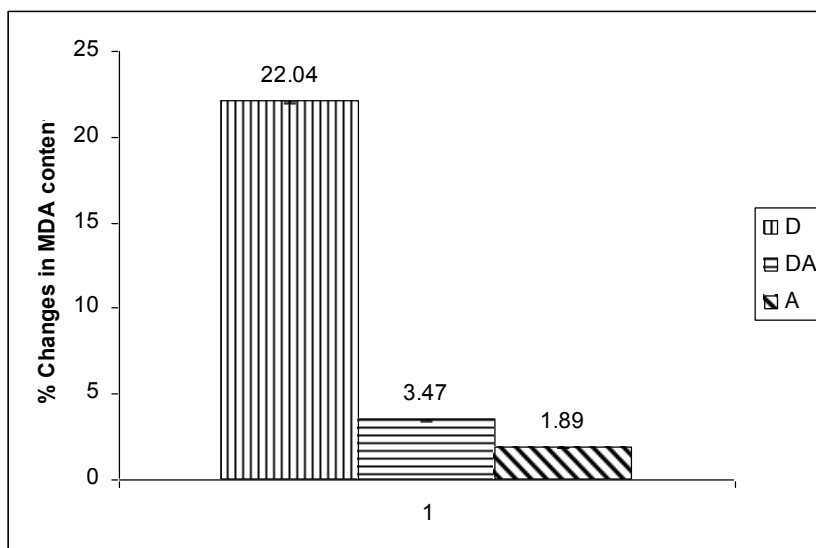


Figure 1: Effects of morin on paclitaxel-induced changes in MDA content (n=3); D, DA & A indicate only paclitaxel-treated, paclitaxel & morin -treated and only morin–treated samples

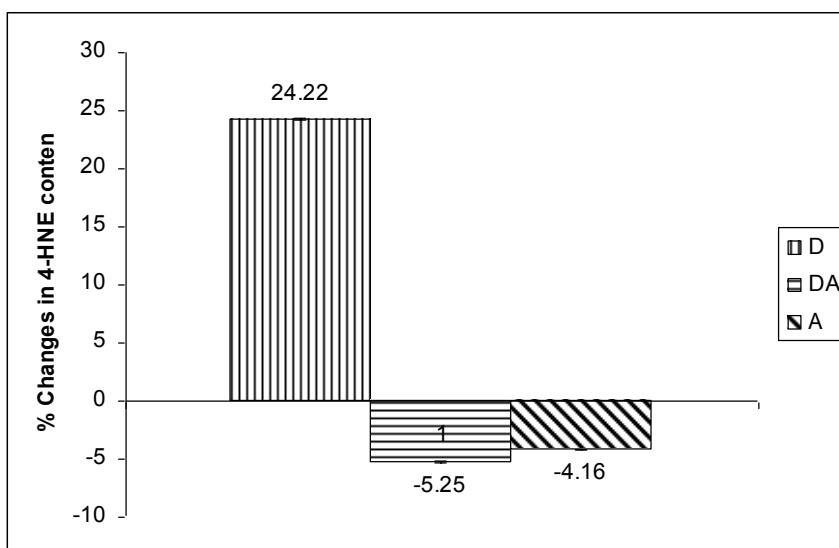


Figure 2: Effects of morin on paclitaxel-induced changes in 4-HNE content (n=3); D, DA & A indicate only paclitaxel-treated, paclitaxel & morin -treated and only morin–treated samples

CONCLUSION

The results of the study demonstrate the lipid peroxidation induction potential of paclitaxel, which may be related to its toxic potential. The results also showed the protective effects of morin and demonstrate its potential to reduce paclitaxel-induced lipid peroxidation and thus to increase therapeutic index of the drug by way of reducing toxicity that may be mediated through free radical mechanisms. However, a detailed study has to be carried out.

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