ABSTRACT
The study was designed with an aim to evaluate the protective effects of naringenin and naringin on docetaxel-induced changes in cholesterol profile in vitro. Goat blood was used as lipid source for the model. In the cholesterol profile total cholesterol (TC) and high density lipoprotein (HDL) cholesterol content of goat blood was determined. The study reveals that docetaxel has the induction capacity to produce changes in cholesterol profile. It was also noticed that naringenin / naringin had the capacity to inhibit docetaxel-induced changes in cholesterol profile. Interpretation of the results is supported by analysis of variance and also by statistical multiple comparison analysis using least significant different procedure.

KEYWORDS: Docetaxel, Naringenin, Naringin, Total cholesterol, HDL.

INTRODUCTION
In cancer chemotherapy, patients in general suffer from fear psychosis. Most of the anticancer drugs produce severe nausea and vomiting. These side effects are unwelcome as well as repulsive to patients. The anticancer drugs are utilized to kill the cancerous cell with little damage to normal cell. But most of the cytotoxic drugs affect the rapidly dividing normal tissue, and produce to a greater or lesser extent toxic side effects such as bone marrow toxicity, impaired wound healing, loss of hair, damage to gastrointestinal epithelium, depression of growth in children, sterility, teratogenicity, kidney damage, reversible liver abnormalities etc.[1] One of the causative factors of anticancer drug mediated damage to normal cells may be drug-induced lipid peroxidation.
Cholesterol is a fatty lipid produced by the liver and is crucial for normal body functioning. It exists in the outer layer of every cell in our body and is transported in the blood plasma of all animals. It is the main sterol synthesized by animals and small amounts are also synthesized in plants and fungi. Several factors like nutrition, diet, weight, physical activity, age, gender, heredity, alcohol etc affect the cholesterol level in blood. Serum cholesterol or its fractions like low density lipoproteins (LDL), high density lipoproteins (HDL) content have been found responsible for many diseases. Cholesterol and lipoprotein levels correlate well with the risk of cardiovascular diseases. Stress in the form of starvation was found to increase lipid peroxidation and alter lipid profile in rabbits.

Docetaxel is a semi synthetic derivative of paclitaxel which is obtained from the rare Pacific yew tree Taxus brevifolia. It is primarily used for the treatment of breast, ovarian and non-small cell lung cancer. As docetaxel is a cell cycle specific agent, it is cytotoxic to all dividing cells in the body and produces several toxic side effects due to damage of normal cell like hair follicles, bone marrow and other germ cells. It was reported that docetaxel has the capability of inducing lipid oxidization and membrane damage in human hepatoma cells.

In view of the above findings and the ongoing search of the present authors for antioxidant that may reduce drug induced lipid peroxidation, the present work has been carried out in vitro to evaluate the antiperoxidative potential of naringenin and naringin on docetaxel-induced changes in cholesterol content in goat blood sample.

MATERIALS & METHODS

Pure sample of docetaxel used in present study was provided by Fresenius Kabi, Kalyani, India. Naringin was from Himedia Bioscience, Mumbai. Naringenin was from Sigma-Aldrich, St. Louis, MO. Cholesterol test kit was from Span Diagnostic Ltd., Surat, India. All other reagents were of analytical grade.

Collection and preservation of goat blood

The goat (Capra capra) blood was collected from Silchar Municipal Corporation approved outlet. Appropriate quantity of blood as per the requirement for determination of a specific parameter was collected in a sterile vessel containing sodium citrate. Then the whole blood was divided equally. The first portion was kept as control (C), while the second portion was treated with docetaxel (D) at a concentration of 0.143μM/g blood. The third portion was
treated both with docetaxel at a concentration of 0.143μM/g blood and antioxidant (Naringenin / Naringin) at a concentration of 0.189μM/g blood (DA). The fourth one was treated only with the above mentioned antioxidant alone at a concentration of 0.189μM/g blood (A). After treatment with docetaxel and / or antioxidant, the different portions of blood samples were initially shaken for 5 hours at ambient temperature and total cholesterol and HDL-cholesterol content of different proportions were estimated. Then the samples were stored at 10-12 °C for 24 hours for next determinations.

**Estimation of total cholesterol and HDL-cholesterol from goat blood**

Determination of cholesterol concentration was performed in one step method \(^{(1)}\) with the help of cholesterol test kit. The determinations were done at 5 and 24 hrs of incubation and it was repeated for three times. In each case there were three samples. After the specified hours of incubation, 2 ml of blood samples were centrifuged at 2000 rpm for 15 minutes and the supernatant (plasma) was separated out. After that total cholesterol and high density lipoprotein cholesterol of the goat blood were determined.

**Total cholesterol**

The Total Cholesterol (TC) was calculated by using the following formula

\[
\text{Total Cholesterol (mg / dL)} = \left( \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \right) \times 200
\]

**HDL cholesterol**

**Step-I**

HDL- cholesterol separation: 0.2 ml of the supernatant was transferred into a centrifuge tube and to it 0.2 ml of reagent 3 from test kit was added. Then it was shaken well to mix and the tubes were kept at room temperature for 10 minutes. It was centrifuged at 2000 rpm for 15 minutes to obtain a clear supernatant.

**Step-II**

HDL-cholesterol determination: The test sample was prepared by mixing 3 ml of reagent 1 from test kit with 0.12 ml of the supernatant obtained from the step-I. The centrifuge tubes were shaken well and the tubes were kept in the boiling water bath exactly for 90 sec. The tubes were cooled immediately at room temperature under running tap water. The O.D. of Standard (S) & Test (T) were measured at 560 nm against reagent 1 as blank.
The content of HDL-Cholesterol was calculated by using the following formula

\[ \text{HDL-Cholesterol (mg / dL)} = \left( \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \right) \times 50 \]

**Statistical analysis**

For *in vitro* model of experiment, interpretation of the result is supported by analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure. \(^{13-14}\)

**RESULT & DISCUSSION**

It was observed from Figure 1 and 2 that goat blood treated with docetaxel caused an increase in total cholesterol content (23.00 and 4.78 %) with respect to corresponding control. But the HDL cholesterol level (-10.23 and -6.88%) was reduced in comparison to control group at 5 and 24 hours of incubation. These observations suggest that docetaxel can change the cholesterol profile. It was further found that incubation of blood sample with docetaxel and naringenin produce a decrease in total cholesterol (-10.48 and -3.20%), but the HDL-cholesterol contents (6.84 and 3.60%) were increased in comparison to both control and docetaxel-treated group respectively. Incubation of blood samples only with naringenin also shows a tendency of decrease in total cholesterol (-9.69 and -3.36%), but HDL-cholesterol contents (5.69 and 2.17%) were increased in comparison to control or docetaxel-treated group respectively.

![Graph](image-url)

**Figure 1:** Effects of naringenin on docetaxel-induced changes in total cholesterol profile (n=3); D, DA & A indicate only docetaxel-treated, docetaxel & naringenin-treated and only naringenin–treated samples
Figure 2: Effects of naringenin on docetaxel-induced changes in HDL-cholesterol profile (n=3); D, DA & A indicate only docetaxel-treated, docetaxel & naringenin-treated and only naringenin–treated samples

From Figure 3 and 4 it was also observed that goat blood treated with docetaxel caused an increase in total cholesterol content (20.01 and 5.89 %) with respect to corresponding control. But the HDL cholesterol level (-8.35 and -5.35%) was reduced in comparison to control group at 5 and 24 hours of incubation. These observations suggest that docetaxel can change the cholesterol profile. It was further found that incubation of blood sample with docetaxel and naringin produce a decrease in total cholesterol (-21.61 and -8.14%), but the HDL-cholesterol contents (4.11 and 2.93%) were increased in comparison to both control and docetaxel-treated group respectively. Incubation of blood samples only with naringin also shows a tendency of decrease in total cholesterol (-19.73 and -7.46%), but HDL-cholesterol contents (3.12 and 1.99%) were increased in comparison to control or docetaxel-treated group respectively. These results suggest that naringenin and naringin could inhibit docetaxel-induced changes in cholesterol profile.
To compare means of more than two samples, multiple comparison analysis along with analysis of variance was performed on the percent changes data of various groups (Table 1-2). It is seen that there is significant differences among various groups (F1) such as docetaxel-treated, docetaxel and naringenin / naringin-treated and only naringen–treated group. But within a particular group, differences (F2) are insignificant which shows that there is no statistical difference in animals in a particular group. If F-test is significant and more than two treatments are incorporated into the experiment it may not be obvious.
immediately which treatments are different. To solve the problem multiple comparison analysis is suggested. We are using least significant different procedure [13-14] on the percent changes data of various groups such as docetaxel-treated (D), docetaxel and naringenin / naringin (DA) and only naringenin / naringin -treated (A) with respect to control group of corresponding time. It was observed that the level of total cholesterol content (Table 1) in docetaxel-treated group is statistically significantly different from docetaxel and naringenin / naringin-treated group as well as only naringenin / naringin-treated group. But there is no statistically significantly different among the docetaxel and naringenin / naringin -treated group and only naringenin / naringin -treated group. In case of HDL-cholesterol content (Table 2) all three groups, i.e. docetaxel-treated, docetaxel and naringenin / naringin / naringin-treated and only naringenin / naringin-treated groups are significantly different from each other.

Table 1: ANOVA & Multiple comparison for changes of total cholesterol

<table>
<thead>
<tr>
<th>Name of the antioxidant</th>
<th>Time of incubation (hrs)</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naringenin</td>
<td>5</td>
<td>F1=530.34[df=(2,4)], F2=2.38[df=(2,4)], Pooled variance ($S^2$) =2.065, Critical difference (p=0.05) * LSD=2.71 Ranked means** (D) (DA, A)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>F1=310.19[df=(2,4)], F2=2.02[df=(2,4)], Pooled variance ($S^2$) =0.209, Critical difference (p=0.05) * LSD=0.86 Ranked means** (D) (DA, A)</td>
</tr>
<tr>
<td>Naringin</td>
<td>5</td>
<td>F1=845.96[df=(2,4)], F2=1.38[df=(2,4)], Pooled variance ($S^2$) =1.958, Critical difference (p=0.05) * LSD=2.63 Ranked means** (D) (DA, A)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>F1=245.53[df=(2,4)], F2=5.62[df=(2,4)], Pooled variance ($S^2$) =0.765, Critical difference (p=0.05) * LSD=1.65 Ranked means** (D) (DA, A)</td>
</tr>
</tbody>
</table>

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 & naringenin / naringin-treated and only naringenin / naringin–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.
Table 2: ANOVA & Multiple comparison for changes of HDL-cholesterol

<table>
<thead>
<tr>
<th>Name of the antioxidant</th>
<th>Time of incubation (hrs)</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1=8678.23 [df=(2,4)], F2=0.63 [df=(2,4)], Pooled variance ($S^2$) = 0.0328, Critical difference (p=0.05) # LSD=0.33 Ranked means** (D) (DA) (A)</td>
</tr>
<tr>
<td>Naringenin</td>
<td>5</td>
<td>F1=2199.01 [df=(2,4)], F2=3.1 [df=(2,4)], Pooled variance ($S^2$) = 0.044, Critical difference (p=0.05) # LSD=0.39 Ranked means** (D) (DA) (A)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>F1=4144.53 [df=(2,4)], F2=0.46 [df=(2,4)], Pooled variance ($S^2$) = 0.0347, Critical difference (p=0.05) # LSD=0.35 Ranked means** (D) (DA) (A)</td>
</tr>
<tr>
<td>Naringin</td>
<td>5</td>
<td>F1=2377.25 [df=(2,4)], F2=1.31 [df=(2,4)], Pooled variance ($S^2$) = 0.0347, Critical difference (p=0.05) # LSD=0.35 Ranked means** (D) (DA) (A)</td>
</tr>
</tbody>
</table>

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 & naringenin / naringin-treated and only naringenin / naringin–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

These findings indicate that docetaxel has the ability to change the cholesterol profile by inducing lipid peroxidation which may be related to its toxic potential. The results also suggest the antiperoxidative effects of naringenin / naringin and demonstrate its potential to reduce docetaxel-induced changes in cholesterol profile and thus to increase therapeutic index of the drug by way of reducing toxicity that may be mediated through free radical mechanisms.

REFERENCES


