Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 6, 2015

Short Communication

PROTECTIVE ROLE OF N-PROPYL GALLATE ON DOCETAXEL-INDUCED CHANGES IN CHOLESTEROL PROFILE

SUPRATIM RAY

Department of Pharmaceutical Sciences, Assam University, Silchar 788011, India
Email: supratimray_in@yahoo.co.in

Received: 31 Jan 2015 Revised and Accepted: 25 Feb 2015

ABSTRACT

Objectives: The aim of the study is to evaluate the protective effects of n-propyl gallate on docetaxel-induced changes in cholesterol profile *in vitro*.

Methods: Goat blood was used as the lipid source for the model. In the cholesterol profile total cholesterol (TC) and high density lipoprotein (HDL) cholesterol content of goat blood was determined. Interpretation of the results is supported by analysis of variance and also by statistical multiple comparison analysis using least significant different procedure.

Results: The study reveals that docetaxel increased the total cholesterol content and decrease the high density lipoprotein (HDL)-cholesterol content at different time of incubation. The results also showed the protective role on these changes in cholesterol content.

Conclusion: The study showed the antiperoxidative effects of n-propyl gallate and demonstrates its potential to reduce docetaxel-induced changes in the cholesterol profile.

Keywords: Docetaxel, n-propyl gallate, Total cholesterol, HDL-cholesterol.

N-Propyl gallate and its analogues are used in foods and other products to prevent oxidation. It also inhibits the growth of HeLa cells by regulating intracellular reduced glutathione (GSH) level [1-2]. It also inhibited gluconeogenesis and stimulates oxygen uptake [3]. In our earlier studies, it is observed that n-Propyl gallate have the capacity to reduce docetaxel-induced lipid peroxidation by reducing malondialdehyde (MDA), 4-hydroxy-2-nonenals (4-HNE) [4, 5]. Reactive oxygen species and other pro-oxidants cause the decomposition of ω_3 and ω_6 polyunsaturated fatty acids of membrane phospholipids leading to the formation of aldehydic end products including malondialdehyde, 4-hydroxy-2-nonenals and 4-hydroxy-2-alkenals (HAKs) of different chain length [6]. 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 damage. 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 [7-8] 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 [9]. Stress in the form of starvation was found to increase lipid peroxidation and alter the lipid profile in rabbits [10].

Docetaxel is a semi synthetic derivative of paclitaxel which is obtained from the rare Pacific yew tree *Taxus brevifolia* [11]. 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 [12] 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 [13]. In view of the above findings and the ongoing search of the present authors for antioxidant that may reduce drug induced lipid peroxidation [14-17] the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of n-propyl gallate on docetaxel-induced changes in cholesterol content in goat blood sample.

Pure sample of docetaxel used in the present study was provided by Fresenius Kabi, Kalyani, India. n-propyl gallate was from SRL, Mumbai. Cholesterol test kit was from Span Diagnostic Ltd., Surat, India. All other reagents were of analytical grade.

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 0.143µmol/g blood. The third portion was treated both with docetaxel at a concentration of 0.143µmol/g blood and antioxidant (n-propyl gallate) at a concentration of 0.189µmol/g blood (DA). The fourth one was treated only with the above mentioned antioxidant alone at a concentration of 0.189µmol/g blood (A). After treatment with docetaxel and/or antioxidant, the different portions of blood samples were initially shaken for 5 h 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 h for next determinations.

Determination of cholesterol concentration was performed in one step method [18] with the help of cholesterol test kit. The determinations were done at 5 and 24 h 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 min and the supernatant (plasma) was separated out. After that total cholesterol and high density lipoprotein cholesterol of the goat blood was determined.

Total cholesterol

The total cholesterol (TC) was calculated by using the following formula

Total Cholesterol (mg/dl) = (O. D. of Test/O. D. of Standard) x 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 min. It was centrifuged at 2000 rpm for 15 min 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 s. 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:

HDL-Cholesterol (mg/dl) = (O. D. of Test/O. D. of Standard) x 50

The interpretation of the result is supported by analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure [19-20]. It was observed from fig. 1 that goat blood treated with docetaxel caused an increase in total cholesterol content (12.68 and 1.65 %) with respect to corresponding control. But the HDL cholesterol level (-7.17 and-5.25%) was reduced in comparison to control group (fig. 2) at 5 and 24 hours of incubation. These observations suggest that docetaxel can change the cholesterol profile. It was further found that incubation of the blood sample with docetaxel and n-propyl gallate produce a decrease in total cholesterol (-1.94 and-1.02%), but the HDL-cholesterol contents (3.51 and 2.20%) were increased in comparison to both control and docetaxel-treated group respectively. Incubation of blood samples only with n-propyl gallate also shows a tendency of decrease in total cholesterol (-2.63 and-0.85%), but HDL-cholesterol contents (2.87 and 1.89%) were increased in comparison to control or docetaxel-treated group respectively.

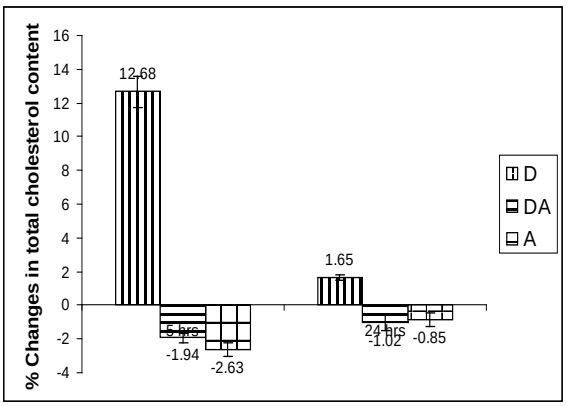

Fig. 1: Effects of n-propyl gallate on docetaxel-induced changes in total cholesterol profile (n=3); D, DA & A indicate only docetaxel-treated, docetaxel & n-propyl gallate-treated and only n-propyl gallate–treated samples

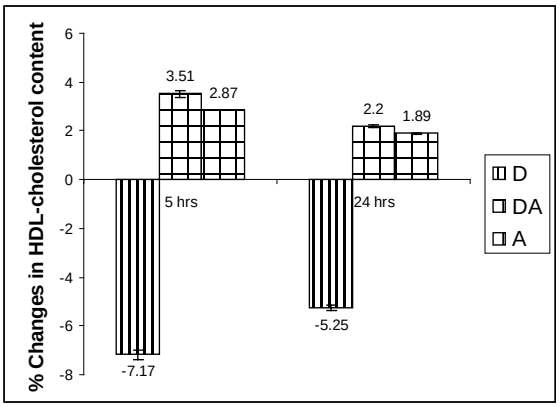

Fig. 2: Effects of n-propyl gallate on docetaxel-induced changes in HDL-cholesterol profile (n=3); docetaxel-treated, docetaxel & n-propyl gallate-treated and only n-propyl gallate–treated samples

To compare means of more than two samples, multiple comparison analysis along with analysis of variance was performed on the percent changes data of various groups (table 1-2). It is seen that there is significant differences among various groups (F1) such as docetaxel-treated, docetaxel and n-propyl gallate-treated and only n-propyl gallate-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. Least significant different procedure [19-20] is applied on the percent changes data of various groups such as docetaxel-treated (D), docetaxel and n-propyl gallate (DA) and only n-propyl gallate-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 n-propyl gallate-treated group as well as an only n-propyl gallate-treated group. But there is no statistically significantly different among the docetaxel and n-propyl gallate-treated group and only n-propyl gallate-treated groups. In case of HDL-cholesterol content (table 2) docetaxel-treated, docetaxel and n-propyl gallate-treated as well as only n-propyl gallate-treated groups are significantly different from each other.

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 showed the antiperoxidative effects of n-propyl gallate and demonstrate its potential to reduce docetaxel-induced changes in cholesterol profile and thus to increase the 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.

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

Name of the antioxidant	Time of incubation (h)	Analysis of variance and multiple comparison
N-propyl gallate	5	F1=213.43[df=(2,4)], F2=1.7[df=(2,4)], Pooled variance $(S^2)^*$=1.05, Critical difference (p=0.05)# LSD=1.93 Ranked means** (D) (DA, A)
	24	F1=32.84 [df=(2,4)], F2=4.61[df=(2,4)], Pooled variance $(S^2)^*$=0.204, Critical difference (p=0.05)# LSD=0.85 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 & n-propyl gallate-treated and only n-propyl gallate–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

Name of the antioxidant	Time of incubation (h)	Analysis of variance and multiple comparison
N-propyl gallate	5	F1=2883.12[df=(2,4)], F2=2.4[df=(2,4)], Pooled variance $(S^2)^*$=0.037, Critical difference (p=0.05)# LSD=0.36 Ranked means** (D) (DA) (A)
	24	F1=6437.63[df=(2,4)], F2=3.21[df=(2,4)], Pooled variance $(S^2)^*$=0.008, Critical difference (p=0.05)# LSD=0.17 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 & n-propyl gallate-treated and only n-propyl gallate–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.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

REFERENCES

1. Eler GJ, Santos IS, de Moraes AG, Mito MS, Comar JF, Peralta RM, *et al.* Kinetics of the transformation of n-propyl gallate and structural analogus in the perfused rat liver. Toxicol Appl Pharmacol 2013;273:35-46.
2. Han YH, Park WH. Propyl gallate inhibits the growth of HeLa cells via regulating intracellular GSH level. Food Chem Toxicol 2009;47:2531-8.
3. Eler GJ, Peralta RM, Bracht A. The action of n-propyl gallate on gluconeogenesis and oxygen uptake in the rat liver. Chem Biol Interact 2009;181:390-9.
4. Ray S, Mondal S, Dey Ray S, Roy PP. Role of antioxidants on docetaxel-induced *in vitro* lipid peroxidation using malondialdehyde as model marker: an experimental and in silico approach. Med Chem Res 2014;23:4436-46.
5. Roy PP, Dey Ray S, Ray S. Combined experimental and in silico approaches for exploring antiperoxidative potential of structurally diverse classes of antioxidants on docetaxel-induced lipid peroxidation using 4-HNE as the model marker. Bioorg Chem 2014;56:1-7.
6. Halliwell B. Drug antioxidant effects-A basis for drug selection? Drugs 1991;42:569-605.
7. Yeagle PL. Modulation of membrane function by cholesterol. Biochimi 1991;73:1303-10.
8. Demonty I, Ras RT, Van der Knaap HC, Duchateau GS, Meijer L, Zock PL. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr 2009;139:271-84.
9. Eliot RS. Stress and the heart. New York: Futura Publishing Company; 1974. p. 41-63.
10. Lata H, Ahuja GK, Narang APS. Effect of starvation stress on lipid peroxidation and lipid profile in rabbits. Indian J Physiol Pharmacol 2002;46:371-4.
11. Clarke SJ, Rivory LP. Clinical pharmacokinetics of docetaxel. Clin Pharmacokinet 1999;36:99-114.
12. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburg: Churchill Livingstone; 2003. p. 666-71.
13. Yang Z, Fong DW, Yin L, Wong Y, Huang W. Liposomes modulate docetaxel-induced lipid oxidization and membrane damage in human hepatoma cells. J Liposome Res 2009;19:122-30.
14. Ray S, Roy K, Sengupta C. *In vitro* evaluation of protective effects of ascorbic acid and water extract of *Spirulina plantesis* (Blue green algae) on 5-fluorouracil-induced lipid peroxidation. Acta Poloniae Pharm Drug Res 2007;64:335-44.
15. Ray S, Roy K, Sengupta C. Evaluation of protective effects of water extract of *Spirulina platensis* (blue green algae) on Cisplatin-induced lipid peroxidation. Indian J Pharm Sci 2007;69:378-83.
16. Ray S, Roy K, Sengupta C. Exploring the protective effect of ascorbic acid and water extract of *Spirulina platensis* (blue green algae) on methotrexate-induced lipid peroxidation. Iran J Pharm Sci 2007;3:217-28.
17. Ray S, Manna AK. QSAR modeling of aldose reductase inhibitory activity of flavonoid compounds using electrotopological state atom parameter (E-state). Asian J Pharm Clin Res 2013;6:140-5.
18. Wybenga DR, Pileqqi VJ, Dirstine PH, Di Giorquio J. Direct manual determination of serum total cholesterol with a single stable reagent. Clin Chem 1970;16:980-4.
19. Snedecor GW, Cochran WG. Statistical Methods. New Delhi: Oxford & IBH Publishing Co Pvt Ltd; 1967.
20. Bolton S. Statistics. In: Gennaro AR. editor. Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 124-58.