

CLINDAMYCIN: EFFECTS ON PLASMA LIPID PROFILE AND PEROXIDATION PARAMETERS IN RABBIT BLOOD PLASMA

PRITESH DEVBHUTI^{1*}, ACHINTYA SAHA² and CHANDANA SENGUPTA³

¹Division of Medicinal and Pharmaceutical Chemistry, Gupta College of Technological Sciences, Ashram More, G. T. Road, Asansol-713301, West Bengal, India

²Department of Chemical Technology, University of Calcutta, Kolkata-700009, India

³Division of Medicinal and Pharmaceutical Chemistry, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India

Abstract: Alteration of plasma lipid profile and induction of lipid peroxidation may take place due to drug effect, which may be correlated with adverse drug reactions and drug-induced toxicity. Considering this fact, the present *in vivo* study was carried out to evaluate the effect of clindamycin on plasma lipid profile and peroxidation parameters alone and in combination with ascorbic acid, a promising antioxidant. After administering drug and antioxidant alone and in combination in rabbit, it was found that clindamycin had mild lipid peroxidation induction and profile alteration capacity, which can be arrested on co-administration of ascorbic acid.

Keywords: clindamycin, adverse drug reactions, lipid peroxidation, lipid profile, ascorbic acid

During the passage of transport, drug has to cross many barriers, most of which are lipoidal in nature. Interaction of drug with membrane lipids may lead to alteration in lipid pattern and composition, as well as lipid peroxidation, which is a measure of the membrane damage. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids (1). It is a highly destructive process that induces a wide variety of alterations in the structure and function of cellular membranes (2), and an important cause of certain diseases or disorders, like diabetes mellitus, gastric ulcer, neurodegenerative diseases (3), atherosclerosis (4), aging (5) etc. Indomethacin-induced gastric mucosal damage (6) and doxorubicin-induced cardiomyopathy (7) are the consequences of drug-induced lipid peroxidation and reactive oxygen species (ROS) formation.

A drug may also induce changes in plasma lipid profile (8-11). A lipid profile is a measure of three components: cholesterol (Ch), triglycerides (Tg), and lipoproteins (LP) (high and low density). Total cholesterol (TCh) comprises all the cholesterol found in various lipoproteins, such as high density LP (HDL), low density LP (LDL) and very low density LP (VLDL). High density lipoprotein cholesterol (HDL-Ch) is believed to play a key role in the

process of reverse cholesterol transport that promotes the efflux of excess cholesterol from vessel wall to the liver for excretion (12, 13). On the contrary, low density lipoprotein cholesterol (LDL-Ch) contains the high percentage of cholesterol, and is responsible for cholesterol deposit on the arterial wall, resulting in coronary symptom like atherosclerosis. Very low density lipoprotein cholesterol (VLDL-Ch) is a large group of macromolecules synthesized and secreted mainly by liver and intestinal mucosal cells, and contains large quantities of Tg (14). Tg is the neutral fat metabolite found in the tissue and blood and may contribute to the disorders related to coronary heart disease (CHD) (15). Phospholipid (PL) is one of the major components of total lipid (TL) present in the biological membrane. Evidence suggests that oxidized PL (a major lipid of LDL-Ch) is formed in atherogenesis and plays an important role in the oxidative modification during LDL-Ch oxidation (16). Elevated lipid profile is associated with many diseases. Diabetic patients have increased level of TCh, Tg, LDL-Ch, VLDL-Ch and decreased level of HDL-Ch (17). CHD is also associated with elevated level of blood TCh, Tg, LDL-Ch and decreased HDL-Ch (18). Blood TCh, HDL-Ch and LDL-Ch are found to be

* Corresponding author: e-mail: priteshdevbhuti@yahoo.co.in

inversely associated with incidence of cancer like leukemia and Hodgkin's disease, but Tg is found to be significantly elevated in patients (19). In AIDS patients, disease progression is accompanied by a decrease in TCh, HDL-Ch and LDL-Ch, and increase in Tg and VLDL-Ch levels (20). Patients with chronic kidney disease (CKD) are at an increased risk for cardiovascular disease and have a higher prevalence of hyperlipidemia (21).

Clindamycin is a lincosamide antibiotic similar in mechanism of action and spectrum of activity to erythromycin (22). It inhibits most gram positive cocci, *C. diphtheriae*, *Nocardia*, *Actinomyces*, *Toxoplasma*, but the distinctive feature is its high activity against a variety of anaerobes specially *Bact. fragilis*. But the use of this antibiotic is restricted due to the development of adverse reactions including pseudomembranous enterocolitis which is potentially fatal (22). In the present *in vivo* study, an attempt has been made to evaluate the lipid peroxidation induction and lipid profile alteration potential of clindamycin and their subsequent control on ascorbic acid co-administration. Ascorbic acid, a promising antioxidant, has free radical scavenging capacity (23, 24). Use of antioxidants as adjuvant therapy may become a promising approach (8, 9, 25) in reducing drug-induced abnormalities. Alteration of lipid profile, which may occur due to drug effect, is also regulated by antioxidant ascorbic acid.

EXPERIMENTAL

Lipid peroxidation induction potential of the drug was measured by estimating laboratory markers, like malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), reduced glutathione (GSH) and nitric oxide (NO) levels, and lipid profile alteration ability was measured by estimating TCh, HDL-Ch, Tg, LDL-Ch, VLDL-Ch, PL and TL levels. New Zealand white rabbits (*Oryctolagus cuniculus*) were used as animal model. All the reagents used in the study were of analytical grade. The design of the study protocol was approved by Institutional Animal Ethical Committee.

Collection of blood

Animals were divided into different experimental groups: control (C), drug treated (D), drug co-administered with antioxidant (DA) and only antioxidant treated (A). The drug, clindamycin was administered intramuscularly at a dose of 30 mg/kg body weight (26) to animal groups marked as D and DA. The antioxidant, ascorbic acid was adminis-

tered at a dose of 40 mg/kg body weight (27) to animal groups marked as DA and A. Blood was collected from marginal ear vein of animal after 3 and 24 h of drug and/or antioxidant administration and the samples were subjected to test for determination of effect of drug and antioxidant on peroxidation parameters and lipid profiles.

Determination of lipid peroxidation

Drug-induced lipid peroxidation was measured by estimating the content of MDA, 4-HNE, GSH and NO in blood sample. Determination was done by precipitating the protein substances using trichloroacetic acid (10% w/v). The protein free samples were used for estimation of lipid peroxidation parameters as follows:

Estimation of MDA

The protein free sample was added to equal volume of thiobarbituric acid (TBA) and heated in a boiling water bath for 30 min. The absorbance of the pink colored sample was measured at 530 nm against a blank (28). The concentration of MDA present in the sample was estimated from the standard curve prepared using tetraethoxypropane (TEP) and TBA (1 : 1).

Estimation of 4-HNE

The sample was mixed (1 : 1) with 2,4-dinitrophenylhydrazine (DNPH) solution (100 mg% in 0.5 M HCl) and incubated at room temperature for 1 h. The mixture was extracted with hexane followed by addition of methanol. The absorbance of the methanol sample was measured at 350 nm (29). The concentration was estimated from the standard curve.

Estimation of GSH

GSH was measured by reacting the sample with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to give a color complex (Ellman's method) (30). The protein free sample was mixed with DTNB (1 : 3) solution (0.01% in phosphate buffer 0.1 M, pH 8) and absorbance of the solution was measured at 412 nm against a blank. Concentration of GSH present in the blood samples was estimated from the standard curve.

Estimation of NO

NO content was determined by reaction with Griess reagent [1 : 1 sulfanilamide (1% w/v in 3 M HCl) and 0.1% w/v N-(1-naphthyl)ethylenediamine dihydrochloride]. The pH of the mixture was adjusted to 6.7 with Na₂HPO₄ and the absorbance of the

solutions was measured at 540 nm (31). The concentration of NO was estimated from the standard curve.

The percent changes in peroxidation parameters, MDA, GSH, 4-HNE, and NO levels of different samples at different hours of interval were calculated with respect to the control.

Determination of lipid profiles

Drug-induced changes in lipid profile were measured by estimating the level of TCh, HDL-Ch, LDL-Ch, VLDL-Ch, Tg, PL and TL in the blood serum. The commercially available enzyme kits used for estimation of lipid profiles were obtained from Span Diagnostics Ltd., Surat, India and Labkit, Barcelona, Spain.

Estimation of TCh

The total cholesterol was estimated by cholesterol oxidase (CHOD) – peroxidase aminoantipyrine

phenol (PAP) method (32, 33). Ten microliters of blood serum was mixed with 1 mL of cholesterol reagent, containing Good's buffer pH 6.7, cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine and stabilizers. The mixture was incubated at 37°C for 10 min. The absorbance was measured at 505 nm against cholesterol reagent as blank. The concentration of TCh was calculated from the standard curve prepared using cholesterol standard samples.

Estimation of HDL-Ch

Estimation of HDL cholesterol was done using CHOD – PAP method (33). Two hundred microliters of serum was mixed with 200 µL of precipitating reagent containing PEG 6000 (200 mM/L), stabilizer and preservative. The mixture was kept at room temperature for 10 min and centrifuged for 15 min at 2000 rpm and the clear supernatant was separated. 100 µL of supernatant was mixed with 1 mL

Table 1. Percent changes in lipid peroxidation parameters with respect to control.

Parameter	Average (\pm SE) % change at time interval							
	3 h				24 h			
	D	DA	A	ANOVA and multiple comparison	D	DA	A	ANOVA and multiple comparison
MDA	8.19 (\pm 0.71)	4.02 (\pm 0.29)	-8.60 (\pm 0.62)	F1 = 295.53 (df 2,8) F2 = 1.83 (df 4,8) Pooled variance = 1.29 LSD = 1.56 Ranked means ^s = (D) (DA) (A)	2.01 (\pm 0.29)	0.55 (\pm 0.24)	-2.34 (\pm 0.17)	F1 = 98.20 (df 2,8) F2 = 1.49 (df 4,8) Pooled variance = 0.25 LSD = 0.69 Ranked means ^s = (D) (DA) (A)
4-HNE	7.14 (\pm 1.02)	3.16 (\pm 0.64)	-8.79 (\pm 0.29)	F1 = 177.69 (df 2,8) F2 = 2.04 (df 4,8) Pooled variance = 1.93 LSD = 1.91 Ranked means ^s = (D) (DA) (A)	2.23 (\pm 0.51)	0.87 (\pm 0.14)	-2.49 (\pm 0.16)	F1 = 64.44 (df 2,8) F2 = 1.42 (df 4,8) Pooled variance = 0.46 LSD = 0.93 Ranked means ^s = (D) (DA) (A)
GSH	-7.56 (\pm 0.67)	-3.77 (\pm 0.42)	4.46 (\pm 0.78)	F1 = 85.49 (df 2,8) F2 = 0.84 (df 4,8) Pooled variance = 2.20 LSD = 2.04 Ranked means ^s = (D) (DA) (A)	-2.05 (\pm 0.15)	0.17* (\pm 0.94)	1.27 (\pm 0.31)	F1 = 9.02 (df 2,8) F2 = 1.19 (df 4,8) Pooled variance = 1.58 LSD = 1.73 Ranked means ^s = (D) (DA) (A)
NO	-10.09 (\pm 1.13)	-5.43 (\pm 0.61)	17.72 (\pm 2.51)	F1 = 86.06 (df 2,8) F2 = 1.11 (df 4,8) Pooled variance = 12.88 LSD = 4.94 Ranked means ^s = (D) (DA) (A)	-4.84 (\pm 0.65)	-4.01 (\pm 0.87)	6.06 (\pm 0.86)	F1 = 74.05 (df 2,8) F2 = 1.91 (df 4,8) Pooled variance = 2.48 LSD = 2.17 Ranked means ^s = (D) (DA) (A)

Average (of 5 animal sets) percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at $p < 0.05$ except marked with* F1 and F2 correspond to variance ratio between samples and between animals, respectively. LSD means critical difference according to the least significant difference procedure. D, DA and A indicate clindamycin-treated, clindamycin and ascorbic acid-treated and only ascorbic acid-treated, respectively. SE = standard error (df = 4); df = degrees of freedom. ^s denotes that two means not included within the same parenthesis are statistically significantly different at $p < 0.05$

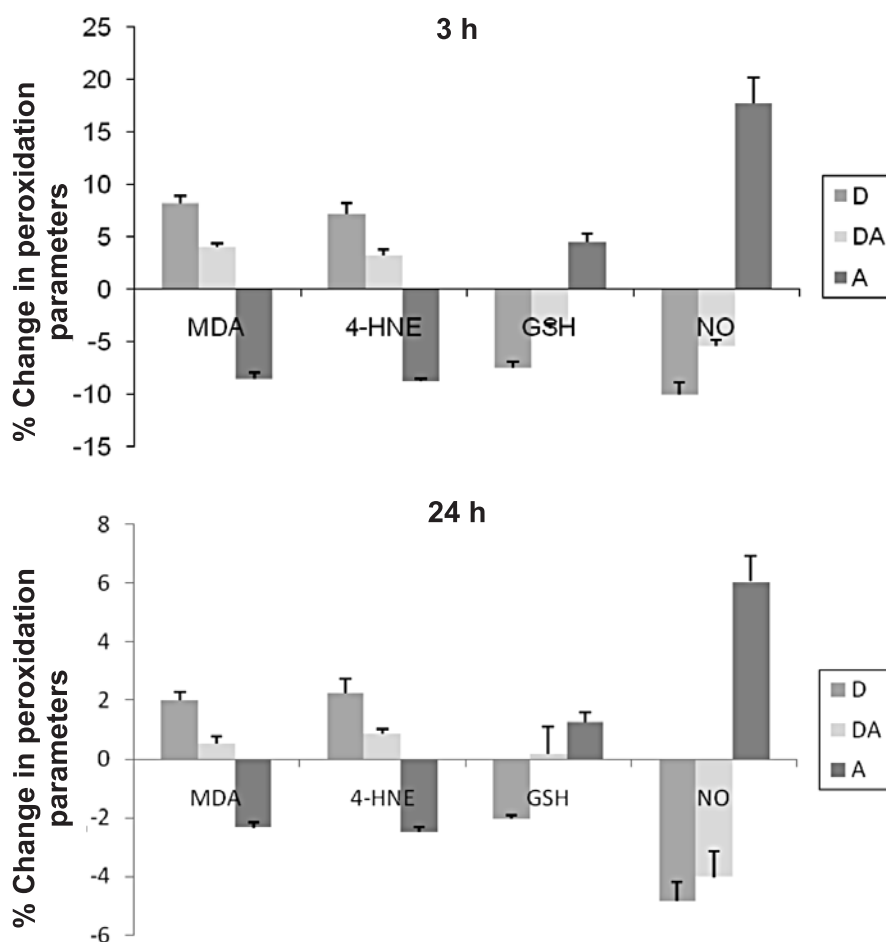


Figure 1. Effect of clindamycin and ascorbic acid on lipid peroxidation parameters. D, DA and A denote clindamycin treated, clindamycin-ascorbic acid treated and only ascorbic acid treated animals, respectively, MDA = malondialdehyde, 4-HNE = 4-hydroxy-2-nonenal, GSH = reduced glutathione, NO = nitric oxide

of cholesterol reagent and incubated at 37°C for 10 min. The absorbance was measured at 505 nm. The concentration of HDL-Ch was calculated from a standard curve prepared using HDL-Ch standard samples.

Estimation of LDL-Ch and VLDL-Ch

Friedewald's equations (34) were used to calculate concentrations of LDL-Ch and VLDL-Ch in the samples.

$$\text{LDL-Ch content} = \text{TCh content} - (\text{Tg content} / 5) - \text{HDL-Ch content}$$

$$\text{VLDL-Ch content} = \text{Tg content} / 5$$

Estimation of Tg

Ten microliters of serum was mixed with 1 mL of Tg mono reagent containing pipes buffer, 4-chlorophenol, magnesium, ATP, lipase, peroxidase,

glycerol kinase, 4-aminoantipyrine, glycerol-3-phosphate oxidase, detergents, preservative and stabilizer. The mixture was incubated at 37°C for 10 min. The absorbance of the solution was measured at a wavelength of 505 nm (33, 35). The concentration of Tg was calculated from a standard curve prepared using Tg standard samples.

Estimation of PL

Ten microliters of blood serum was mixed with 1 mL of reagent containing TRIS buffer pH 7.55, dichlorophenol, phospholipase D, choline oxidase, peroxidase and 4-aminophenazone. The mixture was incubated for 5 min at 37°C and the absorbance of the solution was measured at a wavelength of 505 nm (36). The concentration of PL was calculated from a standard curve prepared using PL primary standards.

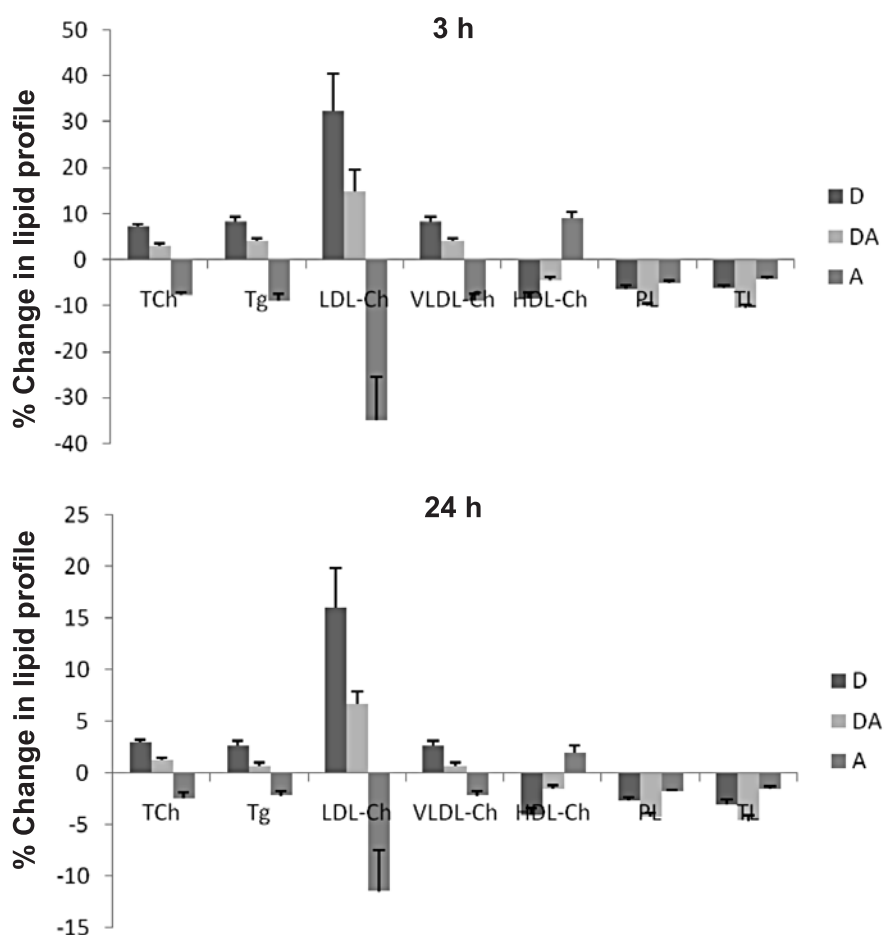


Figure 2. Effect of clindamycin and ascorbic acid on lipid profile. D, DA and A denote clindamycin treated, clindamycin-ascorbic acid treated and only ascorbic acid treated animals, respectively. TCh = total cholesterol, Tg = triglyceride, LDL-Ch = LDL cholesterol, VLDL-Ch = VLDL cholesterol, HDL-Ch = HDL cholesterol, PL = phospholipid, TL = total lipid

Estimation of TL

Hundred microliters of serum was mixed with 2.5 mL of sulfuric acid, heated for 10 min in a boiling water bath (100°C) and then cooled in iced water. Fifty microliters of the acid digested sample was mixed with 1 mL of phosphovanillin reagent and incubated for 15 min at 37°C. The absorbance was measured at a wavelength of 520 nm (37, 38). The concentration of TL present in the sample was calculated from a standard curve prepared using TL primary standards.

The percent changes in TCh, HDL-Ch, LDL-Ch, VLDL-Ch Tg, PL and TL levels of different samples at different time intervals were calculated with respect to the control.

RESULTS AND DISCUSSION

Results of the study are presented in Tables 1, 2 and are further illustrated in Figures 1, 2. The

results were statistically validated by analysis of variance (ANOVA) followed by multiple comparison using a least significant difference procedure (39, 40). From Table 1 and Figure 1, it is evident that clindamycin has low but significant lipid peroxidation induction potential that might cause elevation of MDA and 4-HNE contents, which were found to be the end products of lipid peroxidation (41) and involved in drug-induced toxicity (6, 7). When ascorbic acid was co-administered, it reduced the elevated levels of MDA and 4-HNE. Results (Table 1 and Figure 1) also showed the reduction in GSH and NO contents that might be due to peroxidation induction capacity of clindamycin. GSH and NO are related to the antioxidant defense mechanism (42, 43) of the body. Again, when animals received both drug and antioxidant, the GSH and NO levels are elevated with respect to the drug treated group. Levels of these parameters are increased compared to the control group in animals which

received only ascorbic acid. This increased GSH and NO contents indicate the antiperoxidative potential of ascorbic acid (8, 9, 25).

Drug-induced alteration in lipid profile and their control by ascorbic acid are illustrated in Table

2 and Figure 2. From the results, it is evident that clindamycin slightly elevated the levels of TCh, LDL-Ch, VLDL-Ch and Tg, which are further decreased upon co-administration of ascorbic acid. Both Table 2 and Figure 2 showed a reduced level of

Table 2. Percent changes in lipid profile contents with respect to control.

Parameter	Average ((± SE) % change at time interval							
	3 h				24 h			
	D	DA	A	ANOVA and multiple comparison	D	DA	A	ANOVA and multiple comparison
TCh	7.19 (± 0.63)	3.00 (± 0.49)	-7.78 (± 0.67)	F1 = 125.46 (df 2,8) F2 = 0.31 (df 4,8) Pooled variance = 2.37 LSD = 2.12 Ranked means ^s = (D) (DA) (A)	2.93 (± 0.25)	1.21 (± 0.25)	-2.56 (± 0.60)	F1 = 37.67 (df 2,8) F2 = 0.34 (df 4,8) Pooled variance = 1.04 LSD = 1.40 Ranked means ^s = (D) (DA) (A)
Tg	8.23 (± 1.09)	4.04 (± 0.45)	-9.11 (± 1.62)	F1 = 46.29 (df 2,8) F2 = 0.28 (df 4,8) Pooled variance = 8.84 LSD = 4.09 Ranked means ^s = (D) (DA) (A)	2.56 (± 0.49)	0.62 (± 0.35)	-2.23 (± 0.41)	F1 = 24.81 (df 2,8) F2 = 0.32 (df 4,8) Pooled variance = 1.17 LSD = 1.49 Ranked means ^s = (D) (DA) (A)
LDL-Ch	32.48 (± 7.90)	14.75 (± 4.91)	-34.92 (± 9.36)	F1 = 15.75 (df 2,8) F2 = 0.25 (df 4,8) Pooled variance = 387.33 LSD = 27.12 Ranked means ^s = (D, DA) (A)	16.00 (± 3.84)	6.69 (± 1.14)	-11.55 (± 4.01)	F1 = 13.06 (df 2,8) F2 = 0.14 (df 4,8) Pooled variance = 75.21 LSD = 11.95 Ranked means ^s = (D, DA) (A)
VLDL-Ch	8.23 (± 1.09)	4.04 (± 0.45)	-9.11 (± 1.62)	F1 = 46.29 (df 2,8) F2 = 0.28 (df 4,8) Pooled variance = 8.84 LSD = 4.09 Ranked means ^s = (D) (DA) (A)	2.56 (± 0.49)	0.62 (± 0.35)	-2.23 (± 0.41)	F1 = 24.81 (df 2,8) F2 = 0.32 (df 4,8) Pooled variance = 1.17 LSD = 1.49 Ranked means ^s = (D) (DA) (A)
HDL-Ch	-8.45 (± 1.32)	-4.48 (± 0.71)	9.01 (± 1.28)	F1 = 46.51 (df 2,8) F2 = 0.17 (df 4,8) Pooled variance = 8.99 LSD = 4.13 Ranked means ^s = (D, DA) (A)	-4.18 (± 0.71)	-1.57 (± 0.34)	1.95 (± 0.66)	F1 = 18.56 (df 2,8) F2 = 0.11 (df 4,8) Pooled variance = 2.54 LSD = 2.19 Ranked means ^s = (D) (DA) (A)
PL	-6.30 (± 0.76)	-9.99 (± 0.54)	-5.05 (± 0.50)	F1 = 33.05 (df 2,8) F2 = 3.68 (df 4,8) Pooled variance = 0.99 LSD = 1.37 Ranked means ^s = (D, A) (DA)	-2.74 (± 0.34)	-4.28 (± 0.43)	-1.84 (± 0.17)	F1 = 41.26 (df 2,8) F2 = 7.20 (df 4,8) Pooled variance = 0.18 LSD = 0.58 Ranked means ^s = (D) (DA) (A)
TL	-6.03 (± 0.49)	-10.50 (± 0.79)	-4.20 (± 0.45)	F1 = 94.67 (df 2,8) F2 = 7.73 (df 4,8) Pooled variance = 0.55 LSD = 1.02 Ranked means ^s = (D) (DA) (A)	-3.07 (± 0.49)	-4.67 (± 0.58)	-1.60 (± 0.21)	F1 = 24.70 (df 2,8) F2 = 4.61 (df 4,8) Pooled variance = 0.48 LSD = 0.95 Ranked means ^s = (D) (DA) (A)

Average (of 5 animal sets) percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at $p < 0.05$. F1 and F2 correspond to variance ratio between samples and between animals, respectively. LSD means critical difference according to the least significant difference procedure. D, DA and A indicate clindamycin-treated, clindamycin and ascorbic acid-treated and only ascorbic acid-treated, respectively. SE = standard error (df = 4); df = degrees of freedom. ^s denotes that two means not included within the same parenthesis are statistically significantly different at $p < 0.05$

HDL-Ch in drug treated animals with respect to control. The group receiving both drug and antioxidant showed increased level of HDL-Ch than the drug treated group, while only antioxidant treated group showed elevated level of HDL-Ch in comparison to control animals. Elevation in TCh, LDL-Ch, VLDL-Ch, Tg contents and reduction in HDL-Ch may be linked with drug-induced alteration in lipid profile (8, 9, 44-46), though changes in lipoprotein level may also happen as a result of drug-induced protein metabolism (47). The results further corroborated with lipid peroxidation induction potential of the drug that caused elevation of TCh, Tg, LDL-Ch and VLDL-Ch and reduction in HDL-Ch levels (8, 9). The present study also showed that the liposuppressive effect of the antioxidant ascorbic acid plays a beneficial role to minimize abnormal lipid profile alteration including increased lipid peroxidation (48-51). Ascorbic acid is not only an antioxidant but also an important antihyperlipidemic agent. From Table 2 and Figure 2 it was also found that there is a reduction in PL and TL contents in all animal groups. In case of drug treated group, the reduction in PL and TL contents might be due to binding ability of the drug with lipids (52). The efficient binding capacity of the drugs to PL and TL might cause significant reduction of those parameters, and increased level of peroxidation parameter like MDA (53, 54).

In both figures, similar pattern of changes occurs in both 3 and 24 h time period, but the extent of change is more prominent in the former. This difference may be due to significant elimination of the drug and antioxidant from the body within 24 h.

The drug – clindamycin – is found to be effective in the treatment of many bacterial infections (22) and has also mild lipid peroxidation induction potential. The drug-induced changes in lipid profile may be correlated with its lipid peroxidation induction potential. Some of the toxicities of clindamycin may be linked with its lipid peroxidation induction capacity and that can be effectively controlled on co-administration of ascorbic acid, having free radical scavenging capacity (23, 24). The concept of antioxidant co-therapy may be exploited during future formulation design with an aim of reducing drug-induced adverse reactions and toxicities. Moreover, lipid peroxidation induction as well as lipid profile alteration capacity of a drug may be tested at the individual level to determine the extent of risk from the drug in case of a particular individual in view of variable *in vivo* antioxidant defense and accordingly, the decision about safe use of a drug and necessary co-administration

of antioxidant may be taken. Thus, the antioxidant co-therapy approach will be an effective tool for the physicians to reduce possibilities of drug-related hazards. This may lead to enhancement of patient compliance and improvement of therapeutic index of the drug.

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REFERENCES

- Halliwell B., Gutteridge J.M.C.: in *Free Radicals in Biology and Medicine*, 2nd edn., p. 200, Oxford University Press, Oxford 1989.
- Kale R.K., Sitasawad S.L.: *Radiat. Phys. Chem.* 36, 361 (1990).
- Adibhatla R.M., Hatcher J.F.: *Antioxid. Redox. Signal.* 12, 125 (2010).
- Tappel A.L.: *Geriatrics* 23, 97 (1968).
- Harmann D.J.: *Am. Geriat. Soc.* 17, 721 (1969).
- Naito Y., Yoshikawa T., Yoshida N., Kondo M.: *Drug Dis. Sci.* 43, 305 (1998).
- Dorr R. T.: *Semin. Oncol.* 23, 23 (1996).
- Devbhuti P., Saha A., Sengupta C.: *Int. J. Cur. Pharm. Res.* 2, 76 (2010).
- Devbhuti P., Sikdar D., Saha A., Sengupta C.: *Acta Pol. Pharm. Drug Res.* 68, 15 (2011).
- Ames R.P.: *Am. Heart J.* 114, 998 (1987).
- Weidmann P., Courten M., Ferrari P.: *Eur. Heart J.* 13, 61 (1992).
- Ohashi R., Mu H., Wang X., Yao Q., Chen Q.: *QJM* 98, 845 (2005).
- Lewis G.F., Rader D.J.: *Circ. Res.* 96, 1221 (2005).
- Tzagournis M.: *Am. J. Clin. Nutr.* 31, 1437 (1978).
- Khan S.R., Ayub N., Nawab S., Shamsi T.S.: *J. Coll. Physicians Surg. Pak.* 18, 270 (2008).
- Friedman P., Horkko S., Steinberg D., Witztum J.L., Dennis E.A.: *J. Biol. Chem.* 277, 7010 (2002).
- Suryawanshi N.P., Bhutey A.K., Nagdeote A.N., Jadhav A.A., Manoorkar G.S.: *Indian J. Clin. Biochem.* 21, 126 (2006).
- Ghosh J., Mishra T.K., Rao Y.N., Agarwal S.K.: *Indian J. Clin. Biochem.* 21, 181 (2006).
- Naik P.P., Ghadge M.S., Raste A.S.: *Indian J. Clin. Biochem.* 21, 100 (2006).

20. Khiangte L., Vidyabati R.K., Singh M.K., Devi S.B., Singh T.R., Singh W.G.: *J. Indian Acad. Clin. Med.* 8, 307 (2007).
21. Chan C.M.: *Ann. Acad. Med.* 34, 31 (2005).
22. Tripathi K.D.: in *Essentials of Medical Pharmacology*, 5th edn., p. 678, Jaypee Brothers, New Delhi 2003.
23. Alamed J., Chaayasit W., Mc Clements D.J., Decker E.A.: *J. Agric. Food Chem.* 57, 2969 (2009).
24. Kim D.O., Lee C.Y.: *Crit. Rev. Food Sci. Nutr.* 44, 253 (2004).
25. Devbhuti P., Saha A., Sengupta C.: *Acta Pol. Pharm. Drug Res.* 66, 363 (2009).
26. Paris M.M., Shelton S., Trujillo M., Hickey S.M., Mc Cracken Jr. G.H.: *Antimicrob. Agents Chemother.* 40, 122 (1996).
27. Yousef M.I.: *Toxicology* 207, 81 (2005).
28. Ohkawa H., Ohisi N., Yagi K.: *Anal. Biochem.* 95, 351 (1979).
29. Kinter M.: in *Free Radicals - A Practical Approach*, PUNCHARD N.A., KELLY G.J. Eds., p. 136, Oxford University Press, Oxford 1996.
30. Ellman G.L.: *Arch. Biochem. Biophys.* 82, 70 (1959).
31. Sastry K.V.H., Moudgal R.P., Mohan J., Tyagi J.S., Rao G.S.: *Anal. Biochem.* 306, 79 (2002).
32. Herbert K.: in *Lipids, In Clinical Chemistry: Theory, Analysis and Correlation*, p. 1182, CV Mosby, Toronto 1984.
33. Nader R., Paul B., John A.: *Lipids, Lipoproteins and Apolipoproteins*, in *Tietz Textbook of Clinical Chemistry*, 3rd edn., p. 809, WB Saunders, Philadelphia 1994.
34. Friedewald W.T., Levy R.I., Fredrickson D.S.: *Clin. Chem.* 18, 499 (1972).
35. Mc Gowan M.W., Artiss J.D., Strandbergh D.R., Zak B.: *Clin. Chem.* 29, 538 (1983).
36. Taylor S., Watts R.: *Clin. Chim. Acta* 73, 547 (1976).
37. Woodman D.D., Price C.P.: *Clin. Chim. Acta* 38, 39 (1972).
38. Akins J.R., Waldrep K., Bernert Z.T.: *Clin. Chim. Acta* 184, 219 (1989).
39. Snedecor G.W., Cochran W.G.: in *Statistical Methods*, p. 301, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi 1967.
40. Bolton S.: in *Remington: The Science and Practice of Pharmacy*, 19th edn., Gennaro A.R. Ed., Vol. 1, p. 111, Mack Publishing Co., Pennsylvania 1995.
41. Esterbauer H., Schaur R.J., Zollner H.: *Free Radic. Biol. Med.* 11, 81 (1991).
42. Kosower E.M., Kosower N.S.: in *Glutathione Metabolism and Function*, p. 139, Raven Press, New York 1976.
43. Kelly E.E., Wagner B.A., Buettner G.R., Bums C.K.: *Arch. Biochem. Biophys.* 370, 97 (1999).
44. Ramkumar K.M., Vijayakumar R.S., Ponmannickam P., Velayuthaprabhu S., Archunan G., Rajaguru P.: *Basic Clin. Pharmacol. Toxicol.* 103, 538 (2008).
45. Thounaojam M., Jadeja R., Ansarullah, Devkar R., Ramachandran A.V.: *J. Health Sci.* 55, 413 (2009).
46. Vaidya H., Rajani M., Sudarsanam V., Padh H., Goyal R.: *J. Nat. Med.* 63, 437 (2009).
47. Lu Y., Guo J., Di Y., Zong Y., Qu S., Tian J.: *Mol. Cells* 28, 175 (2009).
48. Das S., Ray R., Snehlata, Das N., Srivastava L.M.: *Mol. Cell. Biochem.* 285, 143 (2006).
49. Das K.K., Gupta A.D., Dhundasi S.A., Patil A.M., Das S.N., Ambekar J.G.: *J. Basic Clin. Physiol. Pharmacol.* 17, 29 (2006).
50. Filis K., Anastassopoulou A., Sigala F., Theodorou D., Manouras A., Leandros E., Sigalas P. et al.: *Int. J. Vitam. Nutr. Res.* 77, 125 (2007).
51. Yousef M.I., Awad T.I., Elhag F.A., Khaled F.A.: *Toxicology* 235, 194 (2007).
52. Stryer L.: in *Biochemistry*, 3rd edn., p. 297, Freeman and Co., New York 1988.
53. Saha A., Roy K., De K., Sengupta C.: *Acta Pol. Pharm. Drug Res.* 59, 65 (2002).
54. Saha A., Roy K., De K., Sengupta C.: *Indian J. Pharm. Sci.* 65, 171 (2003).

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