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Research Article

STUDIES ON AMIKACIN-INDUCED LIPID PROFILE AND PEROXIDATION PARAMETERS AND THEIR CONTROL WITH ASCORBIC ACID

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ABSTRACT

Drug-induced lipid peroxidation and profile alteration can be correlated with drug-induced toxicity. Antioxidants may play a beneficial role to control drug-induced toxicity. In view of this context, the present *in vivo* study was carried out to evaluate the effect of ascorbic acid on amikacin-induced lipid peroxidation and profile alteration. Rabbit was used in the present work and the animals were divided into different experimental groups- control, drug treated, drug and antioxidant treated, and only antioxidant treated. The drug, amikacin and the antioxidant, ascorbic acid were administered to respective groups. After specified time interval, blood was collected and levels of peroxidation parameters, malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), reduced glutathione (GSH) and nitric oxide (NO), and lipid profiles, total cholesterol (TCh), HDL cholesterol (HDL-Ch), LDL cholesterol (LDL-Ch), triglyceride (Tg), phospholipids (PL), and total lipids (TL) were determined and compared to control. The results revealed that amikacin caused enhancement of MDA, HNE, TCh, LDL-Ch, Tg levels and significant reduction in GSH, NO, HDL-Ch, PL, TL levels. Ascorbic acid on co-administration showed significant control of amikacin-induced lipid pattern changes and peroxidation parameters.

Keywords: Amikacin, Lipid profile, Lipid peroxidation, Antioxidant, Ascorbic acid.

INTRODUCTION

During its passage through biomembranes, a drug may interact with membrane lipids that leads to alteration in lipid pattern and composition, as well as lipid peroxidation, which is a measure of the membrane damage. Lipid peroxidation is a highly destructive process that induces a wide variety of alterations in the structure and function of cellular membranes[1], and an important cause of certain diseases or disorders, like atherosclerosis[2], diabetes mellitus, gastric ulcer, neurodegenerative diseases[3], aging[4] etc. Indomethacin-induced gastric mucosal damage[5] and doxorubicin-induced cardiomyopathy[6] are the consequences of drug-induced lipid peroxidation and reactive oxygen species (ROS) generation.

Alteration of blood-lipid profile may also occur due to drug effect[7,8]. 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[9,10]. On the contrary, low density lipoprotein cholesterol (LDL-Ch) contains the high percentage of cholesterol, and is responsible for cholesterol deposit on the wall of the artery, 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[11]. Tg is the neutral fat metabolite found in the tissue and blood and may contribute to the disorders related to coronary heart disease (CHD)[12]. 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[13].

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[14]. CHD is also associated with elevated level of blood TCh, Tg, LDL-Ch and decreased HDL-Ch[15]. Blood TCh, HDL-Ch and LDL-Ch are found to be inversely associated with incidence of cancer like leukemia and Hodgkin's disease, but Tg is found to be significantly elevated in patients[16]. 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[17]. Patients with chronic kidney disease (CKD) are at an increased risk for cardiovascular disease and have a higher prevalence of hyperlipidemia[18].

Amikacin is a semi-synthetic derivative of the aminoglycoside antibiotic, kanamycin. It is used in the treatment of various infections. The outstanding feature of amikacin is its resistance to bacterial aminoglycoside inactivating enzymes. Thus it has the wide spectrum of activity, including many organisms resistant to other aminoglycosides[19]. However, aminoglycoside induced nephrotoxicity and ototoxicity are limiting factors for their clinical use and ROS have been found to be involved in these consequences[20-24]. These toxicities are reported to be free radical associated[25,26]. In the present *in vivo* study, an attempt has been made to explore the effect of ascorbic acid on amikacin-induced lipid peroxidation and profile alteration. Ascorbic acid, a promising antioxidant, has free radical scavenging capacity[27,28]. Use of antioxidants as adjuvant therapy may become a promising approach[8,29,30] in reducing drug-induced abnormalities. Alteration of lipid profile which may occur due to drug effect is also regulated by antioxidant ascorbic acid[31-34].

MATERIALS AND METHODS

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

Collection of blood

Animals, kept in 18 h fasting condition, were divided into different experimental groups– control (C), drug treated (D), drug co-administered with antioxidant (DA) and only antioxidant treated (A). The drug, amikacin was administered intramuscularly at a dose[35] of 15 mg/kg body weight to animal groups marked as D and

DA. The antioxidant, ascorbic acid was administered at a dose[36] of 40 mg/kg body weight to animal groups marked as DA and A. After 3 and 24 h of drug and/or antioxidant administration, blood was collected from marginal ear vein of animal and the samples were subjected to test for determination of drug-induced peroxidation parameters and lipid profiles.

Measurement of lipid peroxidation

Drug-induced lipid peroxidation was measured by estimating the content of MDA, 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[37]

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 coloured sample was measured at 530 nm against a blank. The concentration of MDA present in the sample was estimated from the best fit equation (eq. 1 in Table 1) of standard curve prepared using tetraethoxy propane (TEP) and TBA (1:1).

Estimation of HNE[38]

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. The concentration was estimated from the equation of standard curve (eq. 2 in Table 1).

Estimation of GSH[39]

GSH was measured by reacting the sample with 5, 5/-dithiobis (2-nitrobenzoic acid) (DTNB) to give a colour complex (Ellman's method)[39]. 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 equation of standard curve (eq. 3 in Table 1).

Estimation of NO[40]

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 Na_2HPO_4 and the absorbance of the solutions was measured at 540 nm. The concentration of NO was estimated from the standard curve equation (eq. 4 in Table 1).

The percent changes in peroxidation parameters, MDA, GSH, 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 Limited, Surat, India and Labkit, Barcelona, Spain.

Estimation of TCh

The total cholesterol was estimated by cholesterol oxidase (CHOD) – peroxidase aminoantipyrine phenol (PAP) method[41,42]. 10 µl 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 37^0C for 10 min. The absorbance was measured at 505 nm against cholesterol reagent as blank. The concentration of TCh was calculated from the best fit equation of standard curve (eq. 5 in Table 1) prepared using cholesterol standard samples.

Estimation of HDL-Ch

HDL cholesterol was also estimated by CHOD – PAP method[42]. 200 µl 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 of cholesterol reagent and incubated at 37^0 C for 10 min. The absorbance was measured at 505 nm. The concentration of HDL-Ch was calculated from a standard curve equation (eq. 6 in Table 1) prepared using HDL-Ch standard samples.

Estimation of LDL-Ch and VLDL-Ch

Concentrations of LDL-Ch and VLDL-Ch in the samples were calculated using Friedewald's equations[43].

LDL-Ch content = TCh content – (Tg content / 5) – HDL-Ch content

VLDL-Ch content = Tg content / 5

Estimation of Tg[42,44]

10 µl of serum was mixed with 1 ml of triglyceride mono reagent containing pipes buffer, 4-chlorophenol, magnesium, ATP, lipase, peroxidase, glycerolkinase, 4-aminoantipyrine, glycerol-3-phosphate oxidase, detergents, preservative and stabilizer. The mixture was incubated at 37^0 C for 10 min. The absorbance of the coloured solution was measured at a wave length of 505 nm. The concentration of Tg was calculated from the equation of standard curve (eq.7 in Table 1) prepared using triglyceride standard samples.

Estimation of PL[45]

10 µl 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 37^0 C and the absorbance of the solution was measured at a wave length of 505 nm. The concentration of PL was calculated from the best fit equation of standard curve (eq. 8 in Table 1) prepared using phospholipid primary standards.

Estimation of TL[46,47]

100 µl of serum was mixed with 2.5 ml sulphuric acid, heated for 10 min in a boiling water bath (100^0 C) and then cooled in iced water. 50 µl of the acid digested sample was mixed with 1 ml of phosphovainilline reagent and incubated for 15 min at 37^0 C. The absorbance was measured at a wave length of 520 nm. The concentration of TL was calculated from a standard curve equation (eq. 9 in Table 1) prepared using total lipid primary standards.

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

RESULTS AND DISCUSSION

Results of the study are listed in Tables 2 and 3 and illustrated in Figures 1- 2. Interpretation of the results is supported by Student's t-test and the results are statistically significant. From the Table 2, it is observed that amikacin has significant lipid peroxidation induction potential which might cause elevation of MDA and HNE contents, as these are considered as the end products of lipid peroxidation[48]. But the elevated levels of these parameters are controlled on ascorbic acid co-administration. Further the contents of GSH and NO, which are related to the antioxidant defense mechanism[49,50], are declined probably due to peroxidation induction capacity of amikacin. The antioxidant, ascorbic acid caused significant enhancement of GSH and NO levels in DA group (Figure 1).

Table 1: Standard curves for the estimation of lipid profile (mg/100 ml) and peroxidation (nM/ml) parameters

Equation No.	Parameter	Equation	Statistics
1	MDA	MDA (nM /ml) = (A_{530} – 4.92 x 10^{-4}) / 5.84 x 10^{-3}	r = 0.994, se = 0.005, F = 1.19x10^3 (df 1, 13), n = 15
2	HNE	HNE (nM /ml) = (A_{350} – 5.60 x 10^{-3}) / 3.26 x 10^{-3}	r = 0.999, se = 0.007, F = 1.66x10^3 (df 1, 2), n = 4
3	GSH	GSH (nM / ml) = (A_{412} – 8.55 x 10^{-6}) / 6.82 x 10^{-3}	r = 0.999, se =0.003, F = 5.32x10^4 (df 1, 24), n = 26
4	NO	NO (nM /ml) = (A_{540} – 4.52 x 10^{-4}) / 5.30 X 10^{-2}	r = 0.998, se = 0.002, F = 4.40x10^3 (df 1, 13), n = 15
5	TCh	TCh (mg /100ml) = (A_{505} – 6.36 x 10^{-5}) / 1.532 x 10^{-3}	r = 0.999, se = 0.004, F = 3.79x10^3 (df 1, 5), n = 7
6	HDL-Ch	HDL-Ch (mg /100ml) = A_{505} / 4.574 x 10^{-3}	r = 0.999, se = 0.002, F = 1.92x10^4 (df 1, 8), n = 9
7	Tg	Tg (mg /100 ml) = A_{505} / 1.068 x 10^{-3}	r = 0.999, se = 0.004, F = 6.57x10^3 (df 1,6), n = 7
8	PL	PL (mg /100 ml) = A_{505} / 1.503 x 10^{-3}	r = 0.999, se = 0.004, F = 2.52x10^4 (df 1, 6), n = 7
9	TL	TL (mg /100 ml) = (A_{520} – 4.311 X 10^{-3}) / 3.87 x 10^{-4}	r = 0.998, se = 0.005, F = 3.12x10^3 (df 1, 7), n = 9

MDA: Malondialdehyde, HNE: Hydroxy nonenal, GSH: Reduced glutathione, NO: Nitric oxide, TCh: Total cholesterol, HDL-Ch: HDL Cholesterol, Tg: Triglyceride, PL: Phospholipid, TL: Total lipid, A: Absorbance at λ_{max} in subscript, r: Correlation coefficient, se: Standard error, F: Variance ratio, df: Degrees of freedom, n: No. of standard sample.

Table 2: Percent changes in peroxide contents with respect to control

Parameter	% Change and concentration (nM/mL ± se) at time interval							
	3 h				24 h			
	C	D	DA	A	C	D	DA	A
MDA	---------	18.17	9.67	-8.38	-------	7.38	4.26	-2.95
	(18.28±0.55)	(21.61±0.56)	(20.05±0.52)	(16.75±0.54)	(18.28±0.55)	(19.63±0.67)	(19.06±0.61)	(17.74±0.59)
HNE	----------	14.36	6.75	-7.44	---------	5.91	2.94	-2.93
	(147.67±2.62)	(168.88±3.79)	(157.65±2.97)	(136.67±2.34)	(147.67±2.62)	(156.40±3.54)	(152.02±3.07)	(143.34±2.01)
GSH	----------	-12.77	-5.55	5.96	----------	-4.84	-2.23	2.95
	(84.17±2.86)	(73.41±3.01)	(79.49±2.83)	(89.19±2.63)	(84.17±2.86)	(80.09±2.52)	(82.29±2.67)	(86.66±2.83)
NO	-----------	-22.16	-12.56	18.22	----------	-9.35	-3.69	6.89
	(0.812±0.037)	(0.632±0.034)	(0.710±0.030)	(0.96±0.036)	(0.812±0.037)	(0.736±0.027)	(0.782±0.038)	(0.868±0.037)

C, D, DA and A denote control, amikacin treated, amikacin-ascorbic acid treated and only ascorbic acid treated animals respectively, MDA: Malondialdehyde, HNE: Hydroxy nonenal, GSH: Reduced glutathione, NO: Nitric oxide, se = Standard error. The changes are significant at $p < 0.05$ (n = 25).

Table 3: Percent changes in lipid profile contents with respect to control

Parameter	% Change and concentration (mg / 100mL ± se) at time interval							
	3 h				24 h			
	C	D	DA	A	C	D	DA	A
TCh	---------	10.85	5.46	-6.46	---------	4.77	2.09	-2.59
	(26.22±3.21)	(29.06±3.46)	(27.65±3.33)	(24.52±3.07)	(26.22±3.21)	(27.47±3.42)	(26.76±3.28)	(25.54±3.18)
HDL-Ch	----------	-11.70	-6.36	8.78	---------	-4.75	-2.17	3.22
	(9.17±1.05)	(8.10±0.96)	(8.59±0.98)	(9.98±1.11)	(9.17±1.05)	(8.74±1.00)	(8.97±1.02)	(9.47±1.09)
LDL-Ch	---------	37.66	20.90	-27.19	---------	21.82	9.36	-11.78
	(5.67±0.95)	(7.81±1.05)	(6.86±1.04)	(4.13±0.89)	(5.67±0.95)	(6.91±1.17)	(6.20±1.02)	(5.00±0.93)
VLDL-Ch	---------	15.67	7.31	-8.43	---------	3.95	1.90	-2.70
	(11.37±1.26)	(13.15±1.48)	(12.20±1.34)	(10.41±1.17)	(11.37±1.26)	(11.81±1.28)	(11.58±1.27)	(11.06±1.22)
Tg	---------	15.67	7.31	-8.43	--------	3.95	1.90	-2.70
	(56.85±6.30)	(65.76±7.42)	(61.00±6.70)	(52.05±5.85)	(56.85±6.30)	(59.09±6.43)	(57.93±6.37)	(55.31±6.11)
PL	---------	-6.62	-10.58	-4.27	--------	-2.82	-3.49	-1.67
	(51.04±4.52)	(47.66±4.50)	(45.63±4.46)	(48.85±4.49)	(51.04±4.52)	(49.60±4.46)	(49.25±4.56)	(50.18±4.52)
TL	----------	-11.07	-16.35	-6.28	---------	-3.32	-4.78	-1.77
	(226.89±8.58)	(201.77±14.74)	(189.78±13.25)	(212.62±16.86)	(226.89±18.58)	(219.34±17.36)	(216.04±16.98)	(222.86±18.46)

C, D, DA and A denote control, amikacin treated, amikacin-ascorbic acid treated and only ascorbic acid treated animals respectively, TCh: Total cholesterol, HDL-Ch: HDL Cholesterol, LDL-Ch: LDL Cholesterol, VLDL-Ch: VLDL Cholesterol, Tg: Triglyceride, PL: Phospholipid, TL: Total lipid, se = Standard error. The changes are significant at $p < 0.05$ (n = 25).

Changes in lipid profiles due to drug effect and control by ascorbic acid are illustrated in Table 3 and Figure 2. The results indicated that amikacin caused significant elevation of TCh, LDL-Ch, VLDL-Ch and Tg contents, which are further decreased on co-administration of ascorbic acid. Table 3 and Figure 2 also showed a decreased level of HDL-Ch in D- animals with respect to C-group. The DA-group showed increased level of HDL-Ch than the D-group, while A-group showed elevated level of HDL-Ch with respect to C-group. Enhancement of TCh, LDL-Ch, VLDL-Ch, Tg and reduction in HDL-Ch may be related to drug-induced lipid profile alteration[51-53], though changes in lipoprotein level may also occur due to drug-induced protein metabolism[54]. The results further corroborated with peroxidation induction potential of drug that caused elevated levels of TCh, Tg, LDL-Ch and VLDL-Ch and reduction in HDL-Ch level[32,34]. Studies[31-34] also showed that the liposuppressive effect of the antioxidant; ascorbic acid plays a beneficial role to control abnormal alteration in lipid profile including increased lipid peroxidation phenomena. The results also demonstrated the reduction in PL and TL contents in all animal groups. In case of D-group, the reduction in PL and TL might be due to binding of the drug with lipids[55]. These observations could be corroborated with the work that efficient binding capacity of the drugs to PL and TL might cause significant reduction of those parameters, and vis a vis increased level of peroxidation parameters[56,57].

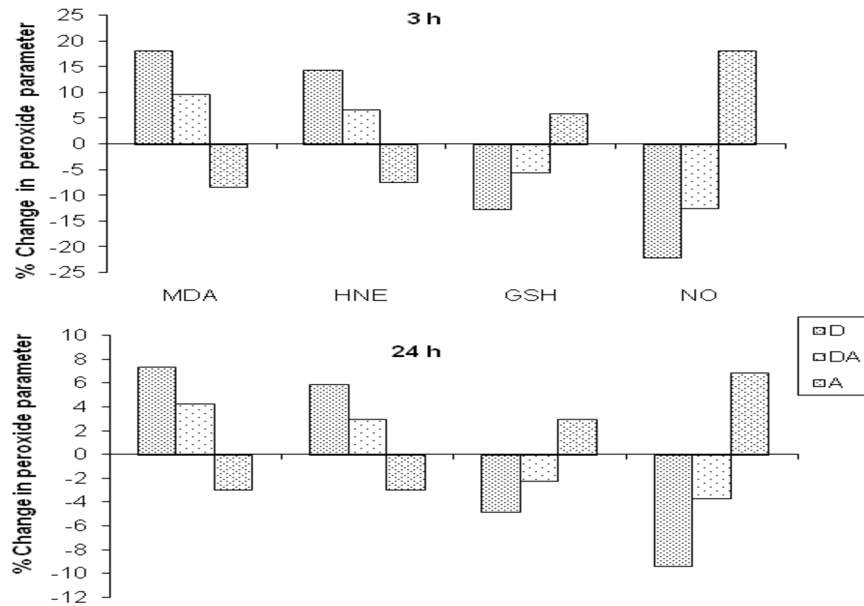

Fig. 1: Effect of ascorbic acid on amikacin-induced lipid peroxidation parameters.

D, DA and A denote amikacin treated, amikacin-ascorbic acid treated and only ascorbic acid treated animals respectively, MDA: Malondialdehyde, HNE: Hydroxy nonenal, GSH: Reduced glutathione, NO: Nitric oxide.

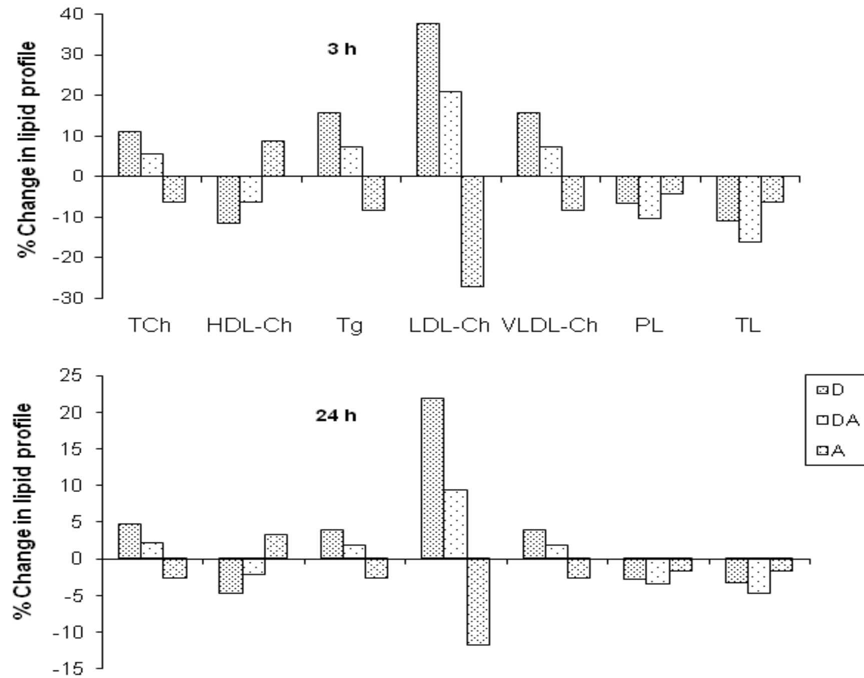

Fig. 2: Effect of ascorbic acid on amikacin-induced lipid profiles.

D, DA and A denote amikacin treated, amikacin-ascorbic acid treated and only ascorbic acid treated animals respectively, TCh: Total cholesterol, HDL-Ch: HDL Cholesterol, Tg: Triglyceride, LDL-Ch: LDL Cholesterol, VLDL-Ch: VLDL Cholesterol, PL: Phospholipid, TL: Total lipid.

The drug, amikacin is a widely used aminoglycoside antibiotic to treat many bacterial infections[19] has potential lipid peroxidation induction capacity. The drug-induced alteration in lipid profile may be correlated with its lipid peroxidation phenomena. Some of the toxicities of amikacin, which are found to be free radical associated[20-24] may occur due to amikacin-induced lipid peroxidation, can be effectively controlled on co-administration of ascorbic acid, having free radical scavenging property[27,28]. Antioxidant co-therapy approach may be beneficial in reducing drug-induced lipo-adverse reactions, and it can be a useful tool for the physicians to enhance patient compliance and to improve therapeutic index of the drug. Any ultimate conclusion can only be drawn after a detail study using more specific parameters.

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