

Research Article**Levofloxacin-induced dyslipidemia in male albino rats**Abiodun Olusoji Owoade¹, Augustine Ikhueoya Airaodion², Adewale Adetutu¹, Oluwatoyin Diana Akinyomi¹¹Department of Biochemistry, Faculty of Basic Medical Science, Ladake Akintola University of Technology, Ogbomoso, Oyo State, Nigeria²Department of Biochemistry, Faculty of Biological Science, Federal University of Technology, Owerri, Imo State, Nigeria

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Abstract

Objective: This study was aimed to investigate whether levofloxacin perturb lipid metabolism. **Materials and methods:** Therapeutic dose (7.14 mg/kg body weight) of levofloxacin was administered intraperitoneally 12 hourly to rats for 5 and 10 days. Twenty-four hours after the last levofloxacin treatment and 7 days after levofloxacin withdrawal (for a group of rats), blood and other tissues (liver, kidney, brain, heart, lung and spleen) were removed from the animals after an overnight fast and analysed for their lipid contents. **Results:** Levofloxacin administration resulted in dyslipidemia in different compartments investigated. Plasma and erythrocyte dyslipidemia were characterised by increased concentrations of phospholipid, free fatty acids (FFA) and depletion of cholesterol. It also resulted in kidney and heart cholesterogenesis, while spleen and lung cholesterol were reduced. Liver cholesterol was unaffected. Administration of the drug equally produced phospholipidosis in the kidney, lung and liver while brain and heart phospholipids decreased. Lipoprotein abnormalities were reflected as up-regulation of HDL triglyceride and phospholipid as well as down-regulation of VLDL-LDL cholesterol and phospholipid. Hypertriglyceremia was the hallmark of dyslipidemia in the plasma, kidney, lung and brain while in the heart and erythrocyte triglyceride level was decreased. **Conclusion:** The distortion observed in the lipid profile in most of the compartments of the animals studied, suggest that induction of dyslipidemia might represent additional adverse effects of levofloxacin.

Keywords: Levofloxacin, lipid metabolism, cholesterogenesis, phospholipidosis, dyslipidemia

Introduction

Levofloxacin [(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid] is a broad spectrum antibiotic of the fluoroquinolone drug class (Nelson et al., 2007). Levofloxacin is active against both Gram-positive and Gram-negative bacteria. Fluoroquinolones interfere with bacterial DNA metabolism by inhibiting two bacterial enzymes—DNA gyrase and topoisomerase IV— which are critical to DNA replication, transcription, repair, and recombination (Hooper, 1999).

Levofloxacin is well tolerated and generally considered safe, but despite this, several adverse effects is associated with it

(Renata, 2004; Gunduz et al., 2006; Domínguez et al., 2009). Adverse effects of levofloxacin range from peripheral neuropathy, tendon damage, heart problems, Stevens-Johnson syndrome and rhabdomyolysis (muscle wasting) to major allergic reactions including photo-sensitivity and anaphylactoid reactions (Renata, 2001; Renata, 2004; Gunduz et al., 2006; Takahama et al., 2005). Other adverse effects include hypoglycemia, myasthenia crisis, acute pancreatitis, loss of vision, abdominal pain, malaise, and fever (Korzets et al., 2006; Renata, 2007; Domínguez et al., 2009).

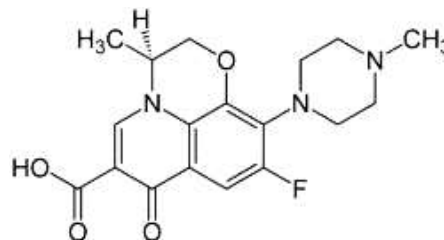


Figure 1. Chemical Structure of Levofloxacin

Although the adverse effects of levofloxacin reported above

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are well documented, but till date, there is dearth of information on the effects of levofloxacin on lipid metabolism. The effect of the drug on lipid metabolism is important because lipids play a major role in the pathogenesis and progression of many disease conditions. In addition, dyslipidemia is presently a major factor to be considered in assessing safety of new drugs and earlier approved drugs. Therefore, the present study was an attempt to investigate the effects of therapeutic doses of levofloxacin on lipid metabolism.

Materials and methods

Chemicals

Levofloxacin was a product of Sigma-Aldrich, Missouri, USA. All other chemicals used in this study were of the purest grade available and were obtained from British Drug House (BDH) Chemicals Limited, Poole, England and Sigma-Aldrich, Missouri, U. S. A.

Animals and treatment

Thirty-five (35) male wistar strain albino rats with body weights between 200 and 220 g were bought from the University of Ibadan animal house. They were housed in Ladoke Akintola University of Technology, (LAUTECH) animal house. They were allowed fourteen (14) days to acclimatize before the commencement of drug administration. The animals were maintained on a standard pellet diet throughout the acclimatization and administration period. At the start of experiments (Day 0), 5 animals were sacrificed to obtain baseline data and the remaining animals were divided into 6 groups of 5 animals each. Three groups were treated with levofloxacin at 7.14 mg/kg body weight, 12 hourly for 5 and 10 days respectively. Levofloxacin was constituted in saline solution, prepared fresh and administered in a total volume of 0.1 ml through the intraperitoneal route. Control animals received saline solution at a dose of 7.14 mg/kg body weight 12 hourly. During the experiment, the animals were allowed free access to food and distilled water. At the end of the levofloxacin treatment and 7 days after the discontinuation of the levofloxacin, blood was collected from the animals into heparinised tubes by cardiac puncture under light ether anaesthesia after an overnight fast. Liver, kidney, brain, heart, lung and spleen were removed from the animals for biochemical analyses. Blood samples were centrifuged to separate plasma and red blood cells. All samples were stored at -20°C until analysed.

Biochemical Analysis

Plasma lipid profiles

Plasma concentrations of total cholesterol and triglycerides were determined with commercial kits (CYPRESS® Diagnostics, Langdorp, Belgium.). HDL cholesterol and

triglycerides were determined in plasma with same commercial kits for total cholesterol and triglycerides after very low density lipoproteins (VLDL) and LDL were precipitated with heparin-MnCl₂ solution (Gidez et al., 1982). Total phospholipids in plasma were extracted with chloroform-methanol mixture (2:1, v/v) as described by Folch et al. (1957). Phospholipid content was then determined as described by Stewart (1980). Briefly, an aliquot of the phospholipid extract was evaporated to dryness at 60°C. After cooling, 2 ml of chloroform was added to the dried lipid extract and vortexed. Ammonium ferrocyanide (2 ml) was then added and the mixture vortexed for 1 min. They were left for 10 min for the phases to separate. The chloroform layer was taken and absorbance read at 488 nm. Phospholipid concentrations were then determined using a phospholipid standard as reference.

Free fatty acids (FFA) in plasma were determined according to the method of Soloni and Sardina (1973) as modified by Brunk and Swanson (1981). Briefly, to 100 µl of plasma was added 300 µl of copper reagent and 2 ml of chloroform. This was shaken with a vertical shaker for 10 min and centrifuged. After centrifugation, the chloroform layer was removed and to this was added 1 ml of cuprizone and 100 µl of ammonia reagent. The contents were shaken briefly by hand and absorbance read at 620 nm 10 min after adding ammonia reagent. A standard curve of palmitic acid taken through the same procedure was used to calculate the concentrations of FFA in the plasma samples.

Erythrocyte lipid profile

Because the Folch extraction (1957) produced lipid extracts which were highly pigmented, an improved procedure for the extraction of lipids from erythrocytes using chloroform-isopropanol (7:11, v/v) described by Rose and Oklander (1965) was employed. For the determination of cholesterol, an aliquot of the chloroform-isopropanol extract was evaporated to dryness at 60°C. Triton X-100/ chloroform mixture (1:1, v/v, 20 µl) was added to resolve the lipids and again the solvent was evaporated. Then 1 ml of commercially available cholesterol kit reagent (CYPRESS® Diagnostics, Langdorp, Belgium.) was added and vortexed. After incubation in the dark at room temperature for 30 min, cholesterol content was determined by colorimetry (Eder and Kirchgessner, 1994). Determination of total phospholipids and free fatty acids in the chloroform-isopropanol extract of the erythrocyte followed the same procedure as described for plasma (Stewart, 1980).

Organ lipid profiles

Lipids were extracted from the organs (liver, kidney, brain,

heart, lung and spleen) as described by Folch et al. (1957). After washing with 0.05M KCl solution, aliquots of the chloroform-methanol extract were then used for the determination of cholesterol, triglycerides and phospholipids concentrations. Cholesterol was determined in an aliquot of the chloroform-methanol extract of each organ as described for erythrocytes while determination of phospholipids followed the same procedure as described for plasma. Triglyceride concentrations in aliquots of the chloroform-methanol extracts of each organ were determined following the procedure described by Kriketos et al. (2003). Briefly, an aliquot of the chloroform-methanol extract in Eppendorf tubes was evaporated to dryness at 60°C. After cooling, 200 µl of ethanol (97%) was added to the tube to re-suspend the triglyceride. Then 1 ml of commercially available triglyceride kit (CYPRESS® Diagnostics, Langdorp, Belgium) was added and vortexed. After incubating in the dark at room

temperature for 20 min, triglyceride content was determined spectrophotometrically.

Statistical Analysis

Results are expressed as mean S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism software Version 5.00 and p values 0.05 were considered statistically significant.

Results

Results of the study presented in figure 2, 3 and 4 depict the effects of levofloxacin on plasma (Figure 2), HDL (Figure 3) and LDL+VLDL (Figure 4) lipid profiles of the animals. Administration of levofloxacin resulted in various degrees of dyslipidemia in these compartments. In the plasma,

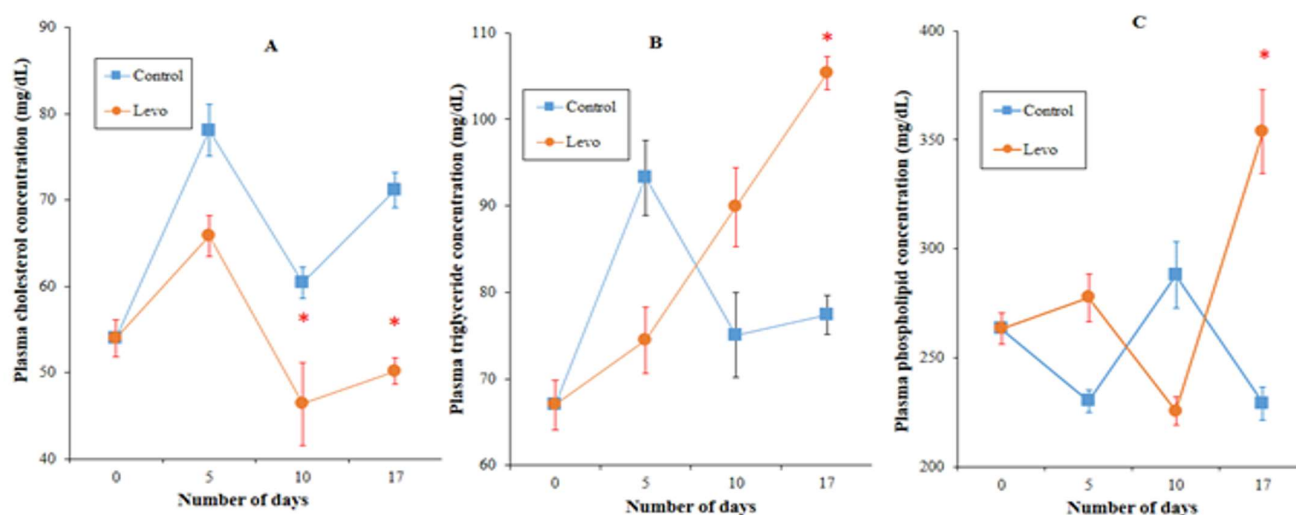


Figure 2: Effect of levofloxacin on plasma lipid profiles of the animals: (A) Plasma cholesterol level, (B) Plasma triglyceride level, (C) Plasma phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

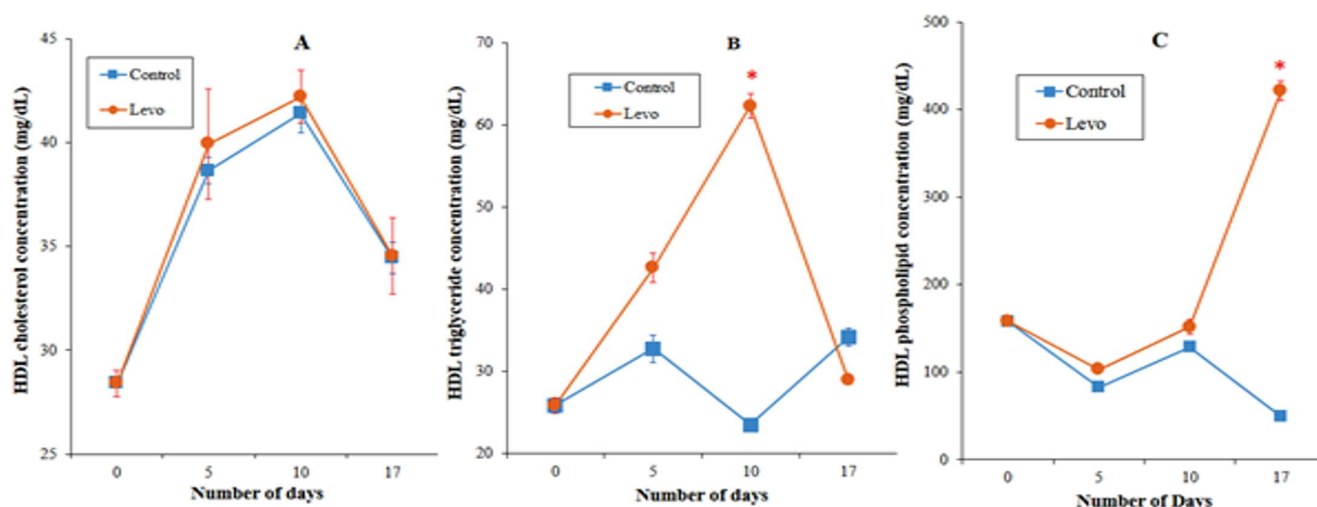


Figure 3: Effect of levofloxacin on HDL lipid profiles of the animals: (A) HDL cholesterol level, (B) HDL triglyceride level, (C) HDL phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

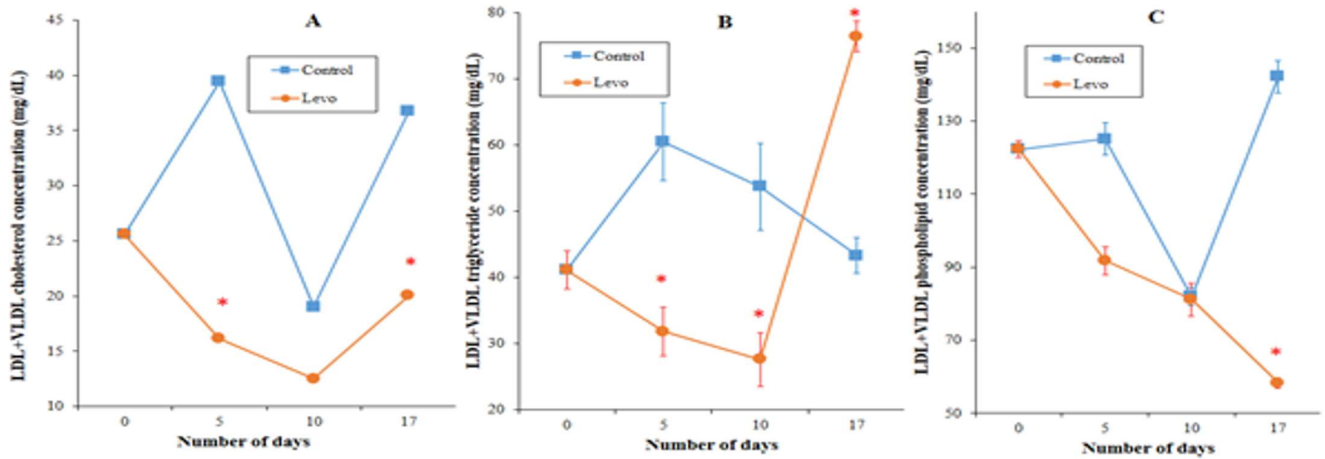


Figure 4: Effect of levofloxacin on LDL + VLDL lipid profiles of the animals: (A) LDL + VLDL cholesterol level, (B) LDL + VLDL triglyceride level, (C) LDL + VLDL phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

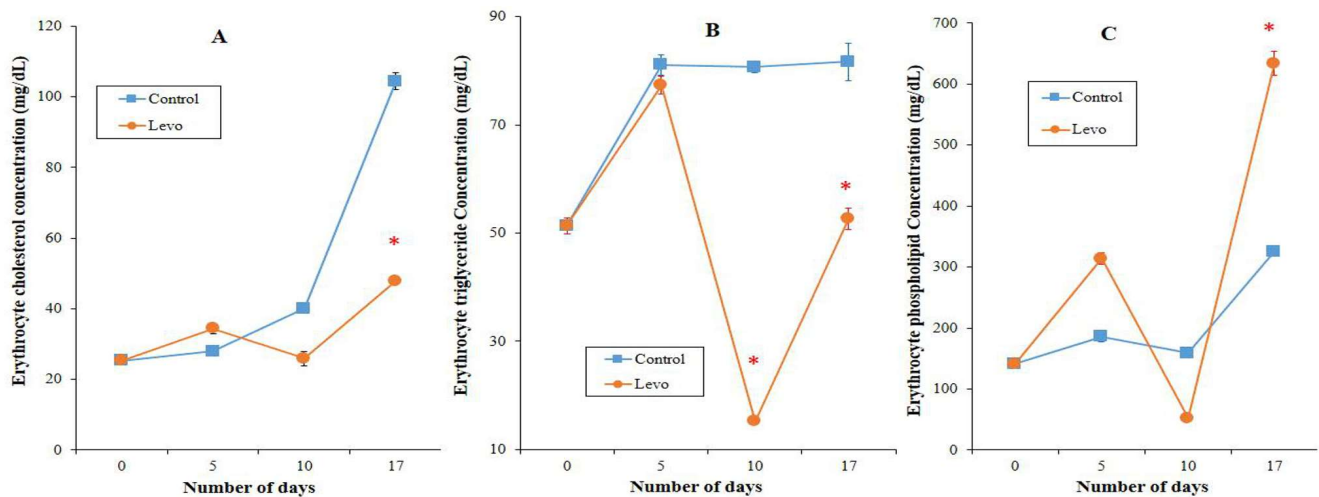


Figure 5: Effect of levofloxacin on erythrocyte lipid profiles of the animals: (A) Erythrocyte cholesterol level, (B) Erythrocyte triglyceride level, (C) Erythrocyte phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

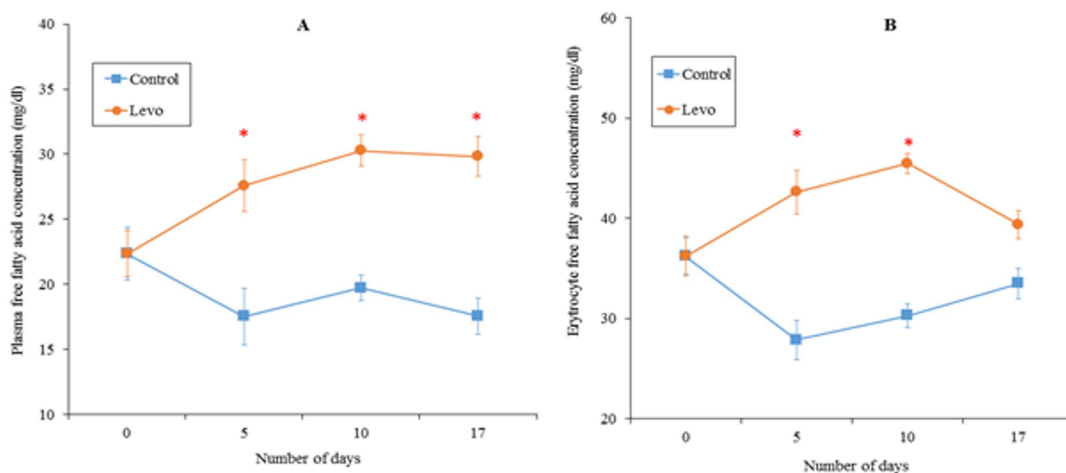


Figure 6: Effect of levofloxacin on plasma (A) and erythrocyte (B) free fatty acid of the animals. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

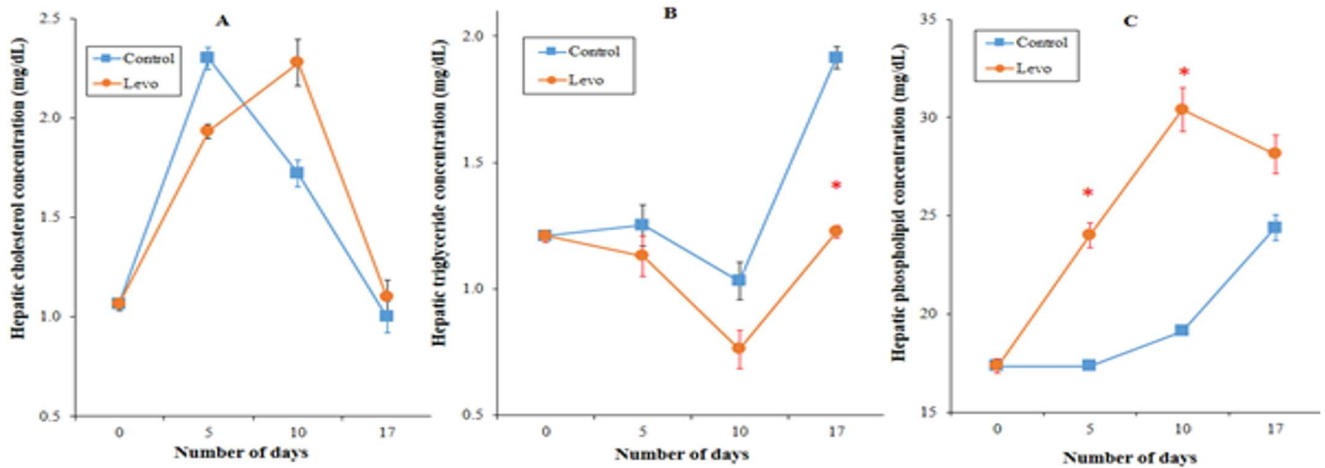


Figure 7: Effect of levofloxacin on hepatic lipid profiles of the animals: (A) Hepatic cholesterol level, (B) Hepatic triglyceride level, (C) Hepatic phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

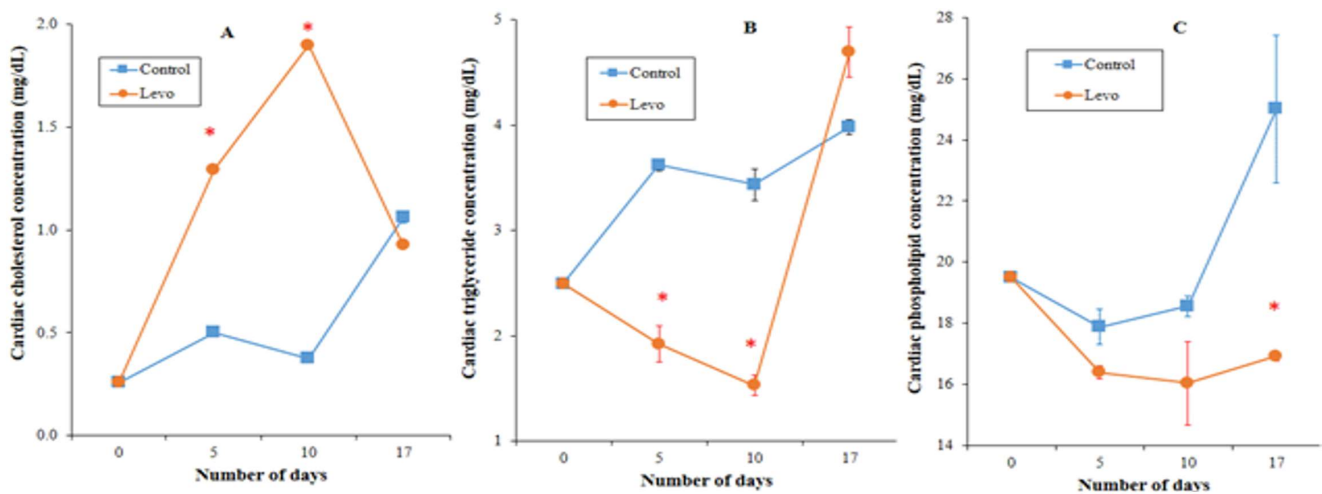


Figure 8: Effect of levofloxacin on cardiac lipid profiles of the animals: (A) Cardiac cholesterol level, (B) Cardiac triglyceride level, (C) Cardiac phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

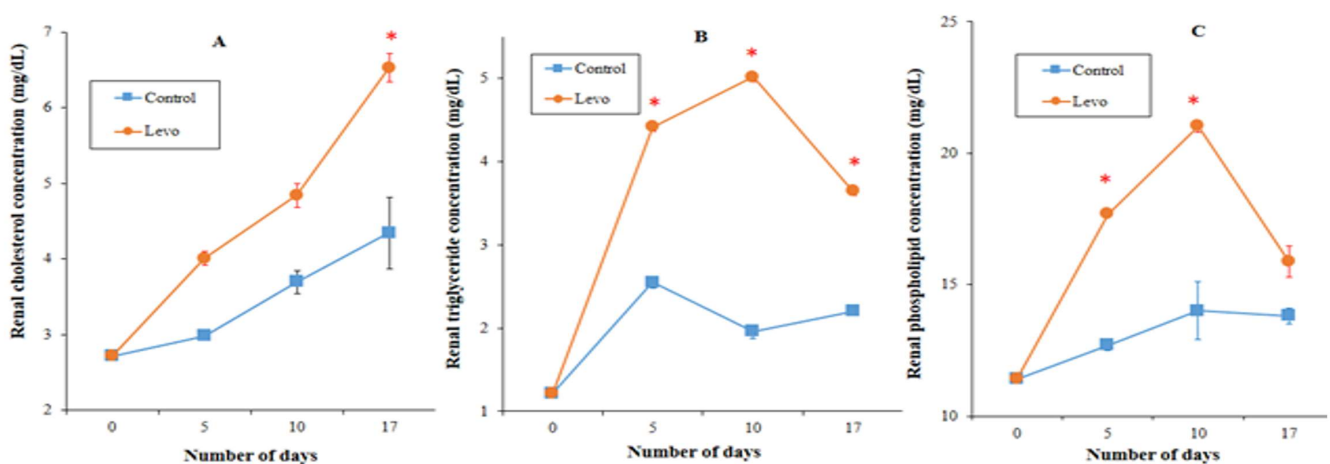


Figure 9: Effect of Levofloxacin on renal lipid profiles of the animals: (A) Renal cholesterol level, (B) Renal triglyceride level, (C) Renal phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

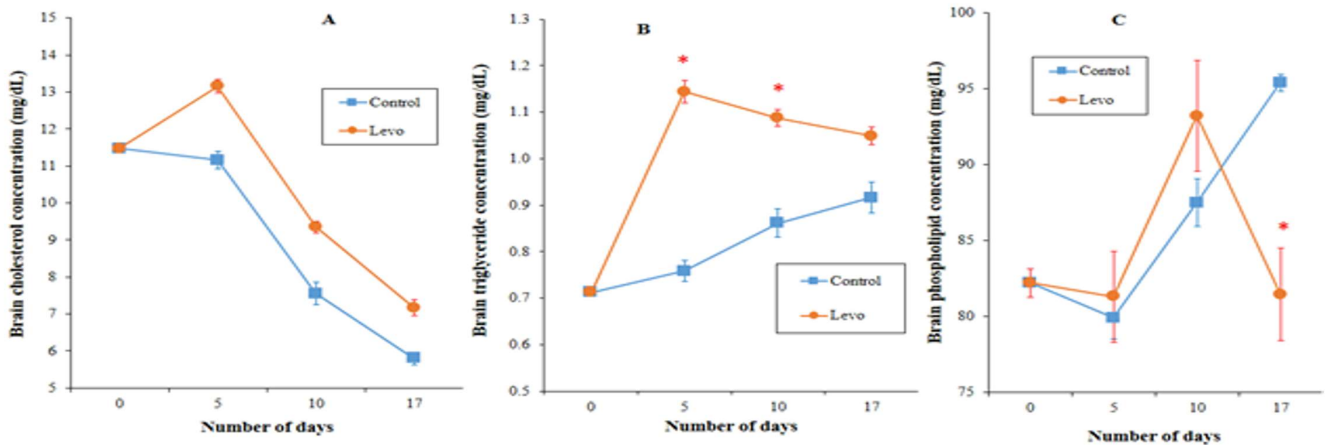


Figure 10: Effect of levofloxacin on brain lipid profiles of the animals: (A) Brain cholesterol level, (B) Brain triglyceride level, (C) Brain phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

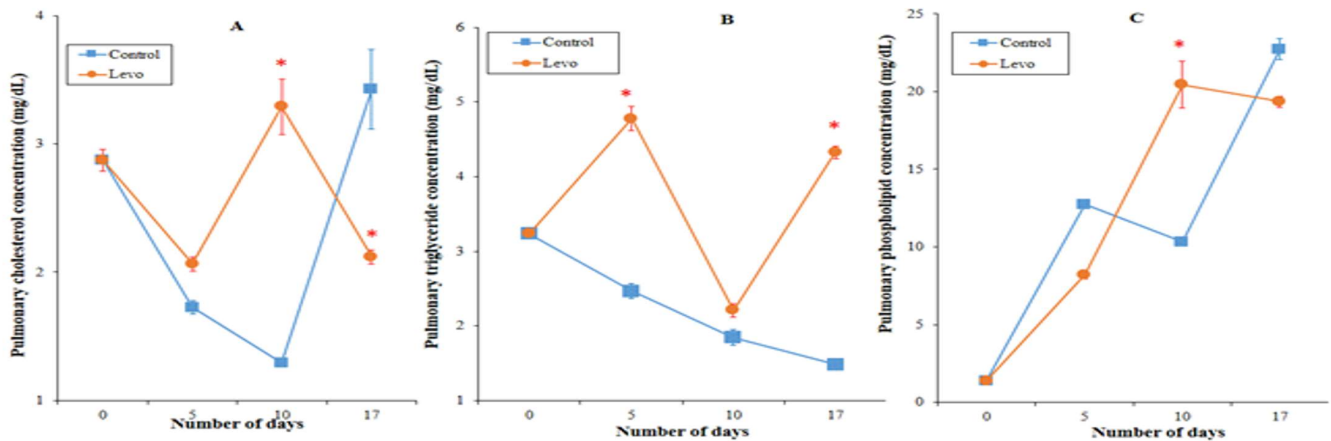


Figure 11: Effect of Levofloxacin on pulmonary lipid profiles of the animals: (A) Pulmonary cholesterol level, (B) Pulmonary triglyceride level, (C) Pulmonary phospholipid level. Each point represents mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

administration of levofloxacin significantly decreases ($p < 0.05$) cholesterol concentrations of all the animals while concentrations of triglyceride and phospholipid were significantly increase in the animals when levofloxacin administration was discontinued for 7 days.

Administration of levofloxacin had no significant effect on HDL cholesterol concentrations. Administration of levofloxacin for five days did not produce any significant change in HDL triglyceride and phospholipid concentrations but produced 2.5-fold increase in HDL triglyceride concentration after 10 days of levofloxacin administration. However, when levofloxacin administration was discontinued for seven days, concentration of HDL triglyceride return to normal while HDL phospholipid concentration was significantly increase by 8 fold.

In the LDL+VLDL fraction (Figures 4A, B and C), administration of levofloxacin resulted in a significant decrease ($p < 0.05$) in cholesterol, triglyceride and phospholipid concentrations. While decrease in cholesterol and phospholipid

concentrations was sustained 7 days after discontinuing the drug (Figures 4A and C), triglyceride concentration rebound and increase significantly by 81.39%, 7 days after the antibiotic was withdrawn (Figure 4B).

The effects of levofloxacin on the lipid profiles of erythrocyte as well as FFA in plasma and erythrocytes are depicted in figures 5 and 6. Levofloxacin treatment for five and ten days did not produce any significant change in erythrocyte cholesterol, triglyceride and phospholipid concentrations except erythrocyte triglyceride concentrations which was decrease significantly by 5 fold after ten days of levofloxacin administration. When the animals were allowed to recover for seven (7) days after levofloxacin treatment, erythrocyte cholesterol and triglyceride concentration were significantly decrease while erythrocyte phospholipid was significantly increase by 2 fold. Levofloxacin administration significantly increased FFA concentration in the plasma and erythrocytes throughout the duration of this study.

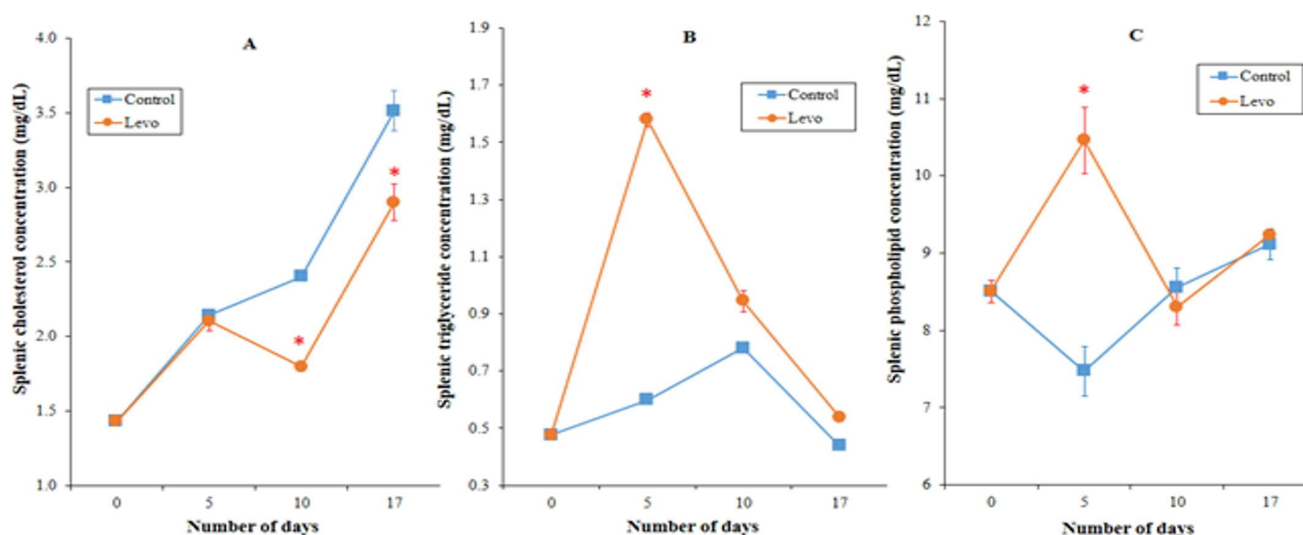


Figure 12: Effect of levofloxacin on splenic lipid profiles of the animals: (A) Splenic cholesterol level, (B) Splenic triglyceride level, (C) Splenic phospholipid level. Each point represents Mean \pm SEM of 5 animals. The significant difference ($p < 0.05$) between the levofloxacin treated groups and their respective control groups at 5, 10 and 17 days are represented by *.

The mean values of the organ lipid profiles are shown in figures 7, 8, 9, 10, 11 and 12. Administration of levofloxacin resulted in a significant increase ($p < 0.05$) in renal, brain, lung and spleen triglyceride concentrations while liver and heart triglyceride concentration were decrease. The increase ranged from 53% in brain, Figure 10B to 300% in spleen, Figure 12B while the decrease ranged from 41% in liver, Figure 7B to 57% in heart, Figure 8B.

Cholesterol concentration was also distorted by administration of levofloxacin. In the liver (Figures 7A), antibiotic treatment did not affect cholesterol concentrations. In the heart, kidney and brain, levofloxacin treatment resulted in the induction of cholesterologenesis (Figures 8A, 9A and 10A), whereas in the lung induction of cholesterologenesis was only at 10 days of levofloxacin treatment (Figures 11A). In the spleen however, levofloxacin treatment resulted in the reduction of cholesterol concentration (Figure 12A).

The mean phospholipid concentrations in the organs as depicted in figures 7, 8, 9, 10, 11 and 12 indicate that levofloxacin treatment resulted in a marked hepatic (Figures 7C) and renal phospholipidosis (Figures 9C) and a transient brain (Figures 10C), lung (Figure 11C) and splenic (Figure 12C) phospholipidosis whereas cardiac (Figures 8C) phospholipid concentrations decreased. Hepatic phospholipids increased by 76% (Figure 7C) while renal phospholipids increased by 65% (Figure 9C) respectively.

Discussion

The findings of this study indicate that levofloxacin administration is associated with perturbations in lipid homeostasis in organs, lipoproteins, plasma and erythrocytes. Compared to control animals, perturbations are characterised by

high circulating FFA, increased renal, brain, splenic and pulmonary lipids, hepatic phospholipidosis and cardiac cholesterologenesis as well as depletion of cardiac triglyceride and depletion of cholesterol in plasma, LDL + VLDL and erythrocyte fraction.

Under physiological conditions, fat reserves of the mammalian body are stored as droplets of triglycerides in the adipose tissue. This triglyceride is then hydrolysed to FFA and glycerol with subsequent mobilisation of FFA into the plasma. This hydrolysis prior to release of FFA occurs within the adipose tissue and is catalysed by a triglyceride lipase distinct from lipoprotein lipase which occurs outside the adipose tissue cell (Newsholm and Start, 1981). Release of FFA into the plasma is followed by FFA uptake into tissues (including liver, heart, kidney, muscle and lung), but not readily by the brain (Botham and Mayes, 2006; Kurokawa et al., 1985), where they are oxidised or used in the synthesis of triglycerides in that tissue (Donnelly et al., 2005; Mermier and Baker, 1974).

In this study, we observed a steady increase in plasma FFA in levofloxacin treated animals. This suggests levofloxacin-induced activation of triglyceride lipase resulting in increased triglyceride hydrolysis in the adipose tissue and subsequent increased mobilisation of the FFA into the plasma. The physiological consequences of this elevated plasma FFA could be viewed from the metabolic roles of FFA. While this elevated plasma FFA should provide an immediate substrate for triglyceride synthesis as well as the source of available fuel for the tissues and also the necessary signal for tissues to oxidise them (Donnelly et al., 2005; Mermier and Baker, 1974), data in figures 9, 10, 11 and 12 indicate that levofloxacin promote uptake of FFA by the

tissues with a considerable amount of the FFA directed towards the synthesis of triglycerides in the levofloxacin treated animals, hence the accumulation of triglycerides in the tissues. Although, liver have a limited capacity for triglyceride storage, tissues like kidney, brain, lung and spleen accumulated triglyceride (Figures 9, 10, 11 and 12). This further suggests that levofloxacin induced a dysfunction of triglyceride degradation resulting from insufficient mitochondrial β -oxidation of FFA, hence compromising energy production in the tissues of the levofloxacin-treated animals.

The key enzyme in the distribution of circulating lipids between organs is lipoprotein lipase (LPL), an enzyme located on the walls of blood capillaries (Goldberg et al., 2012). The role of LPL in lipoprotein metabolism is well known. Since majority of the circulating FFAs are present as triglycerides in lipoproteins, hydrolysis of this triglyceride by LPL is an important determinant of overall fatty acid uptake and β -oxidation in the tissues (Pulinilkunnil and Rodrigues, 2006; Lee and Goldberg, 2007). High circulating FFA (as observed in this study) is known to inhibit the activity of LPL (Saxena et al., 1989). Significant reduction in the activity of LPL probably caused the accumulation of triglycerides in the plasma observed in the study.

Administration of levofloxacin resulted in depletion of cardiac triglycerides and phospholipids in this study. Physiologically, cardiac myocytes have regulatory pathways that regulate lipid metabolism. The myocardium has labile stores of triglyceride that serve as an endogenous source of FFAs (Saddik and Lopaschuk, 1991; Stanley et al., 2005). Intramyocardial triglyceride can be hydrolysed by hormone sensitive lipase and adipose triglyceride lipase (Goldberg et al., 2012; Lopaschuk et al., 2010). Insulin inhibits lipolysis, whereas catecholamines, thyroid hormone and glucagon, accelerate intramyocardial triglyceride degradation (Swanton and Saggerson, 1997). Since the major cardiac triglyceride and phospholipids were depleted as a result of levofloxacin administration, the possibility that levofloxacin might induce overexpression/production of cardiac apolipoprotein B (Apo B) which increased lipid secretion from the heart, cannot be ruled out (Yokoyama et al., 2004).

Administration of levofloxacin resulted in a significant increase of triglyceride in the brain, but the brain cannot take up FFA from the plasma and circulating lipoproteins cannot reach the brain except for small HDL particles due to the blood brain barrier (Kersten, 2014). Most of the lipoproteins in the brain are synthesised by the astrocytes and have been postulated to be responsible for the transfer of lipids within the brain (Postle, 2009; Wang, 2014). Thus, levofloxacin-induced damage to the blood brain barrier might be responsible for the dyslipidemia observed in the brain of the levofloxacin-treated animals (Rosado et al., 2007; Ross et al., 2010).

Results of this study indicate that administration of levofloxacin was associated with renal and pulmonary and cardiac cholesterogenesis. Although, activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase (the rate-limiting enzyme in cholesterol synthesis) was not determined in this study, the enhanced cholesterogenesis may be attributed to levofloxacin-induced activation of HMG-CoA reductase or it may be due to feedback inhibition (Gesquire et al., 1999; Sawada et al., 2005). It may also be due to inhibition of the activity of cholesterol-7 α -hydroxylase, a cytochrome P450 enzyme located in the endoplasmic reticulum. This could limit the biosynthesis of bile acids, which is the only significant route for elimination of cholesterol from the body (Kojima et al., 2004). Since the liver has limited capacity to store lipids, the excess cholesterol and triglycerides are packaged into VLDL particles and secreted into circulation.

Levofloxacin administration resulted in induction of phospholipidosis in liver, kidney and lung in this study. Phospholipidosis is a lipid storage disorder in which abnormal quantities of phospholipids accumulate in various tissues (Joshi et al., 1988; Halliwell, 1997; Reasor and Kacew, 2001; Sawada et al., 2005; Abe et al., 2007). Xenobiotic drugs and chemicals, as well as hormones, cofactors and other agents, may alter the metabolism of the cell and result in phospholipidosis (Joshi et al., 1988; Halliwell, 1997; Reasor and Kacew, 2001; Sawada et al., 2005; Abe et al., 2007). The induction time may be a few days to several months depending on the affinity of the agent for susceptible cells (Halliwell, 1997; Reasor and Kacew, 2001; Sawada et al., 2005; Abe et al., 2007). Induction of phospholipidosis observed in tissues in this study could be as a result of enhanced phospholipid biosynthesis due to enhanced FFA availability, enhanced cholesterogenesis or inhibition of phospholipase (Sawada et al., 2005).

In conclusion, the results of this study indicate that levofloxacin induce a plethora of compartment-specific dyslipidemia and these alterations might contribute to the development of phospholipidosis and cholesterogenesis in tissues which might represent additional adverse effects of levofloxacin.

Conflicts of interest

We declare that we have no conflict of interest.

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