EFFECTS OF TAMOXIFEN ADMINISTRATION ON LIPID PROFILE IN FEMALE ALBINO RATS

ABSTRACT

This study is aimed at investigating the effects of tamoxifen on lipid profile. In order to achieve this, tamoxifen was administered once daily intraperitoneally to the female rats at a dose of 2.07mg/kg body weight for 1 and 2 weeks, while a group of rats was allowed to recover for a week after 2 weeks of tamoxifen administration. Rats in the control group were similarly treated but were given corn oil instead of the drug. Tamoxifen administration caused perturbations of major lipids in the animals. Administration of tamoxifen for 1 week lowered cholesterol and triglycerides levels in the plasma by 23.63 % and 39.74 % respectively and in the low density lipoproteins and very low density lipoproteins (LDL+VLDL) by 79.72 % and 67.59 % respectively, while it increased high density lipoproteins (HDL) cholesterol by 3.44 fold. In the tissues, tamoxifen administration for 2 weeks did not significantly affect cholesterol and triglycerides levels but phospholipid levels in the liver, kidney and heart were lowered by 52.38 %, 42.98 % and 73.82 % respectively, while phospholipidosis was the hallmark of its effect in the spleen. The result of our study affirmed that tamoxifen exerts a favourable effect on lipid profile. The hypocholesterolemic and hypotriglyceridesmia effects of tamoxifen observed in this study may partially explain the decrease in coronary heart disease related mortality seen in patients receiving tamoxifen treatment.

Keywords: Tamoxifen, phospholipidosis, hypocholesterolemic, hypotriglyceridesmia

INTRODUCTION

Tamoxifen, a nonsteroidal anti-oestrogen drug is widely used in the treatment of breast cancer. The anti-oestrogen drug exerts its effect by binding to oestrogen receptors of the tumour cells [l]. It can also be used as an adjuvant treatment in both oestrogen receptor-positive and negative breast tumours [2]. Tamoxifen is a prodrug, that has a relatively little affinity for estrogen receptors. It is metabolized in the liver by the cytochrome P450 isoform CYP3A4 and CYP2D6 into active metabolites, such as N-desmethyl-4-hydroxytamoxifen (endoxifen) and 4-hydroxytamoxifen (afimoxifene) [3], which have 30-100 times further affinity with the estrogen receptor than tamoxifen itself. Tamoxifen has been reported to have various side effects including, hypoglycemia, hypertriglyceridesmia, changes in plasma cholesterol levels, and liver diseases, such as hepatic steatosis and non-alcoholic steatohepatitis [4, 5].

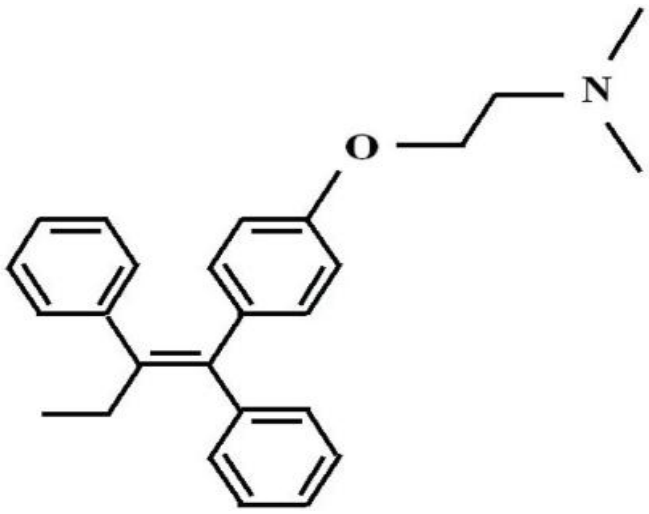

Figure 1. Structure of tamoxifen.

Dyslipidemia is known to be an important risk factor for cardiovascular disease and the effects of tamoxifen therapy on risk factors for cardiovascular disease are of critical importance because myocardial infarction is the major cause of death in women over 60 years of age [6]. Many research studies have reported that adjuvant tamoxifen exhibits different effects on the lipid and liver profile of cancer patients [7-10]. While some studies such as Hernandez *et al.* [7] revealed that there were no associations between tamoxifen and the occurrence of atherosclerotic events, a study by Esteva and Hortobagyi [9] reported that tamoxifen has an overall beneficial effect on lipid profile. However, long-term data from tamoxifen clinical trials have failed to reveal a cardioprotective effect and patients treated with tamoxifen did not experience fewer cardiovascular events compared with those receiving placebo [9]. Till date, information available on the changes in lipid profile induced by tamoxifen is still limited and confusing. Therefore, the present study aims to investigate the effects of tamoxifen administration on risk factors of cardiovascular disease.

MATERIALS AND METHODS

Chemicals

Tamoxifen citrate 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, USA.

Animals and treatment

Twenty–four (24) male Wistar strain albino rats with body weights between 180 and 200 g were obtained from the Experimental Animal Unit of the Faculty of Agriculture, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. All rats were kept in cages in a room maintained at 26–29 °C with a 12-hour light-dark cycle for 3-weeks to acclimatize and were allowed free access to food and water *ad libitum*. The faculty of basic medical science, Ladoke Akintola University of Technology, Ogbomoso, research ethics committee gave ethical approval for the study (FBMS2020/006). All the ethical protocols laid by the committee in line with ARRIVE guidelines, and the national institutes of health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) were followed. The animals were divided into four groups of six animals per group. Three groups were treated with tamoxifen at 2.07 mg/kg body weight, 24 hourly for 7, 14 and 21 days (2 weeks + 1 week recovery), respectively. Tamoxifen was constituted in corn oil and administered in a total volume of 0.1 ml through the intraperitoneal route. Control animals received an equivalent volume of corn oil 24 hourly. During the experiment, the animals were allowed free access to food and distilled water. At the end of the tamoxifen treatment and 7 days after the discontinuation of the tamoxifen, 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 Analyses

Plasma lipid profile

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 the same commercial kits for total cholesterol and triglycerides after very low density lipoproteins (VLDL) and LDL were precipitated with heparin-$MnCl_2$ solution [11]. Total phospholipids in plasma were extracted with the chloroform-methanol mixture (2:1, v/v) as described by Folch *et al.* [12]. Phospholipid content was then determined as described by Stewart [13]. 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 ferrothiocyanate (2 ml) was then added and the mixture vortexed for 1 min. They were left for 10 min for the phases to separate and absorbance of the chloroform layer was read at 488 nm. Phospholipid concentrations were then determined using a phospholipid standard as reference.

Erythrocyte lipid profile

Because the Folch extraction [12] produced lipid extracts that 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 [14] 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 colourimetry [15]. Determination of total phospholipids and free fatty acids in the chloroform-isopropanol extract of the erythrocyte followed the same procedure as described for plasma [13].

Organ lipid profile

Lipids were extracted from the organs (liver, kidney, brain, heart, lung and spleen) as described by Folch *et al.* [12]. 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 the determination of phospholipids followed the same procedure as described for plasma. Triglycerides concentrations in aliquots of the chloroform-methanol extracts of each organ were determined following the procedure described by Kriketos *et al.* [16]. 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 triglycerides. Then 1 ml of commercially available triglycerides kit (CYPRESS® Diagnostics, Langdorp, Belgium) was added and vortexed. After incubating in the dark at room temperature for 20 min, triglycerides 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

Effect of Tamoxifen on Plasma lipid profile of the animals

The results of the study presented in figure 2 depict the effects of tamoxifen on the plasma lipid profile of the animals. Administration of tamoxifen for 1 week did not significantly affect the cholesterol concentration but 2 weeks of administration significantly ($p < 0.05$) increased cholesterol concentration by 49.99 %, when compared with control rats. Also, tamoxifen significantly ($p < 0.05$) decreased triglycerides concentration by 39.74 % and increased phospholipid concentration by 26.58 % after 1 week of tamoxifen administration, when compared with control rats. All the changes in cholesterol, triglycerides and phospholipid concentrations return to normal after tamoxifen administration was discontinued for 7 days.

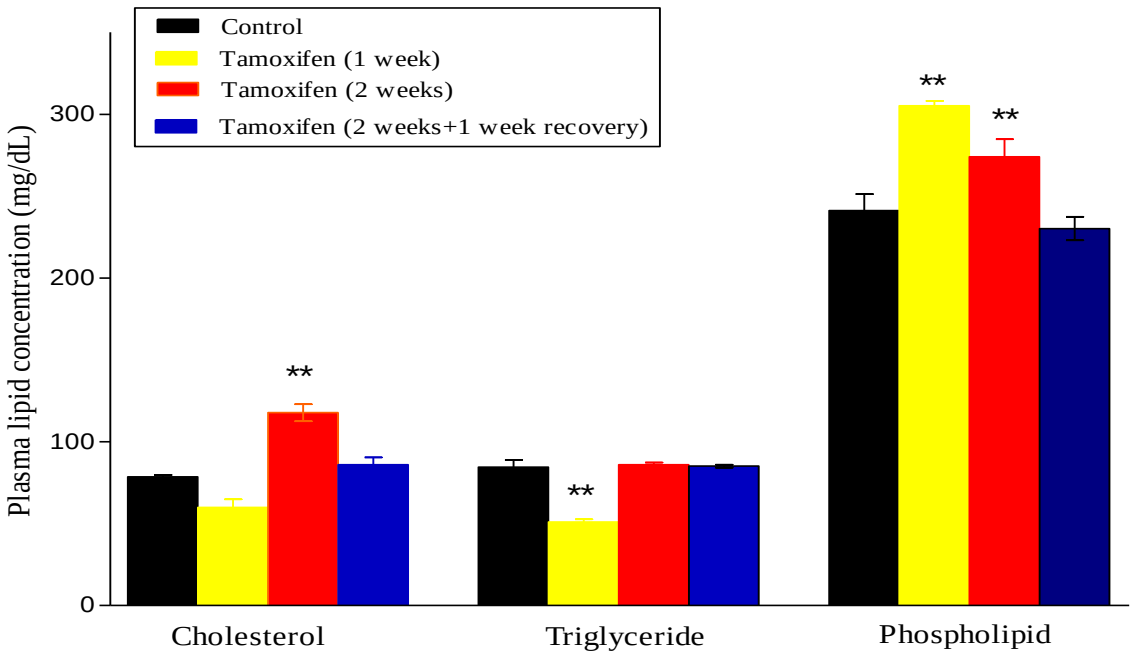

Figure 2. Effect of tamoxifen on plasma lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

Effect of Tamoxifen on HDL lipid profile of the animals

Administration of tamoxifen for 1 week significantly ($p < 0.05$) increased HDL cholesterol concentrations by 3.44 fold when compared with control rats but cholesterol concentration returned to normal after 2 weeks of tamoxifen administration. Also, tamoxifen administration significantly increased HDL triglycerides and phospholipid concentrations. HDL triglycerides concentration increased by 79.17 % and 109.81 % respectively and HDL phospholipid concentration increased by 121.17 % and 76.42 % after 1 and 2 weeks of tamoxifen administration when compared with control rats. The increase in triglycerides and phospholipid concentration was sustained after discontinuation of tamoxifen treatment.

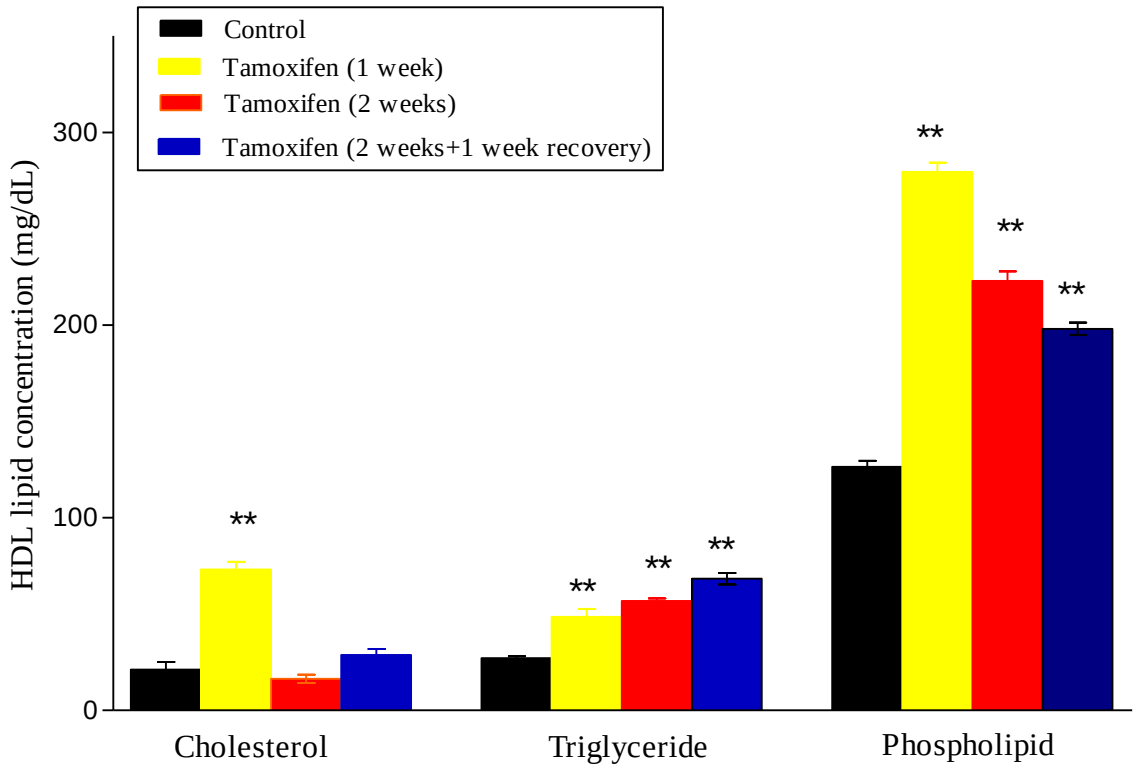

Figure 3. Effect of tamoxifen on HDL lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

Effect of Tamoxifen on LDL+VLDL lipid profile of the animals

The results of the LDL+VLDL fraction is presented in figure 4. Administration of tamoxifen resulted in a significant ($p < 0.05$) decrease in LDL+VLDL cholesterol concentration after 1 week and a significant ($p < 0.05$) increase in cholesterol after 2 weeks when compared with control rats. However, triglycerides and phospholipid concentrations were significantly ($p < 0.05$) reduced after 1 and 2 weeks of tamoxifen administration when compared with control rats. The decrease in LDL+VLDL triglycerides and phospholipid concentrations obtained after 1 and 2 weeks of tamoxifen treatment was sustained while cholesterol concentration returned to normal 1 week after discontinuation of the drug when compared with control rats.

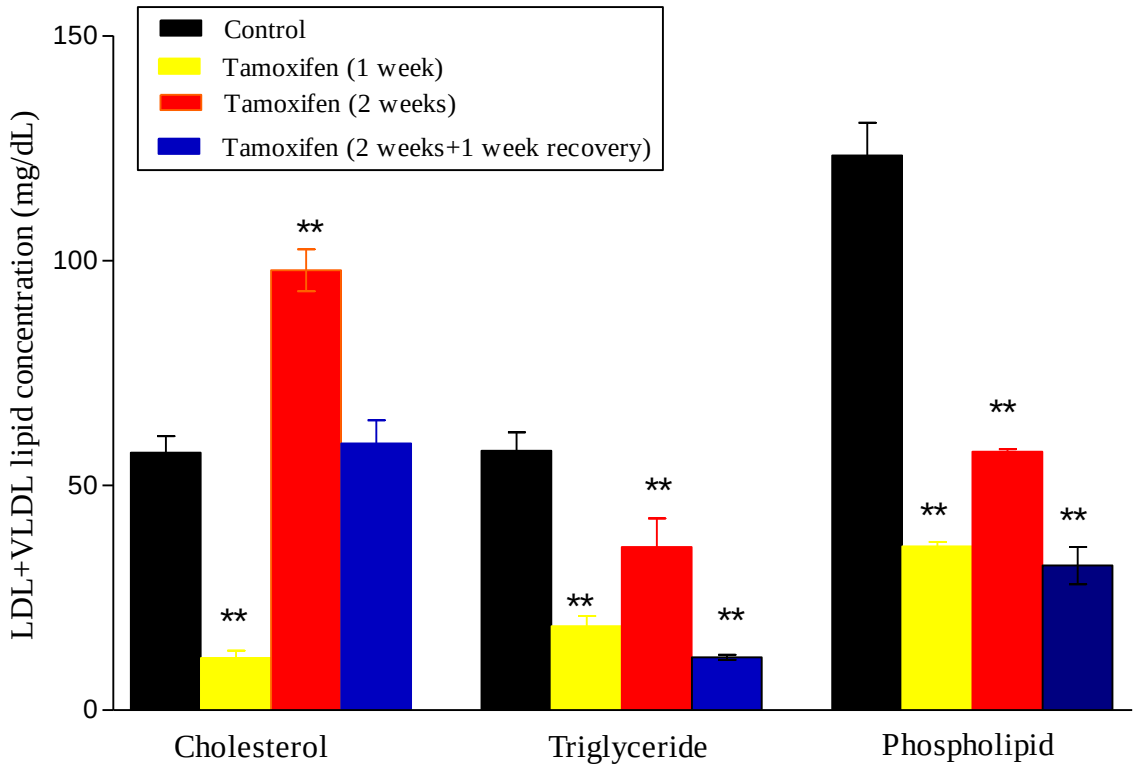

Figure 4. Effect of tamoxifen on LDL+VLDL lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

Effect of Tamoxifen on erythrocyte lipid profile of the animals

The effects of tamoxifen on the lipid profile of erythrocyte is depicted in figure 5. Tamoxifen treatment for 1 and 2 weeks significantly increased cholesterol and triglycerides concentrations while it decreased phospholipid concentration significantly by 9.30 % and 29.35 % respectively when compared with control rats. The increase in cholesterol and triglycerides concentration, as well as decrease in phospholipid concentration, were sustained after 7 days of recovery when compared with control rats.

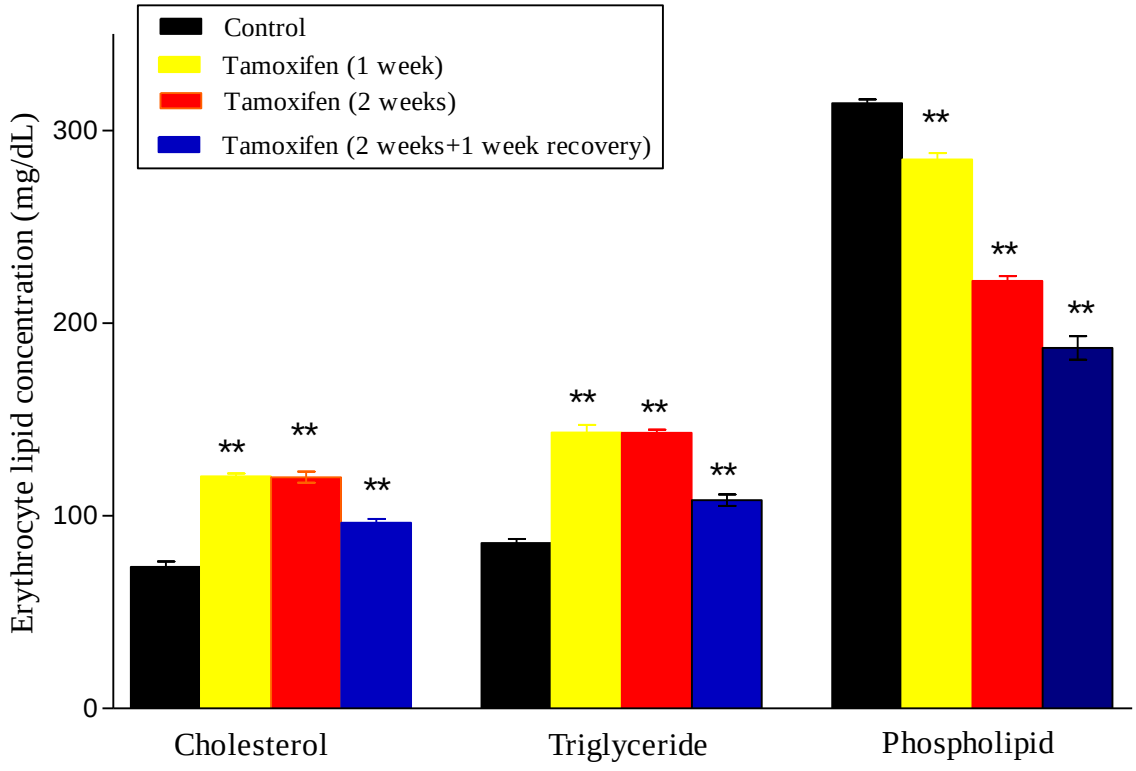

Figure 5. Effect of tamoxifen on erythrocyte lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

Effect of Tamoxifen on liver, kidney and heart lipid profile of the animals

The mean values of the liver, kidney and heart lipid profiles are shown in figures 6, 7 and 8. Administration of tamoxifen for 1 and 2 weeks did not significantly affect cholesterol concentration in the liver, kidney and heart except at 1 week when renal cholesterol was significantly ($p < 0.05$) decreased by 22.58 % when compared with control rats. Tamoxifen administration for 1 and 2 weeks, however, decreased phospholipid concentration significantly ($p < 0.05$) by 53.91 % and 52.38 % in the liver, 40.46 % and 42.98 % in the kidney and 60.65 % and 73.82 % in heart respectively when compared with control rats. The decrease in phospholipid concentration in the 3 tissues was sustained 1 week after discontinuation of the drug when compared with control rats.

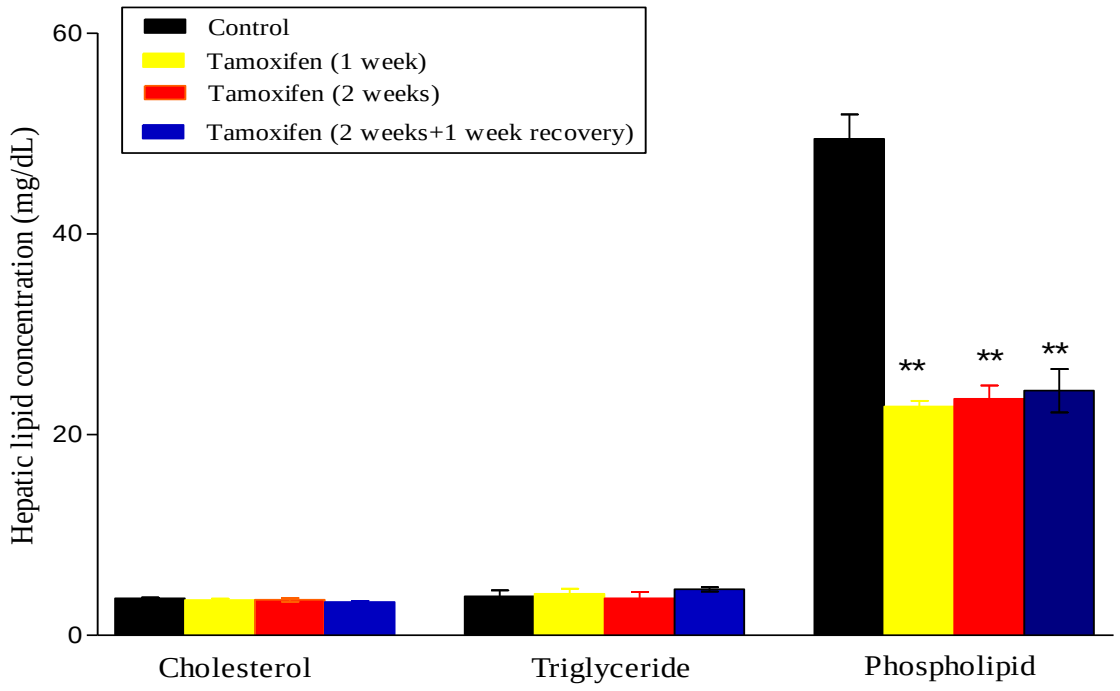

Figure 6. Effect of tamoxifen on hepatic lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

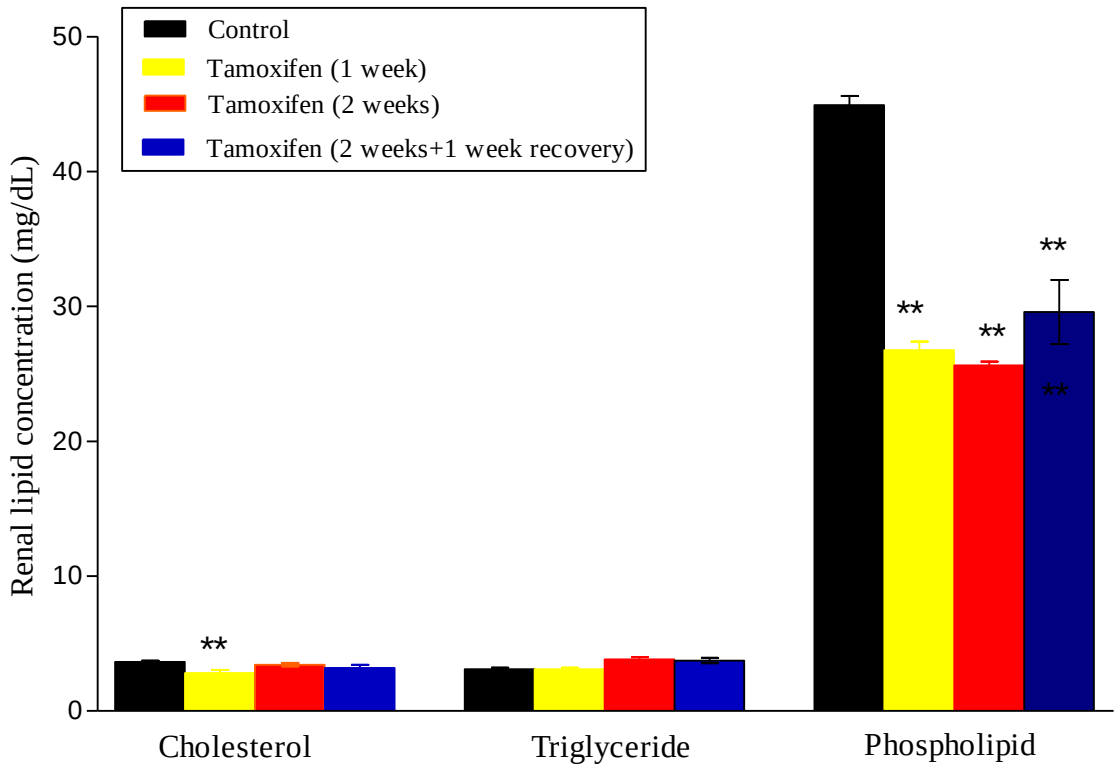

Figure 7. Effect of tamoxifen on renal lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

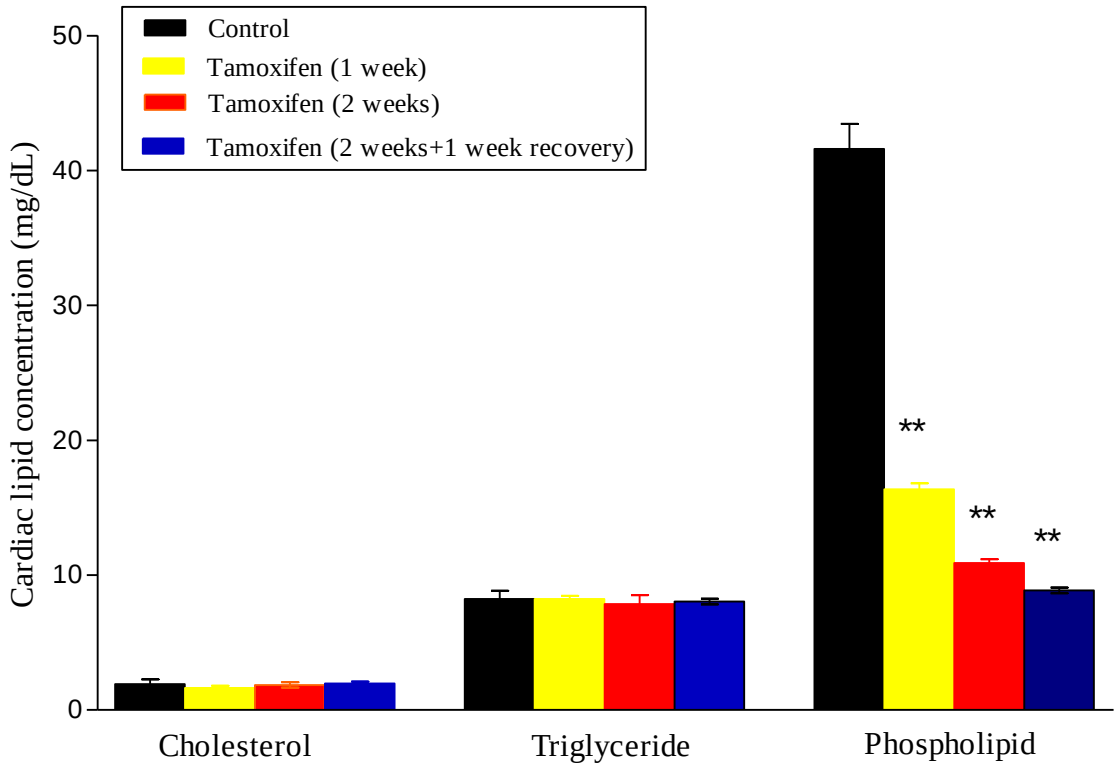

Figure 8. Effect of tamoxifen on cardiac lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

Effect of tamoxifen on brain, lung and spleen lipid profile of the animals

The mean values of the brain, lung and spleen lipid profile are shown in figures 9, 10 and 11. Administration of tamoxifen for 1 and 2 weeks did not significantly affect brain lipids except at 2 weeks when brain phospholipid was significantly ($p < 0.05$) decreased by 27.85 % when compared with control rats (Figure 9). Similarly, pulmonary lipids are not significantly affected by tamoxifen administration except at 2 weeks when cholesterol concentration was significantly ($p < 0.05$) increased by 45.74 % (Figure 10). For splenic lipid, phospholipid concentration was increased by 35.38 % and 41.96 % at 1 and 2 weeks respectively, triglycerides concentration was increased by 25.62 % at 2 weeks while cholesterol was not affected by tamoxifen administration when compared with control rats (Figure 11).

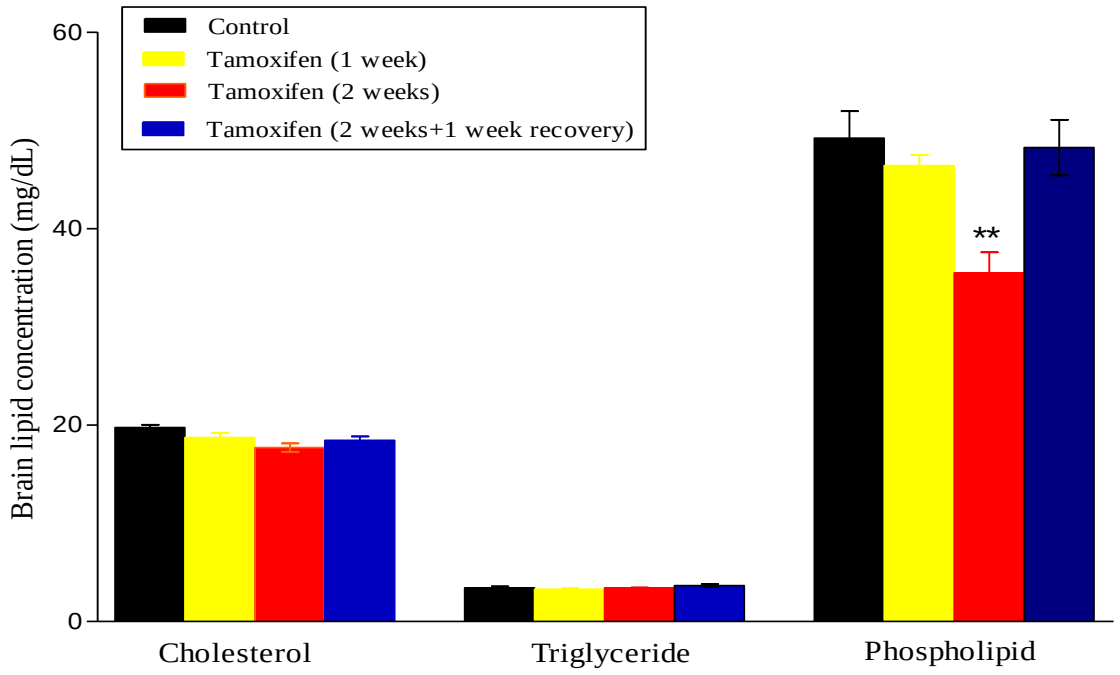

Figure 9. Effect of tamoxifen on brain lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

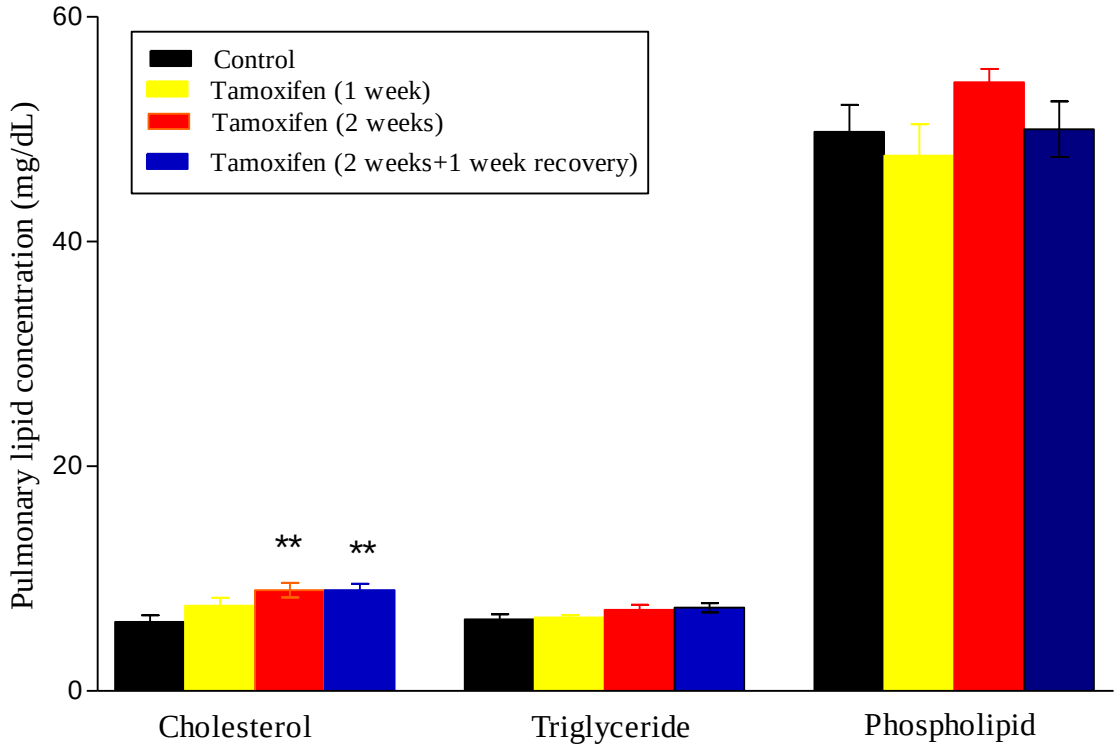

Figure 10. Effect of tamoxifen on pulmonary lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

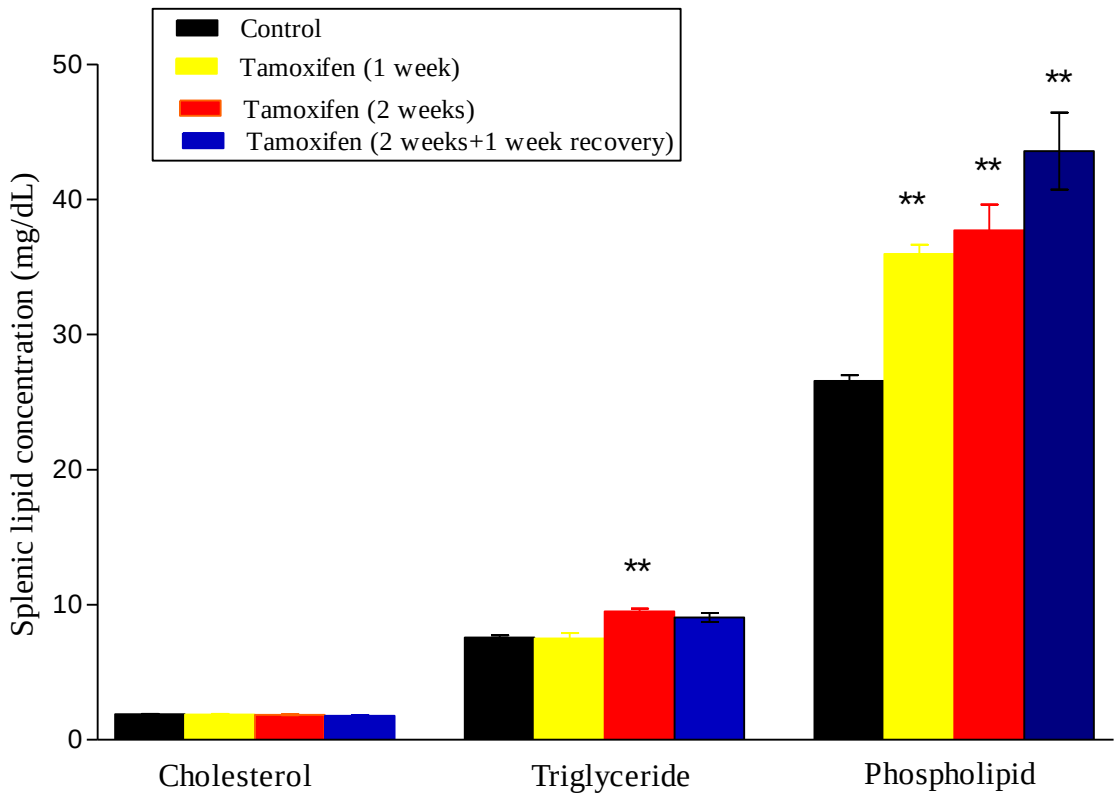

Figure 11. Effect of tamoxifen on hepatic lipid profile of the animals: Each value is mean ± SEM for six rats in each group. The significant difference ($p < 0.05$) between the tamoxifen treated groups and their respective control groups at 7, 14 and 21 days are represented by **.

DISCUSSION

Tamoxifen is an antiestrogenic agent used widely in the treatment and chemoprevention of breast cancer and more recently as a prophylactic for women who have a high risk of breast cancer [5]. However, various side effects including hypoglycemia, hypertriglyceridemia, changes in tissues cholesterol levels, and liver diseases such as hepatic steatosis and non-alcoholic steatohepatitis, have been reported during clinical trials of tamoxifen [4,5]. Many women on chemotherapy for breast cancer are usually placed on tamoxifen for years, this makes them prone to tamoxifen toxicity therefore this work was designed to examine the possible toxicity effect of tamoxifen on lipid metabolism since disruption to lipid metabolism has been postulated to be responsible for the development of several diseases [17,18]. A major finding of this study was that tamoxifen administration perturbs the metabolism of lipids in different compartments of the organism. These perturbations were reflected as up / down regulation of the concentrations of the major lipids (cholesterol, triglycerides, and phospholipids).

Cholesterol is an essential structural component of animal cells that is required to establish proper membrane fluidity and permeability. It serves as a precursor for the biosynthesis of steroid hormones, bile acids and vitamin D [19]. In our work, the administration of tamoxifen resulted in disruption in cholesterol homeostasis in the blood. Tamoxifen after 1 week of administration lowered total cholesterol in the plasma, and LDL+VLDL while it increased HDL cholesterol. This finding is in agreement with previous studies which affirmed that tamoxifen exerts a favourable effect on blood lipid profile [6, 20-23]. The finding is significant because while LDL transports cholesterol to peripheral tissues, HDL in contrast is involved in reverse cholesterol transport, where excess cholesterol in peripheral tissues is transported back to the

liver for excretion into the bile. HDL, in addition, has also been shown to possess other beneficial functions, like antioxidative and anti-inflammatory action [24].

In the tissue, tamoxifen intake significantly lowered renal cholesterol after 1 week, however cholesterol levels return to normal when tamoxifen administration was discontinued for 1 week. Tamoxifen administration does not affect hepatic, cardiac, brain and splenic cholesterol but it results in pulmonary cholesterogenesis. Although the 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 observed in the lung may be attributed to tamoxifen-induced activation of HMG-CoA reductase or it may be due to feedback inhibition [25, 26]. It may also be due to the inhibition of the activity of cholesterol-7α-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 the elimination of cholesterol from the body [27]. Since the liver has a limited capacity to store lipids, the excess cholesterol and triglycerides are packaged into VLDL particles and secreted into circulation.

Triacylglycerols, also called triglycerides are the most abundant dietary lipid compound which is used as storage fuel in the adipose tissue [28]. Administration of tamoxifen significantly increased HDL and erythrocyte triglycerides levels. The key enzyme in the distribution of circulating lipids between organs is lipoprotein lipase (LPL), an enzyme located on the walls of blood capillaries [29]. The role of LPL in lipoprotein metabolism is well known. Since the majority of the circulating FFAs are present as triglycerides in lipoproteins, hydrolysis of this triglycerides by LPL is an important determinant of overall fatty acid uptake and ß-oxidation in

the tissues [30, 31]. Although the level of FFA was not determined in this study, it has been suggested that high circulating FFA can inhibit the activity of LPL [32,33]. Therefore, a significant reduction in the activity of LPL probably caused the accumulation of triglycerides in the HDL and erythrocyte observed in this study.

However, the triglycerides contents of the LDL+VLDL lipoprotein fraction were reduced in tamoxifen administered rats. This may be due to modification of lipid composition induced by tamoxifen and this may explain the reduction in plasma triglycerides levels observed in these animals at 1 week. Inhibition of fatty acid synthesis has been reported to result in decreased formation and secretion of triglycerides in very low density lipoproteins (VLDL) [34]. In addition, a considerable amount of the fatty acid must have been directed into mitochondrial ß-oxidation thereby enhancing hepatic energy production in tamoxifen administered animals at 1 week. Low triglycerides levels observed in the plasma and LDL+VLDL of animals administered tamoxifen may have contributed to the favourable effect of tamoxifen on blood lipid reported by some workers [6, 20-23]. Our study revealed that administration of tamoxifen did not significantly affect the triglycerides content of the tissues except for the spleen triglycerides level which was increased after 2 weeks of tamoxifen administration. Our finding on the effect of tamoxifen on hepatic triglycerides was in complete contradiction to an earlier report by a previous worker who reported that tamoxifen administration resulted in steatosis [35]. The increase in splenic triglycerides content observed in this study returned to normal when the animals were allowed to recover for 1 week suggesting that the dyslipidaemia effect of tamoxifen in the spleen may be related to its prolonged use.

Phospholipids are component of cell membranes where they are organized into bilayer which serves as the framework in which the other components of the membrane are embedded [25]. In this study, administration of tamoxifen resulted in up-regulation of plasma and HDL phospholipid of the animals while its depleted LDL+VLDL and erythrocyte phospholipid levels. Elevation of phospholipid levels in the plasma and HDL may be a result of the stimulation of the endogenous phospholipid synthesis by tamoxifen, through an over-expression of enzymes involved in the synthesis of phospholipids [36]. In addition, there might be an increase in the level of FFA which may result in high availability of fatty acids to form phospholipids.

Administration of tamoxifen did not result in tissue phospholipidosis in this study except in the spleen. Phospholipidosis which is a lipid storage disorder is an abnormal accumulation of phospholipids in various tissues usually caused by xenobiotic drugs, chemicals, hormones, cofactors and other agents [26, 37]. Tamoxifen administration significantly lowered phospholipid levels in the liver, heart and kidney while phospholipid levels in the brain and lung were not significantly affected. Although the activity of phospholipase was not determined in this study, the lowered phospholipid level in the liver, heart and kidney may not be unconnected with the enhancement of phospholipase by tamoxifen which constantly degrades the phospholipid or inhibition of phospholipid biosynthesis due to the unavailability of FFA. The induction of phospholipidosis observed in the spleen in this study could be a result of enhanced phospholipid biosynthesis due to enhanced FFA availability or inhibition of phospholipase [25,38].

Many research studies have reported that tamoxifen exhibits different effects on lipid profile [7-10]. While some studies revealed that there were no associations between tamoxifen and the

occurrence of atherosclerotic events, others reported that tamoxifen has an overall beneficial effect on lipid profile [7, 9]. Our findings in this study revealed that tamoxifen administration caused perturbations of major lipids in the animals. The cholesterol and triglycerides levels of the plasma and LDL+VLDL were lowered by tamoxifen administration although these lipids return to normal levels when rats were allowed to recover for 7 days. The hypocholesterolemic and hypotriglyceridemia effects of tamoxifen observed in this study further give credence to earlier studies that affirmed that tamoxifen administration lower plasma cholesterol and triglycerides levels [6, 20-23]. In addition, our study revealed that tissue's cholesterol and triglycerides levels were largely unaffected by tamoxifen administration while it lowered phospholipid levels in all the tissues except in the spleen compartment. The result of our study affirmed that tamoxifen exerts a favourable effect on lipid profile which is in total agreement with previous studies [6, 20-23].

CONCLUSION

Dyslipidemia is known to be an important risk factor for cardiovascular disease. Several studies have shown a strong independent relation between plasma triglycerides levels, decreased levels of HDL-C, increased FFAs and increased TG-rich lipoproteins and the likelihood of cardiovascular disease. These proatherogenic metabolic abnormalities were not observed in the present study, suggesting that tamoxifen administration may not pose a cardiovascular disease risk.

ETHICAL APPROVAL

The Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Ogbomoso, Research Ethics committee gave Ethical approval for the study (FBMS2020/006). All the ethical protocols laid by the committee in line with the standard accepted principles for laboratory animal handling and care were followed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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