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Dyslipidemic Prospective of Levonorgestrel in the Selected Central Compartments of the Liver, Kidney and Heart

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ABSTRACT

Levonorgestrel, a dedicated emergency contraceptive, is prone to abuse by women of reproductive age group. We evaluated the modulatory effects of varying and repeated doses of the drug on lipid profiles in female rats. Twenty adult female Wistar rats were randomized into four groups. The normal and positive control groups received 0.2 ml/kg normal saline at 48-hour interval and 0.004 mg/kg oral levonorgestrel once at one (1) week interval respectively. While the 2 test groups, received 0.004 mg/kg and 0.008 mg/kg/ oral levonorgestrel at 48-hour interval six (6) weeks. The 48-hour exposure interval was designed to simulate abuse use of levonorgestrel (0.004 mg/kg) at both regular and high (0.008 mg/kg) doses. Levonorgestrel increased significantly hepatic, renal and cardiac total cholesterol irrespective of the administered regimen. Furthermore, there was significant increase in hepatic and cardiac phospholipids but reduced cardiac triglycerides in the test groups. We concluded that frequent use of oral levonorgestrel alters lipid profiles in a murine model.

Keywords: dyslipidemia, levonorgestrel, phospholipids, total cholesterol, triglycerides

INTRODUCTION

Oral levonorgestrel progestin-only pill is principally designed for dedicated emergency contraceptives and specifically to prevent unwanted pregnancy after unprotected sex and/or as a backup when regular contraceptive method fails[1]. It is prescribed to be taken within 72 hours after unprotected sex exposure as a single dose (1.5 mg) at once or in divided doses (0.75 mg) taken 12-24 hours apart after unprotected intercourse in a week and four (4) instances in a month [2,3]. Recurrent administration of levonorgestrel is on the increase among young women of reproductive age. Perhaps, the use of levonorgestrel is adopted as a better alternative to induced abortions which may occur because of unplanned and unwanted pregnancies[4]. This approach has been reported to reduce the number of maternal death by 40% [5,6]. Levonorgestrel tablets are available only by prescription for women younger than age 17, and obtainable over the counter (OTC) for women above 17 year[7]. Since levonorgestrel is classified as OTC, users have unrestricted access to it, which may encourage abuse and increase the possibility of a prolonged pharmacological response and/or drug resistance[7].

As a lipophilic xenobiotic, levonorgestrel diffuses across the plasma membrane and exhibits its action via progesterone receptor, a member of nuclear receptor superfamily, thus competing with the endogenous progesterone[8,9] and modulates the secretion and uptake of its precursor from circulating LDL-cholesterol. Reportedly, levonorgestrel decreases serum high density lipoprotein-cholesterol (HDL-C) level without significant change in serum total cholesterol TC, triglyceride TG, low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) levels[10]. The molecular mechanism proposed was that

levonorgestrel stimulates hepatic lipoprotein lipase which is involved in the degradation of HDL-C [11]. Disposal of levonorgestrel is by liver and kidney but it is metabolized chiefly by the liver[12], thence metabolic effects due to regular and frequent use of levonorgestrel needs to be evaluated. The current study therefore aimed at evaluating potential modulatory effects of varying doses and repeated use of oral levonorgestrel as an emergency contraceptive on lipid metabolism in female Wistar rats.

MATERIALS AND METHODS

Test Substance

Levonogestrel tablets (LNG) produced by Shuangwei Pharmaceutical Company Limited, Ninghai, China was dissolved in vehicle (normal saline) and administered via intragastric tube. While the test animals were given LNG, the regular control animals were given vehicle. The doses used in this study were chosen and modified based on earlier research report[13].

Ethical Approval

All animals were well cared for according to criteria outlined in the "Care on Use of Animals in Research and Teaching" prepared by the U.S. National Institute of Health (NIH) and approved by the University Ethical Review Committee (UERC/ASN/2019/1806), University of Ilorin, Ilorin, Nigeria. The adopted protocol for the research conformed to the provision of declaration of Helsinki 1995 as reviewed by Edinburgh, 2000[14].

Animals

Twenty female Wistar rats with average weight of 150 g were purchased from the University of Ilorin Animal Holding Facility. The animals were kept under standard housing condition (12-hour light/dark cycle, 25 ± 2°C and 65-75% relative humidity) in white plastic cages, fed with standard rat chow and distilled water ad libitum. The rats were acclimatized for 14 days before the commencement of the experiment.

Experimental Design

The rats were randomly divided into 4 groups (n = 5 per group) and were administered normal saline or LNG orally via intragastric gavage for six weeks as described below: the normal control group received 0.2 ml/kg of normal saline alone three times per week at 48 hours intervals, the positive control group was exposed to regular dose of LNG (0.004 mg/kg/week), the two test groups were exposed to regular dose at increased frequency of LNG (0.004 mg/kg; 3 times per week) at 48 hours interval and high dose at increased frequency of LNG (0.008 mg/kg; 3 times per week) at 48 hours interval.

Preparation of Tissue Homogenates

At the end of the experiment, rats were fasted overnight and liver, kidney and heart were excised under light ethyl ether anesthesia and rinsed with 1.15% KCl solution to remove hemoglobin. Tissue samples from each organ were homogenized with potassium phosphate buffer (0.1 M, pH 7.4) in ratio 1:4 w/v and centrifuged at 1600 g for 10 minutes to obtain the supernatants which were scooped into separate bottles and kept at -20°C prior to the biochemical analyses.

Biochemical Assays

Lipids were extracted from liver, heart and kidney as described by Folch *et al*[15]. Briefly, tissues were washed with 0.05 M KCl solution; aliquots of the chloroform–methanol extract were used for the determination of cholesterol, triacylglycerol and phospholipids concentrations in each organ using Randox® commercial kits (Spin React S.A., Santa Colona and Sant Esteve De Bas, Spain respectively).

Statistical Analyses

Statistical evaluations and graphical representation were performed using statistical package for social sciences (SPSS) version 20.0 (IBM Corporation, Armonk, NY) and GraphPad Prism (GraphPad Prism Software version 5.01, San Diego, CA, USA) respectively. All data were expressed as Mean ± standard error of means (S.E.M). Comparisons among the groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison tests. LNG-treated groups were compared to control, while LNG (0.004 mg/kg/3ce/week and 0.008 mg/kg/3ce/week) were compared to LNG (0.004 mg/kg/week). Values of $p< 0.05$ were regarded as significant.

RESULTS

Levonorgestrel Enhanced Cholesterogenesis in Liver, Kidney and Heart

The total cholesterol concentration was significantly elevated in the liver [$F(3,8) = 9.54$, $p< 0.005$]; and heart [$F(3,8) = 27.24$, $p< 0.00$]; by increased dose and frequent LNG (0.004 mg/kg/3ce/week and 0.008 mg/kg/3ce/week) exposure when compared with control and regular LNG (0.004 mg/kg/week) dose. Similarly, the treatment of rats with LNG at all doses and interval significantly [$F(3,8) = 31.10$, $p< 0.00$]

increased renal total cholesterol concentration when compared with control group. Also, LNG (0.008 mg/kg/3ce/week) significantly ($p< 0.05$) increased renal total cholesterol concentration when compared with regular LNG (0.004 mg/kg/week) dose (Figure 1).

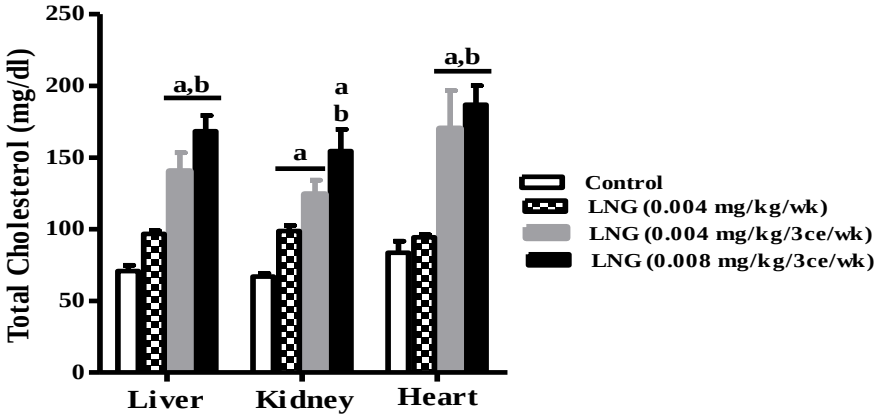

Figure 1: Total cholesterol concentrations in liver, kidney and heart of female Wistar rats administered levonorgestrel at increased dose and frequency. Increased dose and frequency of levonorgestrel caused increased cholesterol levels in the selected organs. [a]Mean values were significantly different compared with control, [b]Mean values were significantly different compared with LNG (0.004 mg/kg/week) at $p< 0.05$. LNG; Levonorgestrel.

Levonorgestrel Lowered Triglycerides Content in the Heart but not in the Liver or Kidney

There was no significant ($p> 0.05$) change in the hepatic [$F (3,8) =7.54$, $p< 0.10$] and renal [$F (3,8)=2.11$, $p< 0.18$] triglyceride concentrations following administration of LNG at all doses and frequencies when compared with control (Figure 2). However, LNG (0.008 mg/kg; 3 times/week) lowered cardiac triglycerides level [$F (3,8) =5.59$, $p< 0.02$] when compared with control group (Figure 2).

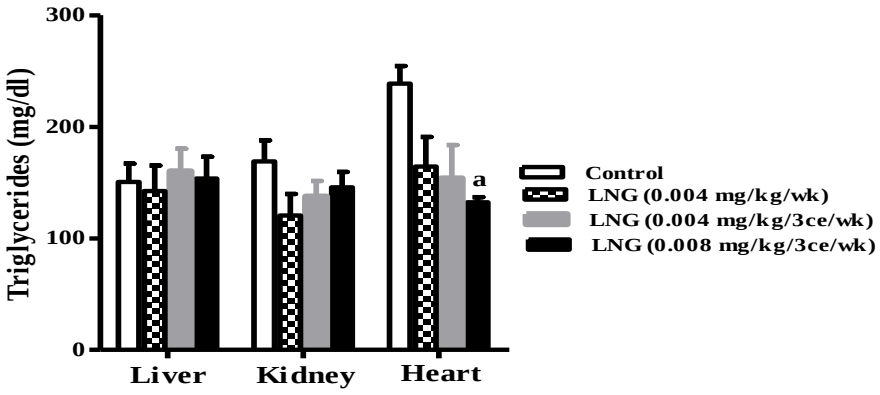

Figure 2: Triglycerides concentration in liver, kidney and heart of female Wistar rats administered LNG. Increased dose and frequency of LNG decreased triglycerides in the heart. [a]Mean values were significantly different compared with control at $p<0.05$. LNG; Levonorgestrel.

Levonorgestrel (LNG) Raised Phospholipids Content in the Liver and Heart but not in the Kidney Phospholipids

The hepatic phospholipids content was significantly [F (3,8) =5.40, p< 0.03] elevated by LNG in a dose-dependent manner and increased frequency (0.004 mg/kg/3ce/week and 0.008 mg/kg/3ce/week) when compared with control. However, all doses of LNG markedly raised [F (3,8) =18.46, p< 0.001] cardiac phospholipids but not in the kidney [F (3,8) =3.41, p< 0.07]; when compared with control (Figure 3).

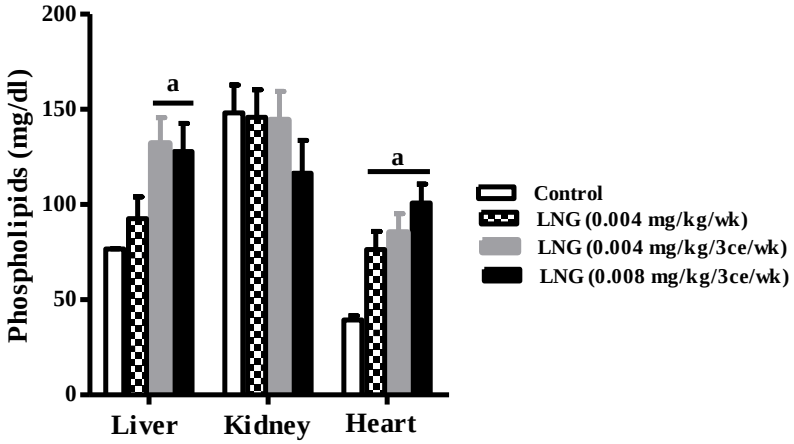

Figure 3: Phospholipids concentration in liver, kidney and heart of female Wistar rats administered LNG at increased dose and frequency. Increased dose and frequency of LNG enhanced phospholipid mobilization in the liver and heart. [a]Mean values were significantly different compared with control, LNG; levonorgestrel.

DISCUSSION

The toxicological potential of levonorgestrel (LNG) is commonly found and evaluated in epidemiologic studies relating to hypercholesterolemic and/or atherogenic conditions[16]. These conditions are robustly linked with dyslipidemic conditions, an independent risk factor in the pathogenesis and progression of atherosclerosis which is characterized by abnormal lipid metabolism and profile [17-19]. Therefore, this present study showed that dyslipidemic conditions could be induced by levonorgestrel. Levonorgestrel has been declared to be safe for all women and in all situations [20,21] and results from earlier studies were at variance with each other. Studies show that levonorgestrel increases plasma TC and LDL-C, but decreased or could not alter TG levels as the case may be [22].

In the present findings, varying doses of oral levonorgestrel administration, at the regular (once per week), repeated and frequent use (three times per week) could possibly cause tissue hypercholesterolemic conditions in rats. The levonorgestrel-induced mild hypercholesterolemic condition as observed in the current study may be due to the administered dose, therapeutic schedule or duration of the experiment. It has been reported that levonorgestrel activates HMG CoA reductase gene, production of NADPH cofactors [23] and hydrolysis of cholesteryl esters by the enzyme cholesteryl ester hydrolase [22]. These are important steps in the synthesis and uptake of free cholesterol from cholesterol-rich lipoproteins (LDL-C) in the cell membrane [21]. In addition, weekly administration of regular dose of levonorgestrel was found to show trend towards the elevation of hepatic total cholesterol, albeit not statistically significant and was comparable to the control. This appears to be in disparity with previous studies where levonorgestrel was shown to decrease intracellular concentrations of cholesterol due to efflux of cholesterol from the membrane to HDL as promoted by lecithin cholesterol acyltransferase, acyl cholesterol acyltransferase and utilization of cholesterol for synthesis of other steroids, such as hormones in the gonads, adrenal cortex and brain, or bile acids in the liver [24, 25]. Notwithstanding, the current study corroborates a previous report that levonorgestrel increased cholesterol deposition on vascular wall and can predispose to arterial vascular diseases due to narrowing of the lumen [24,25].

Triglycerides and phospholipid contents were also examined as a biomarker of the dyslipidemic condition[25,26]. This study revealed that levonorgestrel produced no marked effect on liver and kidney TG concentrations but generally decreased heart triglycerides irrespective of difference in dose and

frequency of administration. These showed that levonorgestrel has potential hypo-triglyceridemic effect on heart. This could be as a result of enhanced mobilization of triglycerides from the cardiac adipose tissues by levonorgestrel levonorgestrel since lipolysis (hydrolysis of triglycerides to free fatty acids (FFA) and glycerol) is a major source of energy in the heart and kidney [27,28]

The hypo-triglyceridemic effect of LNG on heart could stimulate feedback mechanism to enhance biosynthesis of triglycerides in the liver thereby increasing the liver triglycerides as observed in this study. This study did not consider the implication of a very long time administration of LNG on the lipid profile of the selected tissues. Nonetheless, if the tissue hypo-triglyceridemic condition is prolonged as observed in this study, the heart and kidney may suffer damage due to increase need for energy to drive various functions of these organs.

This study has shown that LNG reduced storage of fats TG thereby supporting the report that LNG is appropriate choice in an obese subject[24]. Inconsistently, while LNG deterred dyslipidemic condition by modulating triglycerides at tissue level, the effect on phospholipid was spiky. Levonorgestrel caused mild increase in tissue phospholipids concentrations; however, increase dose and frequency tends to lower phospholipids in the kidney but raised it in the liver and heart. Possibly at regular dose and increase frequency, LNG downregulates the phospholipids synthesis and translocation from their site of production to target tissues [29,30]. In the fed state, lipid biosynthesis favors production of storage fats-triglycerides, suppression of genes encoding phospholipids transporter proteins, decreasing concentration and activities of translocases that are responsible for mobilization from circulation (extracellular space) to target cell and tissues, altered degradation rate of phospholipids, conformational change in the distribution of phosphoglyceride molecules which might also affect the asymmetric distribution of lipids within the cell and lastly, exposure to physical or chemical insult with subsequent cellular adaptive response[27, 31]. Injury to the cell plasma membrane may cause the breakdown and release of a membrane phospholipid called arachidonic acid whose presence is identified as a marker of cellular damage[31]. Decrease in tissue phospholipids may have implication on tissue oxidative damage because it may be consequent upon increased release of phospholipids due to increased tissue membrane damage [27,28]. The slight increase in tissue phospholipids as observed can therefore be linked to activation of endogenous antioxidant system. However, increased concentration of tissue phospholipids at double dose and increased frequency may be ascribed to repressive effect of LNG at high tissue level since van der Linden *et al*. [32] demonstrated that hormones (LNG synthetic analogue of progesterone) have high affinity for their receptors, and very low concentrations are sufficient to produce responses in target tissues[29, 31].

Conclusion

It can be deduced from this study that frequent use or abuse of oral levonorgestrel potentiates dyslipidemic conditions through altered metabolic effect on lipid homeostasis.

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Authors' Contributions

AHA and AAS conceived the study. AHA, AAS, OEB and FJO designed the study. AHA, AAS, FJO, and OEB carried out the study. ONA, NAB, AHA and AAS analyzed and interpreted the data. AHA, ONA, and NAB drafted the manuscript. AAS, FJO, NAB and OEB revised the manuscript. All authors read and approved the final manuscript for publication.

Declaration of Conflicting Interest

Authors have declared that no potential conflicts of interest exist.

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