Original Article

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Assessment of Incessant Intake of Levonorgestrel Oral Contraceptive and the Effect of Drug Withdrawal on Liver Function and Integrity

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Abstract:

Objective: This study used female Wistar rats to evaluate the impact of incessant intake of levonorgestrel on liver function and integrity, and to observe the effect of drug withdrawal on the organs. (organ).

Material and Methods: Sixty rats (110–120g) were randomly placed in 3 groups (n=20). Group A was given the human dose equivalent of 1.83 mg/kg/bodyweight (BW) orally once weekly, Group B was given the same dose orally twice weekly, while Group C, serving as the control, was given the same dose of the vehicle solution. Rats were given food and water *ad libitum*. At the end of 30 days of treatment, 5 rats from each group were sacrificed by cervical dislocation, blood and the liver were harvested, weighed, and processed for biochemical and histological analyses. This was repeated at 60 and 90 days. The last batch of rats was left for another 30 days for possible recovery.

Results: The serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (γGT), and total protein were significantly elevated in the serum. The histopathological examination showed hyperplastic degeneration and extensive tubular necrosis in the treated groups. After the drug was withdrawn, the altered levels of some of the determined biomarkers were not reversed.

Conclusion: This study showed a dose- and time-dependent toxicity of the liver, which was associated with levonorgestrel without improvement after drug withdrawal.

Keywords: contraceptives, hepatotoxicity, levonorgestrel, organ-toxicity

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Introduction

The abuse and misuse of drugs is a serious public health concern worldwide and has become one of the biggest societal issues in several nations of the world[1]. One of the drugs commonly misused among young adults is contraceptives, and this is because, more than ever before, there is an increase in unprotected sexual activities among youths, irrespective of their educational, religious, or socioeconomic background or exposure[2]. Studies have ascertained that many females have knowledge of the existence of emergency contraceptives for use either before or after coitus, and that a large population uses the pills often[3]. Studies have also indicated that a good percentage of these young ladies do not possess adequate knowledge about the proper use of these medications, and they often take these contraceptives without a prescription as they can easily purchase them in pharmacies in sub-Saharan Africa[5]. Over-the-counter dispensation of contraceptives might be one of the reasons for misuse, abuse, and continual use after sex without any awareness of their possible toxic effects on the vital organs (toxicity). There is concern about the chronic, continual use of contraceptives, and we fear that it could result in chronic health issues later in life.

Levonorgestrel (LNG), an emergency contraceptive pill, is a progestin-only hormonal oral contraceptive that a female can take within 72 hours after unprotected sexual intercourse to prevent pregnancy[6]. It is not recommended as a regular family planning method because of its low concentration of active ingredients. It should be used only in cases of emergency, hence the name: emergency contraceptive. It is available commercially as a single dose of 1.5 mg taken once or 2 tablets of 0.75 mg taken 12 hours apart[6]. This drug prevents pregnancy by inhibiting the fertilization of an egg by sperm cells; it prevents sperm migration and function in the genital tract by thickening the cervical mucus, and prevents implantation by thinning the endometrium. Administration within 24 hours after intercourse has been shown to have 95.0 % effectiveness, while 48 and 72 hours after intercourse produced 85.0 % and 58.0 % effectiveness, respectively. The contraceptive's efficacy decreases the longer the interval of use post-intercourse[2]. It is available over-the-counter and can be obtained without a doctor's prescription in many pharmacies, chemist stores, drug stores, or other superstores[7]. Its accessibility has been noted to trigger continuous misuse among adults due to increased sexual activities; however, the toxicity associated with its unabating long-term usage was not taken into cognizance. What was designed for emergencies only (1.5 mg LNG) is now used frequently like a regular family planning method, which contains about 0.1 mg of LNG. Despite clear appeals for LNG's safety, there is limited knowledge about the harmful effects of taking it frequently and for extended periods[8]. Therefore, this study used Wistar rats as an experimental model to investigate the effect of the incessant use of LNG on the main metabolic organs, namely the liver and kidney, and to observe the outcome of drug withdrawal on the biomarkers assessed.

Material and Methods

Sixty female rats of the *Wistar* strain weighing 160 -170 g were purchased from the Physiology Department of the University of Ibadan. They arrived safely at the animal house of the Lead City University and were handled in compliance per the protocols established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals in research (NIH publications No. 8523, revised 2011). They were fed with rat pellets and water *ad libitum.* The animals were acclimatized for 3 weeks before the commencement of the study. They were assigned to 3 groups (n=20). Groups A and B were administered levonorgestrel (1.83 mg/kg bodyweight (BW)) once and twice weekly, respectively, while group C was administered vehicle (normal saline). Administration in all groups was done via oral gavage. Five rats were sacrificed through

cervical dislocation from each group at the end of 30, 60, and 90 days. After the third batch of sacrifice at 90 days, the remaining 5 rats from each group were left untreated for an additional 30 days to monitor recovery. During each batch of sacrifice, blood and the liver were taken for processing and biochemical and histopathological analyses.

Blood was collected into non-heparinized bottles and centrifuged at 3000 revolutions per minute (rpm) for 15 minutes using a table centrifuge, and the serum was obtained. The liver was homogenized separately in a homogenizing buffer (ice-cold phosphate buffer, 0.1M, pH 7.4) to make a 2.0 % homogenate, using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 g for 10 minutes in a Microlfield refrigerated centrifuge (Model: MF – TGL16) at 4 ^{0}C to obtain the post-mitochondrial fraction.

Determination of Liver function biomarkers

Aspartate transaminases (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were determined quantitatively in the serum with Randox kits from Randox Laboratories Limited, United Kingdom. Total protein in the serum was estimated using a standard procedure (Biuret method)[9]. Gamma-glutamyl transferase (γGT) was determined using Spectrum kits produced by the Egyptian Company for Biotechnology, Cairo, Egypt.

Histopathological examination of organ

Sections of about 3 to 5 µm of the liver were fixed in 10.0% phosphate-buffered formalin immediately after excision and used for histopathological analysis, which was done following the standard preparation and hematoxylin-eosin staining method described by Alam et al[10].

Statistical analyses of the data

Data obtained were expressed as mean deviation (S.D.), n=5, using Graph Pad Prism version 9. Treated and control groups were compared using row statistics and 2-way ANOVA (multiple comparison TUKEY test). Statistical significance was set at a 95.0% confidence level (p-value<0.05). α showed a statistical significance when, once weekly, it was compared with the control (p-value<0.05). β indicated a statistical significance when, once weekly, it was compared with those treated twice weekly (p-value<0.05). γ showed significance when, twice weekly, it was compared with the control (p-value < 0.05).

Results

The liver function biomarkers, namely AST, ALT, and ALP, were elevated in the serum of the rats given LNG (Figure 1A-C). AST concentration in the serum (Figure 1A) was elevated, and the increase occurred progressively with treatment days. The ALT concentrations measured (Figure 1B) became significantly elevated in the serum at 90 days, and the increase was not reversed post-treatment. The concentration of γGT determined in this study was elevated significantly in the treated group when compared with the control (Figure 1D). The increase was promptly reduced during the post-treatment period. Twice weekly, there was a significant increase from the first month up until the treatment period. Total protein levels (Figure 1E) showed remarkable improvement in the post-treatment period, after a decrease of about 70.0 % at 90 days of twice-weekly treatment when compared with the control.

The histopathological examinations showed a great aberration of the liver, especially in the rats given LNG twice weekly, when compared with the control (Figure 2). Treatment once weekly (Figure 3) resulted in congestion and mild focal periportal and disseminated infiltration by inflammatory cells. In contrast, treatment twice weekly (Figure 4) caused marked disseminated congestion, mild disseminated periportal infiltration by inflammatory cells, and a lymphoid aggregate in zone 2 and the focal area of the hepatic nodules.

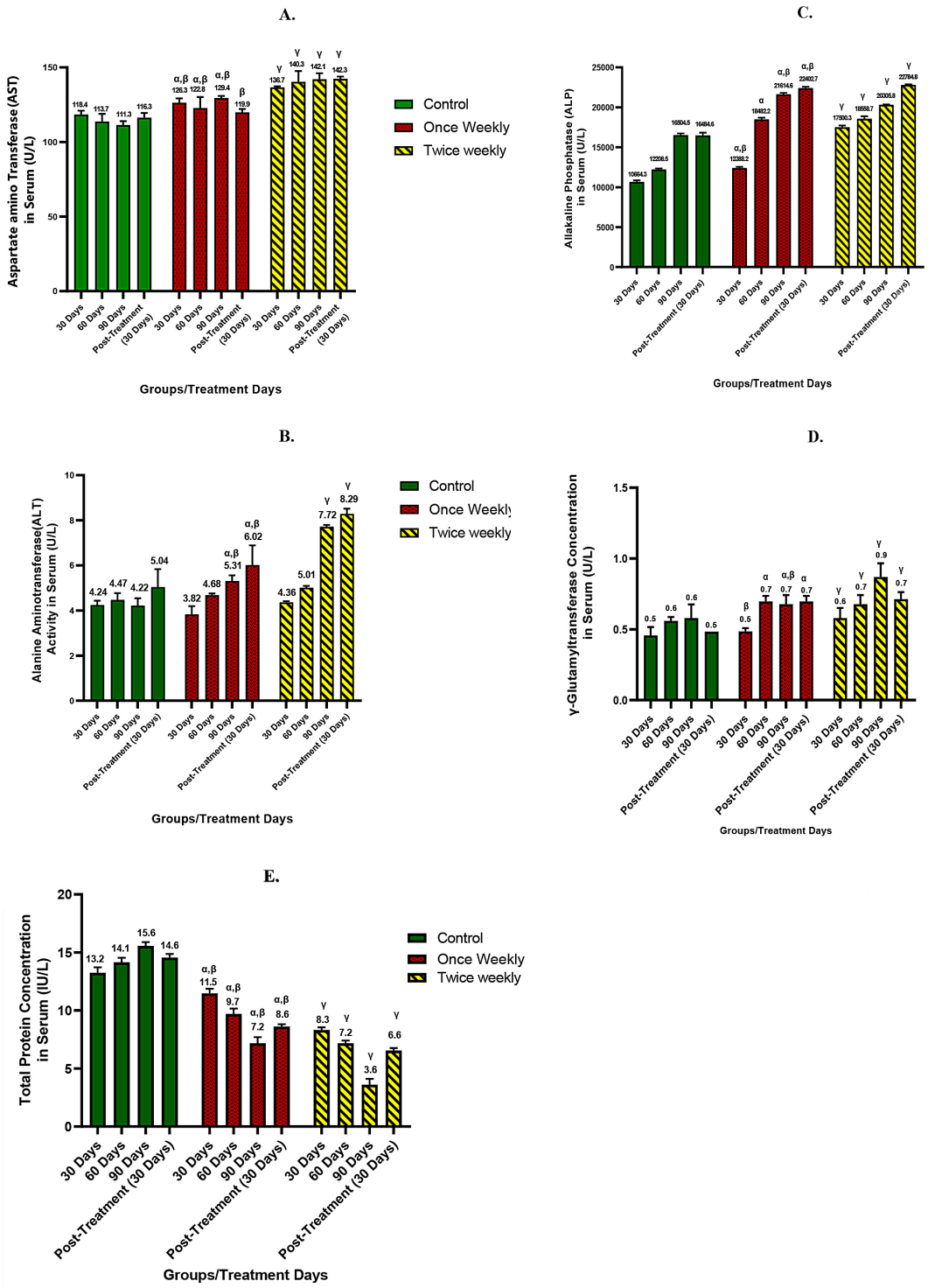

Figure 1 Concentrations of the liver function biomarkers (A – AST, B – ALT, C – ALP, D – ɣGT, E – Total Protein) determined in the serum of rats administered with LNG once weekly and twice weekly compared with the control

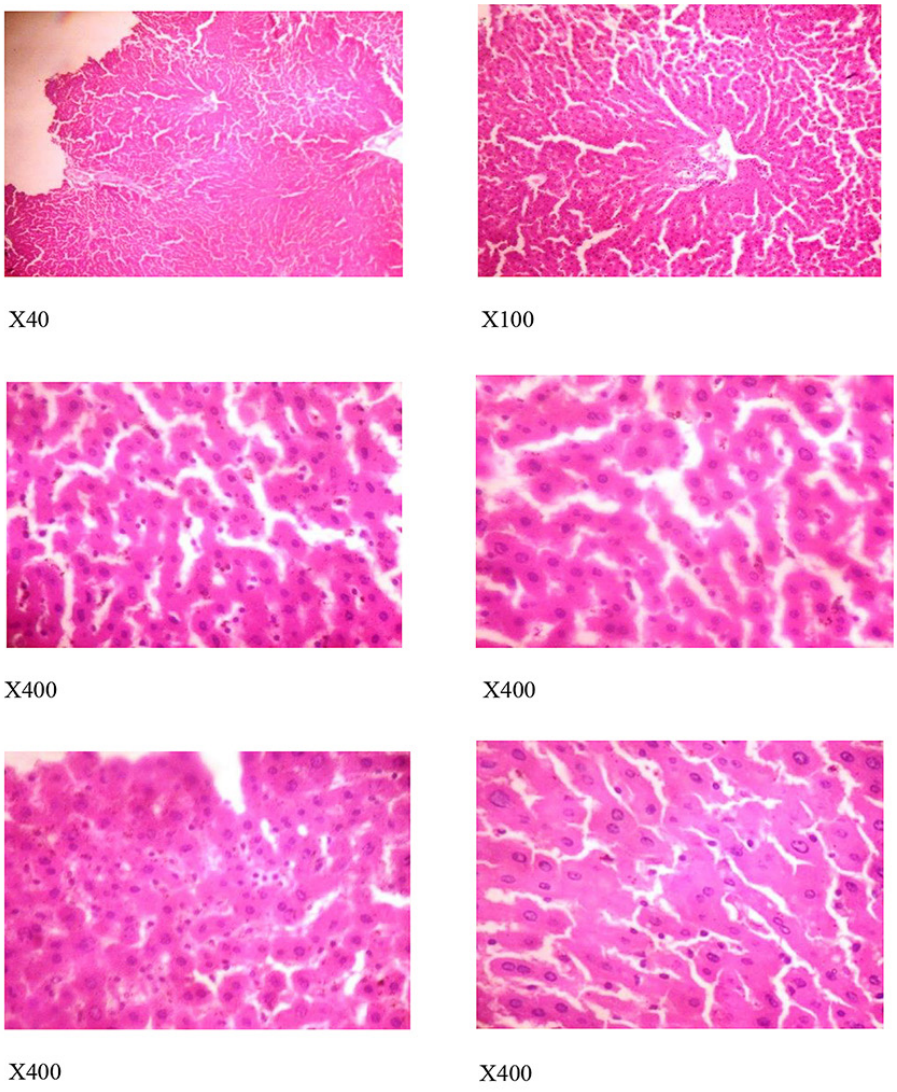

Figure 2 Photomicrophs of the thin Sections of the liver tissues taken from the control group (plates show normal architecture of liver cells)

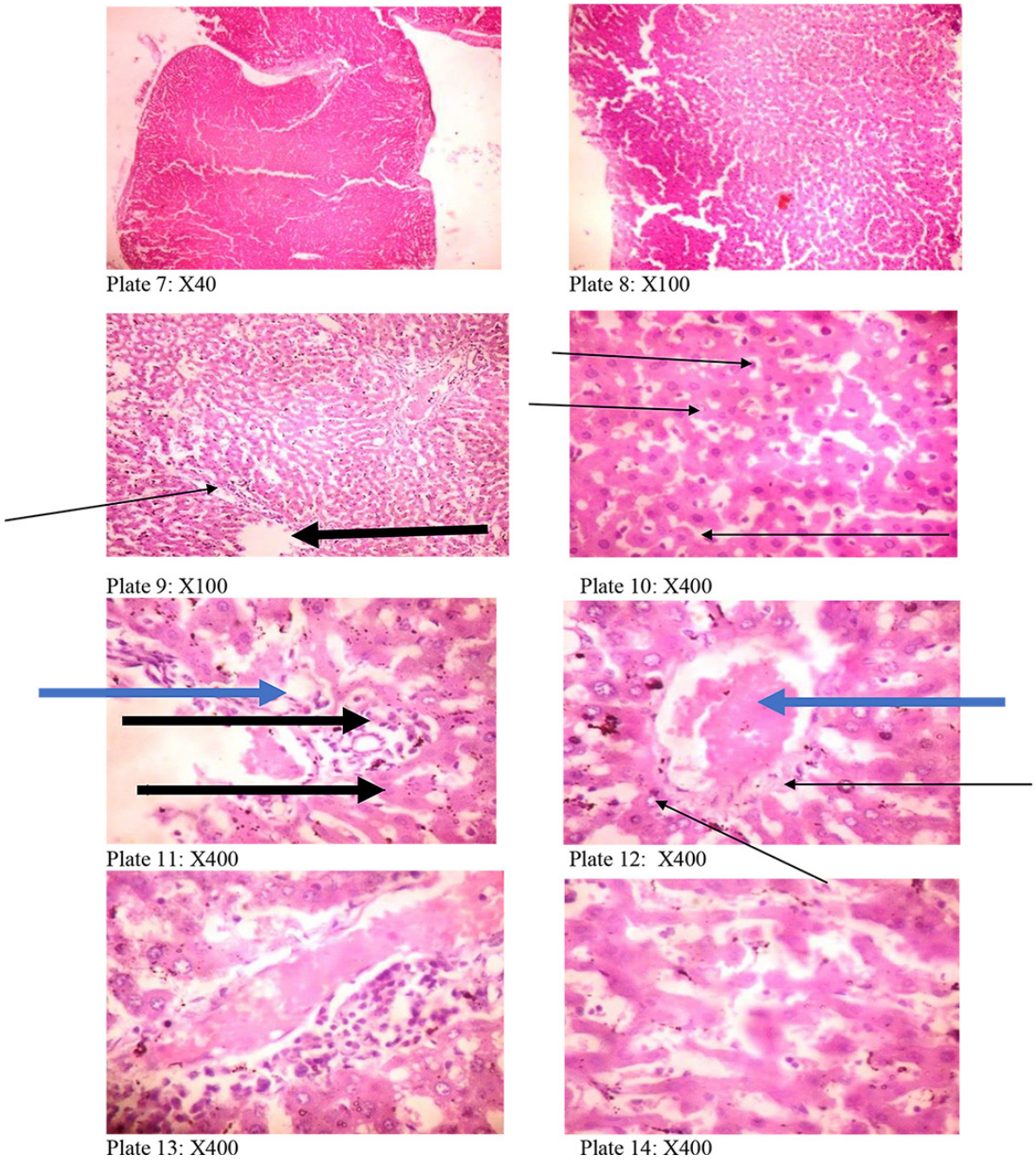

Figure 3 Photomicrophs of the thin sections of the liver tissues taken from the once weekly treated group (plates show infiltration of zone 2 by inflammatory cells (slender arrows, cogestion (blue arrows), mild disseminated periportal infiltration by inflammatory cells (black arrows) and very mild disseminated infiltration of zone2 by inflammatory cells (slender arrows))

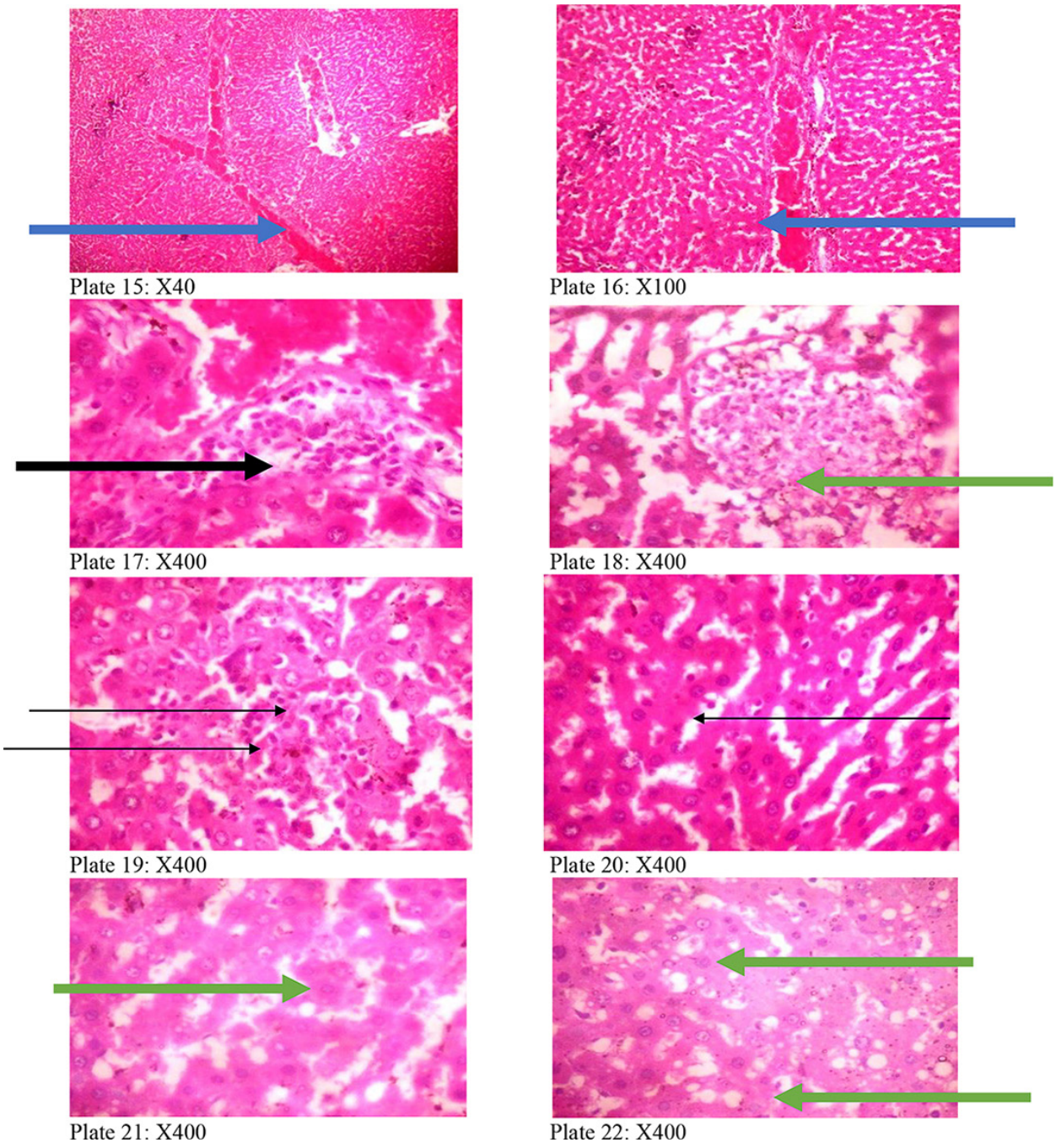

Figure 4 Photomicrophs of the thin sections of the liver tissues taken from the twice weekly treated group. There is marked disseminated congestion (blue arrows), mild disseminated periportal infiltration by inflammatory cells (black arrows), mild disseminated infiltration of zone 2 by inflammatory cells and lymphoid aggregate (slender arrows) and focal area of hepatic nodules (green arrow) mild disseminated infiltration of zone 2 by inflammatory cells (slender arrows), very mild periportal infiltration by inflammatory cells (black arrows) and disseminated microvesicular steatosis (green arrows)

Discussion

The high rise in the use of oral emergency contraceptive pills (ECPs) due to their effectiveness, accessibility, and affordability in a bid to prevent unwanted pregnancies and unsafe abortions is alarming. Several studies are clamoring for increased awareness and use of ECPs without much thought given to the effects of their continuous intake on the metabolic organs[11,12]. The high prevalence of sexual activities has been contributed to by factors such as the early onset of puberty, peer pressure, exposure to pornographic movies, lack of communication between parents and teenagers, adverse opinions on premarital sexual abstinence, social media, a low level of sexual knowledge, late marriage, and increased sexual partner concurrency[12]. The need to prevent unwanted pregnancies and unsafe abortions does not eradicate the fact that these drugs, like every other drug, are toxicants and must be biotransformed by the liver[13].

The liver is a vital organ responsible for numerous metabolic, detoxification, and regulatory functions. The effect of liver injury could manifest as either impairment of liver functions, such as glucose metabolism, excretion of waste substances from the body in the bile, or synthesis of the important body proteins, such as albumin and coagulation factors, or impairment of blood circulation through the liver parenchyma cells. Distortion of the architectural configuration of the liver (lobule) leads to hindrance in blood flow and impairment in function, manifesting in poor glucose maintenance, impaired clearance of toxic wastes, ascites and pleural effusion, excessive bleeding, and escape of liver enzymes into the circulation[14].

Certain liver enzymes such as AST, ALT, ALP, as well as γGT, and total protein, are essential tools for assessing liver health, diagnosing liver disorders, and tracking disease progression and treatment efficacy[15]. Abnormalities in liver enzymes, notably aminotransferases, namely ALT and AST, can classify liver disorders into cholestatic or hepatocellular patterns, and drugs can induce both types of damage. A disproportionate increase in the concentrations of the aminotransferases (Figures 1A and B), as observed in this study, in comparison with ALP (Figure 1C) and GGT (Figure 1D), is considered a hepatocellular pattern of abnormal liver function, which signifies a release of enzymes from the hepatocytes, resulting in elevated serum concentrations. The cholestatic pattern is associated with the elevation of ALP and total protein (albumin and bilirubin) levels[16]. This results from low specificity for liver diseases but high sensitivity, and γGT is often used to clarify the origin of ALP elevation since γGT elevation is also caused by biliary or hepatocyte disease[17].

The abnormal liver function illustrated by the biological biomarkers determined in this research was further corroborated by the increase in relative liver weight and histological aberrations to the liver, which are also important indicators of organ integrity and function. These results are consistent with studies by Chane et al[18] and Kowalska et al[19], who also discovered that oral contraceptive users had higher concentrations of AST, ALT, ALP, and GGT than the control group; however, this conflicts with a study by Odinga et al[20], who claimed that intake of ECP did not significantly alter the concentrations of AST, ALT and ALP.

The damage caused to the metabolic organs by the intake of LNG may be as a result of any of the following: i. Interactions in the pathway of LNG; ii. Metabolism of LNG via reduction, hydroxylation, and conjugation (glucuronidation and sulfation); iii. Modulation of gonadal hormones. Firstly, if LNG interacts with drugs like barbiturates, phenytoin, and carbamazepine that can induce one of the cytochrome P450 liver enzymes, cytochrome P450 3A4 (CYP3A4), it is metabolized faster and lowers effectiveness. An increased rate of metabolism may be overwhelming on the liver, especially if it is a repeated exertion[21]. Secondly, levonorgestrel undergoes extensive reduction of the alpha, beta-unsaturated ketone in its ring

A. Levonorgestrel is metabolized in the liver by hydroxylation at carbons 2 and 16. The metabolites of both compounds circulate predominantly as sulfates and are found in the urine primarily in the glucuronide form. Conjugation of LNG occurs via glucuronidation and sulfation at the 17-beta hydroxyl position to produce conjugates of glucuronides and sulfates, respectively, with glucuronide conjugates being more predominant (because the functional groups, namely phenol, alcohols, arylamines, and N-hydroxyl compounds, required for sulfation are less available). It is important to note that the metabolism of LNG in the liver produces metabolites such as sulfates and glucuronides. Although glucuronides are considered non-toxic, there are morphine-6-glucuronides that are toxic, but the pathway is not yet fully understood. Furthermore, the accumulation of morphine 3- and morphine-6-glucuronides has been reported with severe side effects[22].

The modulation of gonadal hormones by the drug in a bid to prevent pregnancy and implantation may alter certain master regulators of reproduction, such as kisspeptins, which also participate in the control of metabolism and energy balance. After all, there is always a consequential relatedness between endocrine and metabolic diseases. The involvement of this hormone in metabolism may also explain the effect of LNG on body weight. Kisspeptin is encoded by kiss 1 and kiss 1R genes, which are expressed in metabolic tissues like the liver[22]. The modulatory effect of LNG on ovulation may cause a feedback stimulatory effect on kisspeptin, which is associated with metabolic dysfunction-associated fatty liver disease. Subsequently, kisspeptins and their receptor, KISS1R, have been noted to substantially influence the pathophysiology of many organs and to also modulate age-related diseases in these organs[23].

Since the severity of the effects of LNG on the liver occurs with the duration of use, this puts long-term users at a higher risk of liver damage[23]. The harm caused to the liver by repeated drug intake becomes more pronounced and debilitating, especially in susceptible individuals, owing to the genetic and environmental risk factors that are often undiagnosed or untreated. These risk factors alter hepatic metabolism and the excretion of therapeutic drugs, causing cellular stress, cell death, activation of an adaptive immune response, and the inability to adapt progresses to overt liver injury[24].

It has been reported that several prescribed and over-the-counter drugs have been implicated in drug-induced liver injury (DILI). Close monitoring when taking ECPs, especially with other drugs, and in situations of other underlying risks of liver diseases[25]. Several drugs, such as alteplase, ancrod, acenocoumarol, and antithrombin, were listed among several others that can increase the risk or severity of the adverse effects of LNG when taken in combination by modulating metabolism, bioavailability, and the therapeutic effects of LNG. Several users of ECPs are unaware of the health risks associated with the use of LNG in combination with other drugs, especially to the liver[26,27]. This theory is in line with the findings of Sridharan et al[28], who suggested that several excipients in LNG, including silica colloidal anhydrous, cause harmful effects to the liver, such as inflammatory diseases and necrosis, and opposed to Connoly and Zuckerman's[29], who suggested that progestins are safe for use in patients with liver diseases. Drug withdrawal did not immediately reverse the effects of LNG on the liver's function and integrity.

Limitations of the study

Animals were used in this toxicity study; therefore, it may not accurately capture and predict the outcome and adverse effects observable in humans.

Conclusion

Continuous LNG intake was toxic to the liver in a dose-and time-dependent manner, and there was no remarkable reversal with drug withdrawal. Animals that

were subjected to LNG twice weekly experienced greater assaults. This study, therefore, provides evidence that constant use of LNG as a regular form of contraception has the propensity to cause damage to the vital metabolic organs. There is a need to educate females on the difference between emergency contraceptives and other birth control pills safer for use in cases of continuous sexual activities.

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Conflicts of interest

None to declare.

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