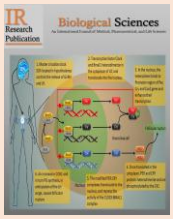
RESEARCH ARTICLE

Biological Sciences
(An International Journal of Medical, Pharmaceutical, and Life Sciences)

Journal home: https://irrespub.com/biolsciences/index.php/1/index



Impact of Prolonged Use of Postinor®-2 and Norinyl®-1/35 on Hepatic Enzyme Levels in Female Wistar Rats: A Biochemical Assessment

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ARTICLE HISTORY

Received: 07-04-2024
Revised: 06-06-2024
Accepted: 07-06-2024
Online: 09-06-2024

KEYWORDS

Contraceptives
Postinor®-2
Norinyl®-1/35
Hepatic Enzymes
Liver Function

ABSTRACT

The liver is prone to inflammation or disease after prolonged exposure to toxins or pharmaceutical agents. This study investigates the impact of prolonged exposure to contraceptive drugs, Postinor®-2 and Norinyl®-1/35, on hepatic enzyme levels in female Wistar rats. Twenty-five (25) adults female Wistar rats were divided into five groups (n = 5), respectively: Group 1 (control; administered 5 mg/kg/bw of normal saline). In groups 2 (Postinor®-2; treated with 0.10 mg/kg/bw), group 3 (Postinor®-2; 0.20 mg/kg/bw), groups 4 (Norinyl®-1/35; 0.10 mg/kg/bw) and group 5 (Norinyl®-1/35; 0.20 0.20mg/kg/bw) all admissions were done orally for fourteen (14) days. On day 15, the animals were anaesthetized, and their livers were harvested for biochemical analysis. After which, the results were analyzed using ANOVA with $p<0.05$ signifying the statistical significance. All the treated groups showed a significant increase in ALT levels compared to the control group; ALT was higher in group 2 (0.10 mg/kg b.wt of Postinor®-2) compared to group 3. While AST levels were reduced in groups 2 and 4 compared to the control group, SOD decreased in groups 1 and 4 when compared to the control group. MDA levels increase in contraceptive-administered groups compared with control group. In conclusion, prolonged intake of Postinor®-2 and Norinyl®-1/35, irrespective of dosage, induces oxidative stress by increasing lipid peroxidation, as observed by the value of MDA, and hence oxidative stress might be the reason by which the contraceptives alter the liver enzyme functions.

Introduction

The liver is a vital organ responsible for metabolism, detoxification, and synthesis of essential proteins, plays a pivotal role in maintaining physiological homeostasis [1]. Its strategic placement exposes it to various xenobiotics, environmental pollutants, and chemotherapeutic agents, making it susceptible to potential damage or stress if natural protective mechanisms are overwhelmed [2]. The

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Email: edacheabah@bsum.edu.ng
DOI: https://doi.org/10.55006/biolsciences.2024.4203
Published by IR Research Publication;

parent drugs and their metabolites, which enter the based contraceptive tablet, is commonly used as an systemic circulation. Some studies have described the incessant abuse of contraceptive drugs among young female of reproductive age [1]. One of the most commonly abused drugs are Postinor®-2 and Norinyl®-1/35 [3]. Postinor®-2, a levonorgestrel-emergency contraceptive in Nigeria, although its safety and efficacy for this purpose remain contentious [4]. With each tablet containing 0.75mg of levonorgestrel, it is utilized to prevent conception after unprotected sexual intercourse, control menstrual disorders, and treat endometriosis [5]. The recommended window for its effectiveness is within three days of unprotected sex, with earlier administration proving more efficacious [6]. Studies reported that it increases the activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes [7].

A combination with Norinyl®-1/35, a birth control pill containing ethinyl estradiol and norethindrone, acts by preventing ovulation and altering cervical mucus and uterine lining conditions, hindering sperm transport and impeding egg attachment [7]. Besides being a contraceptive, it is prescribed to treat moderate acne in women aged 15 and older who use birth control pills [3]. Hence the frequent abuse of these drugs by female of reproductive age has the tendency to increase hepatic activities and alters hepatic enzymes [8]. Understanding the impact on liver function is crucial, as the liver's central role in metabolism and detoxification makes it susceptible to adverse effects from pharmaceutical agents.

Hence this study focuses on assessing the concentration of hepatic enzyme levels, such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) in female Wistar rats after prolonged exposure to Postinor®-2 and Norinyl®-1/35.

Materials and Methods

Experimental animals

A total of twenty-five (25) adult female Wistar rats weighing average of 100grams used for this study were obtained from the animal house, College of Health Sciences, Benue State University, Makurdi. The rats were divided randomly into five groups of five rats each and kept in spacious, well-ventilated plastic cages and allowed to acclimatize for fourteen (14) days in the animal house, while being fed with standard supreme feed and tap water ad libitum.

At the end of the fourteen days acclimatization period, they were treated with normal saline and the test drugs for the next 14 days (two weeks).

Experimental Drugs

The drugs Postinor®-2 (20 tablets of 0.75mg levonorgestrel each) and Norinyl®-1/35 (100 tablets of 0.15mg ethinyl estradiol/norethindrone each) were obtained from Mernax Pharmacy and Veterinary Drugs Store, shop four, Vaipama plaza, opposite College of Health Sciences, Gboko Road, Makurdi, Benue State and stored at room temperature.

Preparation of Drug Solution

20 tablets of Postinor®-2 (0.75mg each) making 15mg of Levonorgestrel was dissolved in 500mls of distilled water to obtain a concentration of 0.03mg/ml [12]. 100 tablets of Norinyl®-1/35 (0.15mg each) making 15mg of ethinyl estradiol was also dissolved in 500mls of distilled water, making a concentration of 0.03mg/ml [4]. The two drug solutions were stored in the refrigerator for optimum temperature regulation at 0°C and ready for use.

Experimental Design

The total of 25 adult female Wistar rats were weighed and randomly divided into five groups of five rats in each group labeled 1 – 5, and then acclimatized for fourteen (14) days, being fed once daily with standard supreme feed and tap water.

After the period of acclimatization, the rats were treated for two weeks (fourteen days) with doses of Postinor®-2 and Norinyl®-1/35 as follows:

Group 1 (Control Group):100mg/kg body weight of Normal Saline once daily for 14 days

Group 2: 0.10mg/kg body weight of Postinor®-2 once daily for 14 days

Group 3: 0.20mg/kg body weight of Postinor®-2 once daily for 14 days

Group 4: 0.10mg/kg body weight of Norinyl®-1/35 once daily for 14 days

Group 5: 0.20mg/kg body weight of Norinyl®-1/35 once daily for 14 days

Sample collection

At the end of day 14 of administration, all animals were anaesthetised by chloroform inhalation, and vital tissues were harvested. Blood was collected through the left ventricle of the heart of the animals in heparinized centrifuge tube under a deep anesthesia with chloroform. The blood collected was

centrifuged using centrifuge machine at 10,000 rmp for five minutes and the serum collected was subjected to liver function test (AST, ALP and ALT) and estimation of oxidative stress enzymes (SOD and MDA).

Liver function tests

The liver enzymes analysis; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) was carried out using an auto-analyzer.

Alanine aminotransaminase (ALT) Activity

This was done with Reitman-Frankel colorimetric method using a Quimica Clinica Applicada (QCA) test kit. ALT activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine which is proportional to its concentration at 505nm.

Aspartate aminotransferase (AST) Activity

This parameter was done using the Reitman-Frankel colorimetric method [15] for invitro determination of GOT/AST in serum using a QuimicaClinicaApplicada (QCA) test kit. I measured AST activity by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 – dinitrophenylhydrazine spectrophotometrically at 505nm.

Alkaline Phosphatase (ALP) Activity

Phenolphthalein monophosphate method for the invitro determination of alkaline phosphatase in serum using QuimicaClinicaApplicada (QCA) test kit. Alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate. Addition of the alkaline reagent stops the enzyme activity and simultaneously develops a blue chromagen which can be measured photometrically at wavelength of 550nm.

Assay of Oxidative Stress Enzymes

Estimation of Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation in the tissue was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) method of [16]. A principal component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue in Tris-HCl buffer, Ph 7.5 was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x105 M-1 cm-1 and expressed as nmol/mg protein.

Assay of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was measured according to the method as described by [9]. The principle of the assay was based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT). Briefly, the reaction mixture contained 2.7 ml of 0.067M phosphate buffer, Ph 7.8, 0.05 ml of 0.12Mm riboflavin, 0.1 ml of 1.5Mm NBT, 0.05 ml of 0.01M methionine and 0.1 ml of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air aluminum foil in a box with a 15W fluorescent lamp for 10 minutes. Control without the enzyme source was included. The absorbance was measured at 560nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions. Activity of enzyme was expressed as units/mg protein.

Ethical Clearance

All experimental procedure was duly followed in agreement with the guidelines on animal experiment as prescribed by the Ethics committee of College of Health Sciences, Benue State University Makurdi, Nigeria. A copy of the proposal was submitted to the ethical committee to be examined for approval.

Statistical Analysis

Statistical data were analyzed using the statistical software, Statistical Package for the Social Sciences (IBM SPSS version 23.0) and results were expressed as Mean±SEM (Standard Error in Mean). Group means were compared using one-way ANOVA, and mean differences among groups were determined using LSD Post-Hoc test. Mean differences across groups were considered statistically significant at $P<0.05$.

Results

Liver Function Enzymes Levels

Figure 1 shows the mean liver function enzyme levels of rats across groups compared on one-way ANOVA. For ALT; groups 2 - 5 all showed a statistically significant increase at $p<0.05$ in mean when compared to the control group (group 1). For ALP;

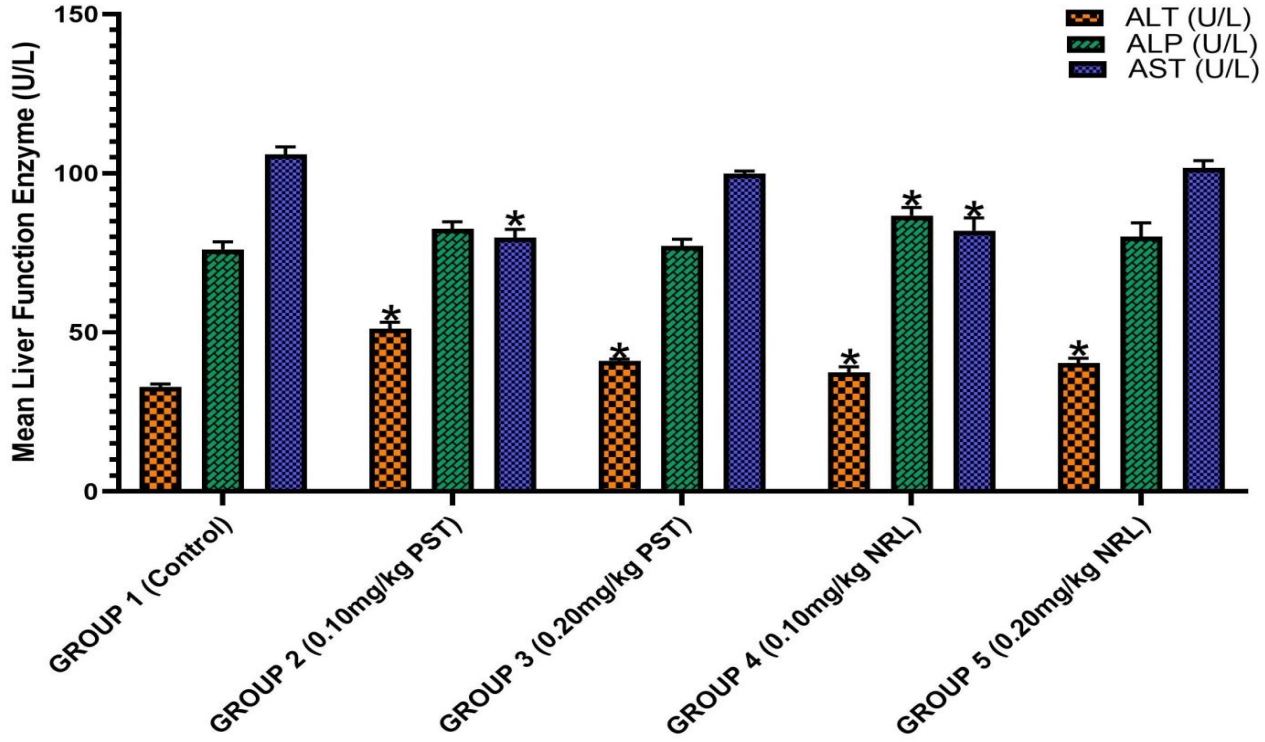

Figure 1. Shows the Mean Level of Liver Function Enzymes across Groups. * = Statistically Significant Difference at p<0.05 in Mean when compared to the Control Group. PST = Postinor; NRL = Norinyl; N = 5.

only group 4 showed a statistically significant increase in mean compared to the control group while for AST, groups 2 and 4 showed a statistically significant decrease in mean when compared to the control group on one-way ANOVA (* = significant at p-value<0.05 when compared to group 1).

This result shows the significant increasing effect of the drugs Postinor® - 2 and Norinyl®-1/35 on the serum level of the liver function enzymes (ALT and ALP), signaling a palpable distortion in the liver function and the utilization of these enzymes for the metabolism of the drugs and xenobiotics.

Oxidative Stress Markers: Superoxide Dismutase (SOD) and Malondialdehyde (MDA)

Figure 2.0 shows the mean oxidative stress markers across groups compared on one-way ANOVA. For the mean SOD levels; groups 2 - 4 showed a statistically significant decrease at p<0.05 in mean compared to the control group. For MDA; groups 2 - 3 showed a statistically significant increase in mean when compared to the control group.

The observed significant decrease in Superoxide dismutase (SOD) and elevation in antioxidant marker Malondialdehyde (MDA) in the experimental groups in this study implies an increasing effect of the test drugs; Postinor® - 2 and Norinyl®-1/35 on the oxidative stress level of the hepatocytes of experimental animals in this research.

Discussion

This research investigated the impact of prolonged administration of Postinor®-2 and Norinyl®-1/35 on hepatic enzyme levels in female Wistar rats, focusing on alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST). Two doses (high and low) of each of the contraceptives was chosen and administered to different groups of the experimental animals to check the possible dose – dependent effects of the drugs by comparing the high and low doses of each drug in the groups administered. One-way ANOVA analysis (Figure 1) facilitated a comparison among groups for liver function enzymes.

Alanine aminotransferase (ALT) levels were notably elevated across groups, indicating potential liver damage induced by the administered drugs. The highest elevation was observed in groups 2 and 3, receiving 0.10mg/kg and 0.20mg/kg body weight of Postinor®-2 respectively, suggesting a dose-dependent effect. ALT, being liver-specific, serves as a marker for hepatotoxicity and liver diseases [10]. These findings align with previous studies by [11] and [12], validating the observed increase in ALT levels.

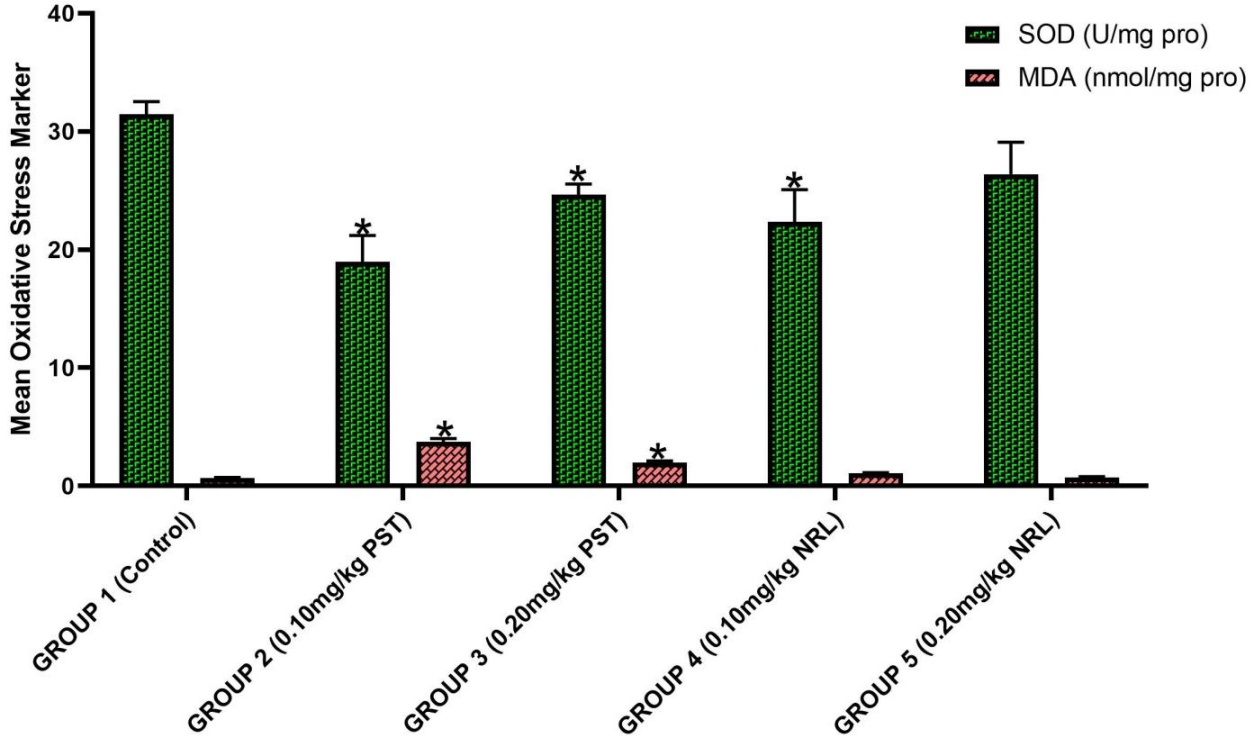

Figure 2. Shows the Mean Oxidative Stress Markers across Groups. * = Statistically Significant Difference at $p<0.05$ in Mean when compared to the Control Group. PST = Postinor; NRL = Norinyl; N = 5.

Similarly, alkaline phosphatase (ALP) levels exhibited a significant increase, particularly in group 4, which received 0.10mg/kg body weight of Norinyl®-1/35. Elevated ALP levels indicate liver and bone pathologies, among others [10]. Consistent with prior research by [11], [4], and [12], these results reinforce the impact of contraceptive drugs on ALP levels in rats.

Conversely, aspartate aminotransferase (AST) levels showed a decreasing trend, with the most significant decrease observed in group 2, receiving Postinor®-2. While AST is a liver enzyme, its decrease could be attributed to the concurrent elevation of ALT, indicating liver damage [10]. This finding contrasts with [11] study but supports [4] observations, possibly influenced by varying experimental conditions.

Moreso, the study explored oxidative stress markers, superoxide dismutase (SOD), and malondialdehyde (MDA). SOD levels decreased significantly across groups, particularly in those administered Postinor®-2, indicating cellular toxicity induced by the drugs. These findings corroborate [13] research, suggesting a potential effect of hormonal contraceptives on oxidative stress in rats.

Furthermore, MDA levels significantly increased, indicative of lipid peroxidation and oxidative stress. This elevation, particularly pronounced in groups 2 and 3 receiving Postinor®-2, aligns with previous studies by [14] and [12], emphasizing the detrimental impact of contraceptive drugs on oxidative stress markers.

In light of these observations, the study demonstrated that prolonged use of Postinor®-2 and Norinyl®-1/35 in female Wistar rats led to significant alterations in hepatic enzyme levels and oxidative stress markers, indicating potential hepatotoxicity and cellular damage. These findings underscore the importance of monitoring liver function and oxidative stress parameters in individuals using contraceptive drugs, highlighting the need for further research to elucidate underlying mechanisms and potential therapeutic interventions.

Conclusion

In conclusion, this study provides valuable insights to the deleterious effect of the impact of prolonged use of Postinor®-2 and Norinyl®-1/35 on hepatic enzyme levels and biomakers of oxidative stress in female Wistar rats. Hence prolonged use of contraceptive irrespective of dosage alters liver function, and hence may contribute to metabolic disorder which could be as a result of oxidative stress induced by the prolonged intake. Hence, there is need for vigilant monitoring and thoughtful consideration of alternative options to safeguard the liver health of individuals using these medications.

Contribution of authors

The authors of this research article contributed as thus: Edache Daniel Abah: Conceived research idea, mobilized for, and undertook the study. Okwute Michael Ochayi: Composed and drafted the manuscript. Somke P. Madueke: Edited the manuscript

Acknowledgments

The authors wish to acknowledge the following individuals for their efforts and contributions towards the actualisation and success of this research: Professor Linus Chia Saalu, Provost, College of Health Sciences, Benue State University, Makurdi, Benue State, Nigeria. Dr. Akunna Gabriel Godson, Head of Department, Department of Anatomy, College of Health Sciences, Benue State University, Makurdi, Benue State, Nigeria.

Conflict of Interest

The authors state that there are no conflicts of interest to this research, and that all reference sources have been duly cited and listed in the bibliography section.

Funding

This study did not receive any specific grant for funding. It was completely funded by the authors.

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