Toxicological Assessment of Levonorgestrel (Postinor 2) Intake in Selected Reproductive and Vital Organs in Animal Model

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Certification

This is to certify that this research was carried out and the thesis written by Catherine Bosede ADENIJI with the Matriculation number LCU/PG/002032 under my supervision as part of the requirement for PhD in Environmental Biochemistry and Toxicology, Department of Chemical Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria.

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Dedication

This research work is dedicated to Tony, Jason and Charis Adeniji whose love and support constantly inspires me to dream and to dare.

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Abstract

Increased sexual activities among adolescents have resulted in the indiscriminate use of contraceptives. This study assessed the toxicological effects of *levonorgestrel* (LNG) intake on reproductive and vital organs in a rat model. Sixty Wistar rats weighing between 110 and 120 g were randomized into three groups (n = 20). Groups A and B were administered 1.83 mg/kg BW of LNG once and twice a week respectively, and group C served as the control. Five animals from each group were sacrificed at 30, 60, and 90 days, while the last batch was left for 30 days for post-treatment recovery. The effect of LNG on the estrous cycle was assessed through physical and cytological evaluations. The uterus, ovary, liver, and kidney were investigated for possible anomalies through histopathological examination and biochemical analyses. Hormonal changes were assessed by measuring the serum levels of progesterone (P4), estradiol (E2), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) using the ELISA technique. Effects of LNG on fertilization, implantation, and tissue inflammation were assessed by measuring progesterone-associated endometrial protein (PAEP), leukemia inhibitory factor (LIF), prolactin (PRL), sex hormone-binding globulin (SHBG), Nuclear Factor Kappa B (NF-κB), and Tumor Necrosis Factor α (TNF-α) using ELISA. Hepatotoxicity was determined by measuring aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) using standard spectrophotometric methods. Similarly, nephrotoxicity was determined via cystatin C, urea, creatinine, BUN, and the BUN-creatinine ratio levels. Oxidative damage was assessed by determining the tissue activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), including the concentrations of reduced glutathione (GSH) and malondialdehyde (MDA), using standard procedures. LNG prolonged the metestrus phase of the estrous cycle. Hyperplastic degeneration, disseminated congestion, and extensive tubular necrosis were found in the uterus, liver, and kidney of the treated groups. P4 was normal, LH, FSH, TNF-α, and NF-kB were markedly elevated with LNG treatment, while E2, PAEP, LIF, PRL, and SHBG were decreased. Serum AST, ALT, ALP, GGT, Cystatin C, creatinine, and urea were significantly elevated. GSH level and CAT, GPx, and SOD activities were significantly lowered, whereas MDA level was increased. LNG treatment caused time- and dose-dependent hormonal modulation and organ toxicity. The uterus and liver were particularly affected, and animals exposed to LNG twice a week apparently suffered more assaults. The ability of the animals to recover following drug withdrawal was more evident in the once-weekly treated group, particularly with respect to hormonal, liver, and kidney aberrations.

Keywords: *Levonorgestrel*, estrus cycle, uterus, ovary, hormonal imbalance

Word Count: 402

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List of Acronyms

Acronym	Meaning
LNG	Levonorgestrel
NSDUH	The National Survey on Drug Use and Health Substance Abuse
SAMHSA	Mental Health Services Administration
SUD	Substance Use Disorder
UNODC	United Nations Office on Drugs and Crime
COCs	Combined oral contraceptives
OTC	Over the Counter
ECPs	Emergency Contraceptive Pills
UPA	Ulipristal Acetate
IUD	Intrauterine Contraceptive Device
RIF	Recurrent Implantation Failure
DES	Estrogen Diethylstilbestrol
NDLEA	National Drug Law Enforcement Agency
STDs	Sexually Transmitted Diseases
WHO	World Health Organization
ELISA	Enzyme Linked Immunosorbent Assay
PAEP	Progesterone Associated Endometrial Protein
LIF	Leukemia Inhibitory Factor
PRL	Prolactin

SHBG	Sex Hormone - Binding Globulin
FSH	Follicle-Stimulating Hormone
LH	Luteinizing Hormone
PRAC	Pharmacovigilance Risk Assessment Committee
LIF	Leukemia-inhibitory factor
IVF-ET	In Vitro Fertilization, and Embryo Transfer
PCOS	Polycystic Ovary Syndrome
MFI	Multiple Failures of Implantation
GnRH	Gonadotropin-releasing hormone
GST	Glutathione-S–Transferase
DTNB	Dinitrothiocyanobenzene
GSH	Reduced Glutathione
GPx	Glutathione Peroxidase
CAT	Catalase