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Original Research Article

Potential Reproductive Toxicity Of Lacatomtom Drink In Male Wistar Rats

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ABSTRACT

Stimulant consumption among Nigerian youths has emerged as a critical public health concern with fatal concoctions like methylated alcohol with soda and Lacatomtom (LTT) replacing drug abuse. This study investigated the impact of lacatomtom drink on reproductive parameters of male Wistar rats. Twenty- five male Wistar rats weighing 190g were randomly grouped into 5 groups of 5, for the following treatments: Group A: (normal control) 0.5 ml distilled water, Group B: (positive control) 0.5 ml *Lacasera*, Group C: 125 mg/kg LTT, Group D: 250 mg/kg LTT, Group E: 500 mg/kg LTT, orally daily for 30 days. At end of experiment, the animals were anaesthetized, and sampled: Blood was evaluated for testosterone, sperm count and sperm cell characteristics were analyzed from the caudal epididymis, and testes, processed for histomorphology. Lacatomtom had no significant ($p > 0.05$) effect on sperm count, morphology and viability relative to the control. Significant ($p < 0.05$) increase in sluggish sperms was observed in animals treated with LTT at 125, 250 and 500 mg/kg dosages relative to the control. There was a significant ($p < 0.05$) decrease in serum testosterone levels in animals treated with LTT at 125 mg/kg and 500 mg/kg relative to the positive control and a significant increase in the serum testosterone levels of the positive control group relative to the normal control. None of the LTT-treated rats had aberrant testicular histoarchitecture. Lacatomtom may reduce testosterone levels and increase sluggish sperm cells, which might impede sperm's capacity to reach and fertilise the egg, affecting male reproductive function.

Keywords: Lacatomtom, testosterone, sperm motility, sperm count

Introduction

Psychotropic drug use among children and adolescents in Europe has increased, particularly in the Netherlands (1995-2000), Denmark (1996-2010), Iceland (2003-2007), and Norway (2004-2010 for 15-16 year old).[1–4] In the US, psychotropic drug use among children and adolescents increased twofold to thrice between 1984 and 1996, with a further increase between 2000 and 2002. Despite the rise in stimulant usage, consumption varies widely by country. In 2007, Icelanders aged 7-15 were roughly five times more likely than Finns to use attention deficit hyperactivity disorder. (ADHD) medicines, according to a Nordic comparison research by Zoëga et al.,[3]

However, stimulant consumption among Nigerian youths has emerged as a critical public health concern, with far-reaching implications spanning psychological, physiological, and socioeconomic dimensions.[5] Various studies,[6,7] have underscored the profound psychological effects resulting from stimulant consumption. The physiological impacts include alterations in feeding and sleeping habits, disregard of personal hygiene, shaking sensations in arms and legs, reddened eyes, unexplained money demands, excessive quest for privacy, lack of coordination, and kidney, lung, and liver diseases.[6]

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The physiological impacts include alterations in feeding and sleeping habits, disregard of personal hygiene, shaking sensations in arms and legs, reddened eyes, unexplained money demands, excessive quest for privacy, lack of coordination, and kidney, lung, and liver diseases.[6] The socioeconomic effects of the consumption of these stimulants are seen in societal volatility, which includes increased armed robbery, kidnapping and rape cases.[8] These societal challenges paint a complex picture of the far-reaching impact of stimulant use on public safety and well-being. The resulting economic burden and height in healthcare expenditures incurred in the management of stimulant-induced health issues places strain on already constrained public health resources.[9]

Recent studies indicate an alarming increase in the misuse of psychoactive drugs among juveniles. Fatal concoctions like methylated alcohol mixed with soda, Tramadol, and "Lacatomtom" are now replacing previously documented drugs of abuse, as highlighted by Nwala et al.[10] An example illustrating the consumption of the psychoactive beverage "lacatomtom" was documented by Emmanuel et al.[11]

"Lacatomtom" (LTT), often known as 'gigabyte,' is a dark-brown solution made from *tom-tom* candy and *lacasera* drink. Its dark-brown colour and peculiar smell come from the tom-tom component. Menthol, carbonated water, sodium benzoate, and aspartame accelerate carbon dioxide gas nucleation, making it bubbly.[12] Some of the reasons Nigerian youths abuse Lacatomtom more are as a result of the ban on codeine imports and the need for cheaper alternatives. The prevalence of lacatomtom at 11.5% as reported in a study by Nwala et al.[10] suggested a potential preference for this beverage among students over alcoholic drinks. It is speculated that *lacasera* drink may be easier to conceal from uninformed parents and guardians compared to outright alcoholic beverages, thus potentially contributing to its popularity among this demographic.

Recognizing the increasing prevalence of Lacatomtom consumption and the possible potential health risks it poses, it becomes imperative to delve into its potential toxicological implications. This study adopts

male Wistar rats as a model system, aiming to comprehensively investigate the effects of Lacatomtom on reproductive parameters. This research design aligns with the need for knowledge regarding the health impact of Lacatomtom drink and adds to the discussion on its use.

Materials And Methods

Study Design
The study is an experimental animal model research involving the use of LTT drink to determine its impact on reproductive parameters in male Wistar rats.

Sample
Lacasera beverages are formulated from a composite of ingredients, including carbonated water, apple juice concentrate (5%), sugar, acidulants (malic acid and citric acid), caramel coloring, apple flavoring, and a preservative (sodium benzoate).[11] Conversely, *tom-tom* candies, a popular confectionery in Nigeria, known for its strong menthol flavor, are comprised of ingredients such as sugar, glucose syrup, water, menthol, flavoring, and color.[10,11] Both *Lacasera* beverages and *Tom-Tom* candies were purchased on August 24, 2023, from Choba market in Obio Akpor Local Government Area of Rivers State, Nigeria.

A Sample Preparation (LTT Solution)
The samples were designed to replicate the dosage commonly consumed in Lacatomtom drinks. Sample A consisted of distilled water, while sample B contained *Lacasera* alone. Samples C, D, and E were formulated by dissolving three tom-tom candies, which collectively weighed 11.45 g, in 350 mL of *Lacasera* drink. As a result, each milliliter (ml) of *Lacasera* drink contained approximately 32 mg of tom-tom (i.e. 32 mg/mL).

Ethical Approval
The University of Port Harcourt's Centre for Research Management and Development Research Ethics Committee accepted the study procedures (Ref. No: UPH/CEREMAD/REC/MM93/034). Humane treatment of all experimental animals was in compliance with university-approved ethics and regulations for research animal usage.

Acute Oral Toxicity Testing
A total of eighteen rats were used to determine the LD_{50} of the Lacatomtom drink based on the research, by Lorke.[23] The experiment involved administering doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg of LTT drink via oral gavage to three groups of three rats each in the first phase. The animals were observed for a day. In the second phase, three groups of three animals each were given doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of LTT. The animals were then monitored for any signs of toxicity or mortality, over a 24-hour period.

Animal housing
A total of 25 male Wistar rats, weighing approximately 200 g, were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. They were acclimatized for 2 weeks and fed standard commercial diet (Top Feed finisher) and water.

Dosage Determination
The study's dosage levels were set using Lorke's approach to ensure safety below the predicted LD_{50}. Lorke's approach estimated the LD_{50} of Lacatomtom drink at 1000 mg/kg. The study used fractions of the LD_{50} for safety. According to Lorke's technique, the study's doses are 125, 250, and 500 mg/kg.

Treatment Protocol
A total of 25 male Wistar rats with average weight of 190 g were randomly grouped into 5 groups of 5 rats each, for the following treatments: Group A (normal control) received 0.5 ml distilled water, Group B (positive control) received 0.5 ml *Lacasera* Drink, Groups C, D and E received 125, 250 and 500 mg/kg Lacatomtom Drink respectively. These treatments were administered daily via oral gavage for a period of 30 days. On the 30th day the animals were sacrificed and anaesthetized with Diethyl-ether. Blood samples were collected for serum testosterone analysis by cardiac puncture into a plain sample bottle. The testes were excised, cleared of fatty and connective tissue, and fixed in Bouin's solution for histomorphology examinations.

Analysis of Spermatozoa Characteristics
The sperm Cell count
Sperm characteristics analysis was conducted on the spermatozoa collected from caudal epididymis. The caudal epididymis was dissected, lacerated, and pressed on a clean glass slide. Two drops of regular saline were added to create a suspension. The suspension was diluted with formal saline (1:20 ratio). A drop of diluted sperm was charged into the upgraded Neubauer counting chamber (Hemocytometer). To see the counting chamber, it was put on a light microscope slide stage and magnified to ×40. The count was measured in million/ml suspension.[14]

Sperm Cell Motility
Sperm motility was done by placing 10 ul of sperm suspension on the slide for microscopic evaluation at a magnification of ×400. About 100 sperm cells were examined and classified as either active, Sluggish or dead and expressed as a percentage.
Sperm Cell Morphology
Sperm morphology was determined using the Eosin and Nigrosine stain. Briefly, 10ul of eosin and Nigrosine was mixed with 40 ul of sperm suspension. The sperm suspension was incubated at 40°C for 5 min and then re-suspended with a micro-pipette. About 100 sperm cells/rats were morphologically examined under the microscope at ×400 magnification. Morphological abnormalities were classified as Normal, abnormal and viability.

Sperm Cell Viability
To create a suspension, one drop of sperms from the caudal epididymis was placed on a clean glass slide and two drops of normal saline were added. The suspension was combined with one drop of 0.5% eosin solution. A light microscope at ×40 magnification was used to view the slide few seconds later. Eosin-stained non-viable sperm differed from viable sperm

Testosterone Assay
The concentration of testosterone was analysed from the blood samples collected using Accu-bind ELISA Microwells (Testosterone Test System Product Code: 3725-300) from Monobind, USA.

Histological examination of the tissues
The testicular samples underwent histological processing following the methodology described by Nasution et al.,[15] After fixation, the tissues were dehydrated using ethanol and subsequently embedded in paraffin wax to facilitate cellular extraction. Sections of 5 µm thickness were then prepared and stained with hematoxylin and eosin (H&E) dyes following paraffin infiltration and embedding. The treated tissues were mounted onto glass slides, covered with a cover-slip, and examined under a light microscope (Olympus CX23, Olympus Corporation, Japan) at an objective magnification of x400. Photomicrographs were captured using an Olympus CX23 digital camera.

Statistical Analyses
Statistical analyses were done with SPSS 21; the data were represented as mean ± SEM, and assessed using one-way Analysis of Variance (ANOVA) and Tukey post-hoc test. The significance level was set at $p<0.05$.

Results And Discussion

Acute Toxicity Testing
At the doses of LTT administered, acute toxicity testing revealed no mortality, morbidity, or other apparent signs of toxicity. This

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demonstrated that LTT drink was not toxic at the maximal concentration of 5,000 mg/kg.

Sperm cell count and characteristics

The results in figure 1-3 summarized the impact of Lacatomtom drink on sperm cell count, sperm cell motility, viability and morphology after 30 days of ingestion. LTT drink had no significant ($p > 0.05$) effect on sperm count, morphology and viability relative to the control (figure 1 &2). However, there was a significant ($p < 0.05$) increase in number of sluggish sperms in the animals administered Lacatomtom drink at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg relative to the normal control.

Testosterone Level

Figure 4 shows the impact of LTT drink on testosterone level at end of experiment. Lacatomtom drink caused a significant ($p < 0.05$) decrease in serum testosterone levels of 125 mg/kg and 500 mg/kg test animals relative to the positive control (0.5 ml *Lacasera*). However, the testosterone level of group B (positive control) rats significantly ($P < 0.05$) increased in comparison with group A.

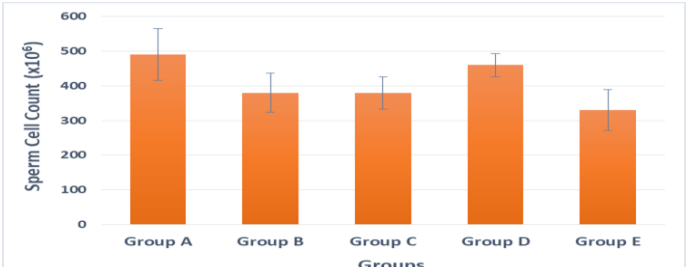

Figure 1: Effect of Varied doses of Lacatomtom drink on Sperm cell count.

Results are given as Mean ± SEM for each group. Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test. Experimental groups are compared with Group A (Control). No significant difference at a 95% confidence interval ($p > 0.05$).

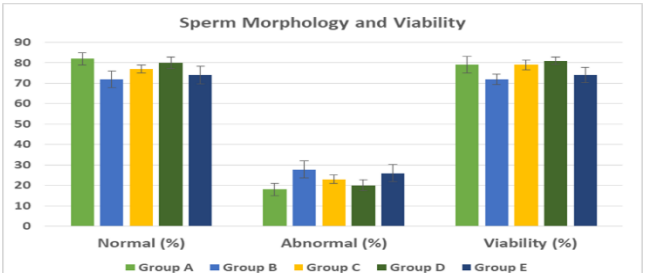

Figure 2: Effect of varied doses of Lacatomtom drink on Sperm morphology and viability

Results are given as Mean ± SEM for each group. Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test. Experimental groups are compared with Group A (Control). No significant difference at a 95% confidence interval ($p > 0.05$).

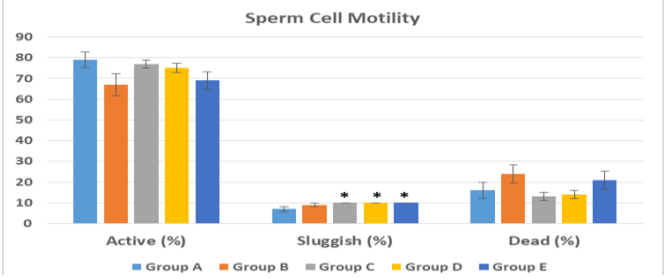

Figure 3: Effect of varied doses of Lacatomtom drink on Sperm cell motility

Results are given as Mean ± SEM for each group. Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test. Experimental groups are compared with group A (Normal Control) and group B (vehicle). *$p < 0.05$ was considered as significant versus the Normal control (Group A).

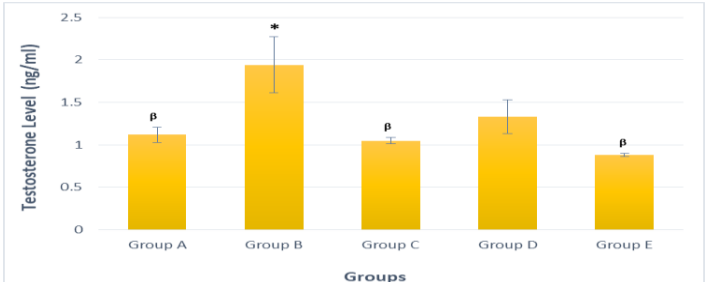

Figure 4: Effect of varied doses of Lacatomtom drink on serum Testosterone level

Results are given as Mean ± SEM for each group. Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test. Experimental groups are compared with group A (Normal Control) and group B (vehicle). *$p < 0.05$ was considered as significant versus the Normal control (Group A); $^{\beta}p < 0.05$ was considered significant versus the vehicle (Group B).

Testicular Sections

Photomicrographs of testicular sections of LTT drink ingested test groups are not different from that of the control. No obvious change was observed (plate 1).

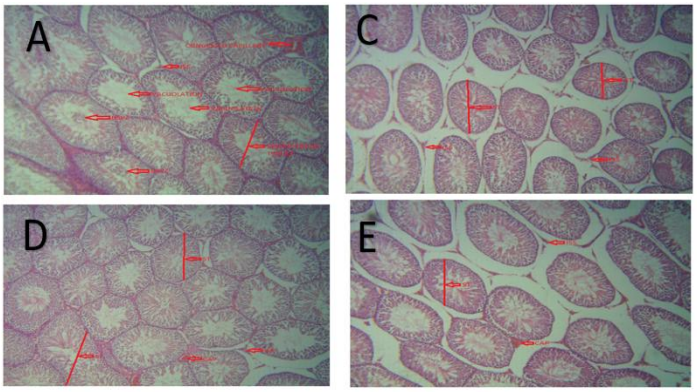

Plate 1: Photomicrographs of testicular sections from rats in control (A) and LTT ingestion groups (C, D and E) (125, 250, and 500 mg/kg dosages, respectively) on day 30 of treatment stained with H&E (×400).

There were no abnormalities in the testes of experimental rats compared to controls. The interstitial spaces have Leydig cells (LC), the seminiferous tubules have spermatogenic cells (SPG), and the lumen has spermatozoa (SPZ).

Psychostimulants such as Cocaine, amphetamines, and marijuana can impair sperm and cause infertility.[16] Previous studies have shown that cocaine usage harms male fertility and spermatogenesis.[17] The degree and particular effects of psychostimulants on reproductive parameters depends on dose, duration, and individual physiology. Possible implications of psychostimulants on reproductive parameters include Low libido, erectile dysfunction, infertility, hormonal imbalances.[18]

This study observed no significant impact of lacatomtom drink on sperm count, morphology and viability, in male wistar rats after 30 days of ingestion. However, there was a significant increase in the number of sluggish sperms seen in the test groups relative to the normal control. This implies that Lacatomtom may reduce sperm

motility of male wistar rats due to the large increase in sluggish sperms despite no effect on count, morphology, or viability. By possibly preventing sperm from fertilizing the egg, this may reduce fertility. This finding is in tandem with findings by El-Shennawy et al.,[19] who reported that sodium benzoate (SB) (at dose of 1000 mg/kg) substantially reduced sperm motility and count, and increased aberrant sperm count, indicating toxicity to sperms. Sodium Benzoate is a popular food ingredient. It is a major compound found in *Lacasera* drink. It is employed as a preservative in food and drinks due to its bacteriostatic and fungistatic qualities, as well as its high solubility and stability in water.[20-22] Contrary to what El-Shennawy et al.,[19] found, which indicated a decrease in sperm count and viability, our results showed no impact on these parameters. Possible explanations for this discrepancy could be as a result of variations in the dose of sodium benzoate used, and the duration of treatment (90 days in the latter research).

The decline, in testosterone levels observed in test groups C (125 mg/kg of LTT drink) and E (500 mg/kg of LTT drink) in comparison with the positive control (group B) might be connected to the inhibition of the enzymes or pathways needed for its production or the hormone like impact produced by the LTT drink. This suggests that the LTT drink probably influenced the Leydig cells and/or the hypothalamus pituitary gonadal axis by limiting hormone release or altering their control. The functionality of testosterone synthesis could be impacted by abnormalities in Leydig cells. Studies have indicated that testosterone has predominant control over testicular function and the male reproductive system.[23] Testosterone also plays a role in germ cell development, sperm production and overall male fertility regulation.[24] Previous research has highlighted that spermatogenesis heavily relies on a testosterone concentration within the testes, which is stimulated by gonadotropins from the pituitary gland.[25] Intra testicular testosterone triggers Sertoli cells to aid smooth transition of germ cells through stage VII of spermatogenic cycle. Therefore, it is believed that testosterone supports spermatogenesis by assisting in meiosis. Without testosterone, in Leydig cells or if Sertoli cells lack expression of androgen receptors, spermatogenesis cannot progress beyond meiosis stage. [26-29]. The observed significant increase in serum testosterone levels seen in the positive control group (group B) relative to the normal control (group A) was an unexpected outcome, considering the fact that previous studies have shown that sodium benzoate, a preservative found in the positive control, can significantly reduce serum testosterone levels in male rats when administered at doses of 400 and 800 mg/kg for 70 days.[30] However, the observed increase in serum testosterone levels in our study's positive control group may be attributed to differences in dosage, treatment duration, and the potential influence of other compounds present in the positive control. This observed unexpected finding would need further investigation to properly understand and explore.

This study observed no discernible alterations in the histomorphology of testicular sections in male rats that consumed lacatomtom drink compared to the control group. Despite this absence of morphological changes, there was evident impairment in reproductive physiology, evidenced by an increase in sluggish sperms and a decline in serum testosterone levels. This suggests the potential presence of a component in lacatomtom drink capable of modulating testosterone levels and sperm motility without manifesting morphological changes in the testes. Furthermore, the effects observed may be contingent on dosage, as indicated in previous reports. The study found that sodium benzoate at dosages ranging from 10 to 1000 mg/kg for 90 days led to decreased semen quality, changed testicular structure, and disruption of spermatogenic processes. Research indicates that sodium benzoate at 100 mg/kg administered for 28 days in rats, and at doses of 140-280 mg/kg for 60 days in mice, significantly reduced sperm count, viability ratio, and testicular spermatogenic activities.[19] The differences in results between the latter study and this one may be due to differences in exposure dosage, duration, and pharmacokinetics and pharmacodynamics of lacatomtom drink in rats.

Conclusion

The findings of this study suggests that lacatomtom drink may harm male reproductive functioning by decreasing Testosterone, which is essential for sperm production and maturation, and significantly increasing the number of sluggish sperm cells, which can impair sperm's ability to reach and fertilize the egg thus, potentially impacting fertility. It is therefore recommended that informational programmes targeting consumers and healthcare providers are needed. Awareness and effective policies can reduce damage and safeguard male reproductive health.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Liu YY, Hsiao HT, Wang JC, Liu YC, Wu SN. Parecoxib, a selective blocker of cyclooxygenase-2, directly inhibits neuronal delayed-rectifier K+ current, M-type K+ current and Na+ current. Eur. J. Pharmacol. 2019 ;844:95-101.
2. Hartz I, Skurtveit S, Steffenak AK, Karlstad Ø, Handal M. Psychotropic drug use among 0–17 year olds during 2004–2014: a nationwide prescription database study. BMC psychiatry. 2016;16:1-0.
3. Zoëga H, Baldursson G, Hrafnkelsson B, Almarsdóttir AB, Valdimarsdóttir U, Halldórsson M. Psychotropic drug use among Icelandic children: a nationwide population-based study. J Child Adolesc Psychopharmacol. 2009;19(6):757–64.
4. Steffenak AKM, Wilde-Larsson B, Nordström G, Skurtveit S, Hartz I. Increase in psychotropic drug use between 2006 and 2010 among adolescents in Norway: a nationwide prescription database study. Clin Epidemiol. 2012;225–31.
5. Obadeji A, Kumolalo BF, Oluwole LO, Ajiboye AS, Dada MU, Ebeyi RC. Substance use among adolescent high school students in Nigeria and its relationship with psychosocial factors. J Res Health Sci. 2020;20(2):e00480.
6. Pennings EJM, Leccese AP, Wolff FA de. Effects of concurrent use of alcohol and cocaine. Addiction. 2002;97(7):773–83.
7. Zou Q, Huang Y, Cao J, Zhao H, Wang G, Li Y, Pan Q. Effects of four feeding stimulants in high plant- based diets on feed intake, growth performance, serum biochemical parameters, digestive enzyme activities and appetite- related genes expression of juvenile GIFT tilapia (Oreochromis sp.). Aquacult. Nutr. 2017 ;(5):1076-85.
8. Adamu H, Ahmad MM, Mudi K, Dakani KM, Bakare AT. Predictors of stimulants use among physicians in a Nigerian tertiary health institution in Sokoto, Northwest Nigeria. J Neurosci Behav Heal. 2018;10(2):9–17.
9. Clemow DB, Walker DJ. The potential for misuse and abuse of medications in ADHD: a review. Postgrad Med. 2014;126(5):64–81.
10. Nwala G, Ibeneme C, Ojinnaka N, Ugolee J. Changing trend of psychoactive drug abuse among adolescent students in South Eastern Nigeria. J Drug Abus. 2021; 7:30.
11. Emmanuel FT, Akor SE, Momoh S, Owemidu IO, Akor SE. Investigation of the Hepatotoxicity of Lacatomtom Drink in Albino Rat. Niger J Biochem Mol Biol. 2022;37(1):26–31.

12. Kudirat MB, Danraka A, Abdulmujeeb A. The Public Health Consequences of Substandard Medicines in Nigeria: https://doi. org/10.51412/psnnjp. 2022.43. Niger J Pharm. 2022;56(2).
13. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54(4):275–87.
14. Cheesebrough M. District laboratory practice in tropical countries, part 2 second edition CUP. United Kingdom. 2006;149–54.
15. Nasution MH, Kalanjati VP, Abdurachman A, Aditya D, Pamungkas DB, Syamhadi MR. The Effects of Methotrexate, Moringa oleifera Leaf Extract, and Andrographis paniculata Leaf Extract on the Testes of Hyperglycemic Wistar Rats. Trop. J. Nat. Prod. Res.2023 ;7(6).
16. Verstegen RHJ, Wang G, Langenberg- Ververgaert KPS, Ren LY, Nulman I. Paternal exposure to recreational drugs before conception and its effect on live- born offspring: A scoping review. Birth defects Res. 2020;112(13):970–88.
17. Hamed MA, Ekundina VO, Akhigbe RE. Psychoactive drugs and male fertility: impacts and mechanisms. Reprod. Biol Endocrinol. 2023 28;21(1):69.
18. Semet M, Paci M, Saïas- Magnan J, Metzler- Guillemain C, Boissier R, Lejeune H, Perrin J. The impact of drugs on male fertility: a review. Andrology. 2017 ;5(4):640-63.
19. El-Shennawy L, Kamel MAE naby, Khalaf AHY, Yousef MI. Dose-dependent reproductive toxicity of sodium benzoate in male rats: Inflammation, oxidative stress and apoptosis. Reprod Toxicol. 2020;98:92–8.
20. Lennerz BS, Vafai SB, Delaney NF, Clish CB, Deik AA, Pierce KA, Ludwig DS, Mootha VK. Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Molecular genetics and metabolism. 2015; 1;114(1):73-9.
21. Ren L, Meng M, Wang P, Xu Z, Eremin SA, Zhao J, Yin Y, Xi R. Determination of sodium benzoate in food products by fluorescence polarization immunoassay. Talanta. 2014 :121:136-43.
22. Khan IS, Dar KB, Ganie SA, Ali MN. Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. Drug Chem Toxicol. 2022;45(3):1345-54.
23. Adedokun KI, Oladokun OO, Abraham TF, Osuntokun OS, Atere TG, Baiyewu ST. Ameliorative Potential of Thymoquinone on Male Reproductive Functions in Lead Acetate-Induced Reproductive Toxicity in Male Wistar Rats. Trop J Nat Prod Res. 2023;7(6).
24. Obinna VC, Kagbo HD, Afieroho OE, Agu GO. Haematological profile of albino rats exposed to polar leaf extracts of Portulaca oleracea Linn. GSC Biol Pharm Sci. 2019 (1):075–85.
25. McLachlan RI. The endocrine control of spermatogenesis. Best Pract Res Clin Endocrinol Metab, 2000 ;14(3):345-62.
26. Walker WH. Testosterone signaling and the regulation of spermatogenesis. Spermatogenesis. 2011;1(2):116–20.
27. Chakraborty A, Singh V, Singh K, Singh R. Testosterone signaling in spermatogenesis, male fertility and infertility. In Molecular Signaling in Spermatogenesis and Male Infertility 2019 (pp. 77-84). CRC Press.
28. Oduwole OO, Huhtaniemi IT, Misrahi M. The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. Int. J. Mol. Sci.. 2021 ;25;22(23):12735.
29. Ge RS, Li X, Wang Y. Leydig cell and spermatogenesis. Molecular Mechanisms in Spermatogenesis. 2021:111-29
30. Al-Ani BT, Al-Saadi RR, Wassef HF. Toxic effects of sodium benzoate on the rat testes. Indian J Public Heal Res Dev. 2019;10(10).