


African Journal of Tropical Medicine and Biomedical Research (AJTMBR)



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ISSN: 2141-6397

Vol. 8, No. 2, December 2025



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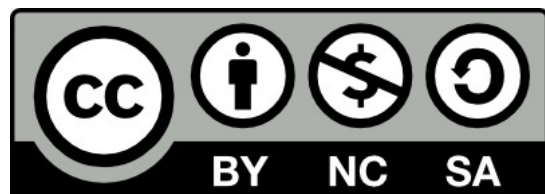


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The Role of Forensic Medicine in Criminal Justice Delivery in Nigeria

¹Dr. U. Umeaku

Department of Morbid Anatomy/ Histopathology, Faculty of Basic Clinical Sciences, Delta State University, Abraka, Nigeria.

Introduction

Forensic medicine is the use of medical knowledge for the purposes of the law.^{1,2} Like in other sub-Saharan African nations, Nigeria's criminal justice system functions within intricate institutional, social, and economic frameworks. One area of the judiciary dedicated to enforcing justice and granting its citizens rights is criminal justice.¹ By using medical expertise in court investigations, forensic medicine—also known as legal medicine—offers a scientific basis for justice. Its function is especially important in situations when the public's trust in legal systems is brittle, investigative resources are scarce, and eyewitness reports may not be trustworthy. Through the promotion of accuracy, justice, and accountability, effective forensic medical practice enhances the delivery of criminal justice. Clinical forensic medicine, toxicology, forensic pathology, and laws pertaining to medical practice are the four primary subfields of forensic medicine.^{1,2} The coroner system, which was in place after British colonisation, is the method of providing forensic pathology services in Nigeria just like other commonwealth countries.²⁻⁴

Determination of Cause, Manner, and Time of Death

Coroner autopsy is one of forensic

medicine's most well-known functions. According to forensic pathologists, cause of death refers to things such as poisoning, physical force trauma, or gunshot wound.^{2,3} Manner of death refers to whether the death is natural, accidental, suicide, homicide, or unidentified. The mode (or mechanism) of death is the specific physiological derangement resulting in death, such as asphyxia, syncope (heart failure), or coma.^{2,3} The use of post-mortem changes like rigor mortis, livor mortis, algor mortis, and decomposition to estimate the time of death. Precise certification of death avoids false allegations, separates criminal from non-criminal fatalities, and directs the focus of investigations.^{2,3}

Coroner Autopsy and Death Investigation

In Nigeria, medicolegal problems frequently centre on deaths resulting from terrorism, traffic accidents, violence, and incarceration. Forensic pathologists are essential to:

- Identifying the cause and manner of death in cases of accidental fatalities, suicide, and suspected homicide,
- Distinguishing between criminally caused and natural deaths, especially in circumstances that are unexpected or unexplained,
- Looking into deaths that occur while

a person is in custody, or in gatherings like hostels, schools, camps e.t.c. as these are of great public and human rights importance.^{4,5}

When carried out correctly, coroner autopsies help police investigations, avoid false allegations, and give judges impartial proof. However, there are still many obstacles to overcome, including a lack of qualified personnel, poor mortuary facilities, and cultural and religious opposition to autopsies.⁵

Examination of Victims of Violence and Abuse

The recording of injuries sustained by victims of assault, armed robbery, child abuse and neglect, domestic violence, and sexual and gender-based violence (SGBV) depends heavily on forensic medicine.⁶ This falls under the area of clinical forensic medicine.

Due to inadequate evidence, SGBV cases are usually underreported and poorly punished in Nigeria and other sub-Saharan African nations. The chances of a successful prosecution are significantly increased when forensic examination, prompt injury documentation, and biological evidence preservation are done while protecting the rights and dignity of the victims.⁶

Injury Interpretation and Crime Reconstruction

By analysing injury trends and connecting them to purported incidents, forensic

specialists support law enforcement organisations. This comprises:

- Separating self-inflicted wounds from defensive injuries,
- Recognising injuries brought on by clubs, machetes, firearms, or homemade weapons,
- Distinguishing post-mortem artefacts from ante-mortem injuries.^{7,8}

These interpretations are especially crucial in areas where narratives may be contradictory and politically sensitive due to insurgency, vigilante violence, and communal conflicts.⁸

Forensic Toxicology and Substance-Related Offences

In Nigeria, forensic toxicology is becoming more and more important because of:-

- An increase in drug trafficking and abuse cases,
- Road traffic accidents caused by alcohol,
- Possible poisoning in political, professional, or domestic settings.

Toxicological analysis aids in proving drug use, poisoning, or intoxication as causes of death or criminal activity. Unfortunately, the usefulness of toxicological evidence in court is frequently jeopardised by inadequate laboratory capacity and delayed sample analysis.⁹

Forensic Psychiatry and the Criminal Justice System

Like many African nations, Nigeria lacks adequate mental health services, yet forensic psychiatry is essential in:

- Evaluating the ability to stand trial,
- Assessing criminal culpability
- Giving judges advice on risk management, treatment, and punishment.¹⁰

By ensuring that mentally ill offenders are treated in compliance with legal requirements and human rights norms, these evaluations help to decrease unwarranted incarceration and enhance rehabilitation results.^{10,11} Forensic psychiatry also falls under clinical forensic medicine.

Expert Testimony and Judicial Decision-Making

As unbiased witnesses, forensic medical specialists help courts make sense of complicated medical data. Clear and impartial expert testimony in Nigeria and other jurisdictions can:-

- Make defences and prosecutions stronger,
- Lessen the dependence on potentially forced admissions,
- Reduce the number of erroneous convictions and acquittals.¹²

Although judicial understanding of forensic evidence is growing, judges, attorneys, and investigators still need ongoing training.¹²

Forensic Medicine and Human Rights Protection

In Nigeria, forensic medicine is essential to the defence of human rights since it examines claims of police brutality and torture, records injuries in situations involving wrongful detention and helps to

handle large deaths during diseases, conflicts, or disasters. The rule of law is upheld and accountability is encouraged by impartial and skilled forensic investigations.¹³

Laws pertaining to Medical Practice

Here it pertains to the functions of Medical and Dental Council of Nigeria which was first established in 1963. Its work include among other things the protection of the public from medical practitioners. This it does through The Medical and Dental Investigating Panel and The Medical and Dental Disciplinary Tribunal.^{14,15} These bodies serve to investigate and discipline the doctor for various types of misconduct including negligence with the tribunal equal in status to that of a high court.^{14,15} The laws pertaining to medical practice also guides the issuance and use of medical certificates including practicing licence. Medical certificates may play a role in criminal justice delivery. Lastly the law relating to medical practice also play a role in how the doctor carries out his duty. The most relevant to this is the importance of proper documentation and as the saying in law goes anything that is not documented never happened. So lack of proper documenting can be considered as the doctor being negligent in the discharge of his duty. It also helps the doctor to remember that each case is a potential medico-legal case.¹³

Challenges to Effective Forensic Medicine Practice

Nigerian forensic medicine service delivery has several major obstacles, such as insufficient funds since the government should bear the expense, subpar mortuary

facilities, lack of qualified forensic pathologists and experts, inadequate cooperation between the police, courts, and health services, limited availability of contemporary forensic labs and religious and cultural objections to autopsies.^{1,9} Legal changes, capacity building, and consistent government commitment are needed to address these issues.

Conclusion

Forensic medicine is a vital component of legitimate criminal justice delivery in Nigeria. It improves investigations, promotes fair trials, defends human rights, and boosts public trust in legal systems by offering unbiased, scientifically supported evidence. To promote justice, accountability, and social stability in the area, forensic medical services must be funded through training, infrastructure development, and institutional independence.

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Umeaku U. Forensic Medicine in Criminal Justice Delivery in Nigeria. *Afr. J. Trop. Med. & Biomed. Res.* 2025; 8(2) 7-11
<https://dx.doi.org/10.4314/ajtmbr.v8i2.15>

Differential Alterations In Behavioural Phenotypes, Brain Biochemical Profiles And Histomorphology In Mice After Prolonged Administration Of Sildenafil-dapoxetine Formulation

¹Omogbiya A, ^{*1}Orovwigho O, ¹Moke EG, ¹Oboh M, ¹Emudainohwo JOT, ²Awhin PE, ^{3,4}Saviour GU

ABSTRACT

Introduction: Cognition encompasses the brain's processes for learning, memory and decision making - functions highly dependent on balanced neurotransmission and oxidative stability. In recent years, aphrodisiac misuse - particularly sildenafil, a phosphodiesterase-5 inhibitor, and dapoxetine, a short-acting selective serotonin reuptake inhibitor - has become prevalent among young adults seeking sexual enhancement without medical supervision. However, the neurocognitive implications of their prolonged use are poorly understood. Hence, this study investigated the cognitive and biochemical effects of prolonged sildenafil-dapoxetine (SIL-DAP) exposure in experimental mice.

Materials and Methods: Adult male mice were divided into seven groups and orally given distilled water, VEH (10 mL/kg), scopolamine (1 mg/kg), sildenafil (1 mg/kg), dapoxetine (1 mg/kg), SIL-DAP (2.5 mg/kg and 5.0 mg/kg), or donepezil (1 mg/kg), respectively, for 60 days (at 2-day intervals). Twenty-four hours after the last treatment, behavioural paradigms (Barnes maze and Rota rod tests) were assessed, followed by biochemical assays of oxidative stress indices (SOD and MDA) and histopathological evaluations in the brain - the prefrontal cortex and hippocampus.

Results: Results show that 30-day SIL-DAP administration enhanced spatial memory, evident as increased escape latency in the Barnes maze, increased coordination in the Rota rod test and enhanced antioxidant enzyme activity. However, prolonged exposure produced dose-dependent neuronal degeneration and reduced antioxidant defence. Histological alterations revealed necrosis within the prefrontal and hippocampal regions, indicating oxidative neurotoxicity.

Conclusion: Collective findings suggest that while acute SIL-DAP use may transiently improve cognition, chronic misuse disrupts redox balance and neuronal integrity, predisposing users to cognitive decline.

¹Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria.

²Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria

³Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

⁴Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Cross River State, Okuku Campus, Yala, Cross River State, Nigeria.

*Corresponding author: oruesedelsu@gmail.com

ORCID Details for Authors:

Orovwigho Oruese – <https://orcid.org/0009-0001-6420-9220>

Omogbiya Adrian Itivere - <https://orcid.org/0000-0002-2731-9287>

Introduction

Cognitive functions such as memory, learning, and decision-making rely on the precise interplay of neurotransmitters, cerebral blood flow, and neural structural integrity. Key brain regions involved in cognition include the

hippocampus (critical for memory and spatial awareness), the prefrontal cortex (responsible for planning, decision-making, and short-term memory), the amygdala (processing emotions like fear and stress), and the basal ganglia (coordinating movement and procedural

memory)¹. Neurotransmitters like acetylcholine, dopamine, serotonin, and glutamate are crucial for nerve cell communication, influencing learning, memory, attention, and mood, particularly in mammals². These disruptions can impact personality, attention, and focus. Cognitive problems can arise from various underlying causes, including impaired neurotransmitter production, nerve cell damage, brain inflammation, reduced blood flow, metabolic imbalances, genetic predispositions, and psychological factors³. Current research is focused on neurotransmitter issues, oxidative stress/inflammation within the nervous system, and genetic abnormalities as significant causes of cognitive impairment^{4,9}. Lifestyle factors and pharmacological agents can disrupt this balance, resulting in cognitive impairment. The increasing off-label and recreational use of aphrodisiacs, particularly sildenafil and dapoxetine raises concerns regarding their long-term neurocognitive effects. Erectile dysfunction (ED) and premature ejaculation (PE) are prevalent sexual dysfunctions affecting millions of men worldwide.

Sildenafil, a phosphodiesterase type (PDE5) inhibitor, increases cyclic guanosine monophosphate (cGMP) levels in penile smooth muscle, facilitating vasodilation and improving erectile function¹⁰. The presence of phosphodiesterases in various parts of the brain and the necessity for maintained levels of cyclic guanosine monophosphate (cGMP) suggest potential roles for PDE-5 inhibitors in treating neurological problems¹¹. Sildenafil has demonstrated the ability to elevate cGMP levels in the brain through the activation the NO/cGMP pathway. This activity may protect against neuroinflammation, improve memory, and prevent nerve cell death in some¹¹⁻¹². Research suggests that cyclic GMP (cGMP)

plays a crucial role in memory formation¹³. Consequently, inhibiting phosphodiesterase type 5 (PDE5), an enzyme that breaks down cGMP, presents a promising approach to improve the initial stages of memory consolidation¹⁴. Studies on healthy young men have demonstrated that sildenafil, a PDE5 inhibitor, can lead to noticeable changes in brain activity patterns associated with improved focus¹⁵. Furthermore, animal studies using mice have shown that sildenafil enhances both the acquisition and retention of memories in tasks like maze navigation and inhibitory learning¹⁶. The mechanism behind these memory-enhancing effects may involve increased blood flow and glucose metabolism, as PDE5 inhibitors promote vasodilation, likely through pathways involving cGMP.

Dapoxetine, a selective serotonin reuptake inhibitor (SSRI), delays ejaculation by increasing serotonin levels in the synaptic cleft, thereby modulating ejaculatory reflexes¹⁷. Research indicates DAP's ability to modulate potassium channels, which influences neurotransmitter release, thus explaining its therapeutic benefits¹⁸. While SSRIs are generally prescribed for depression¹⁹, research has shown potential benefits in neurodegenerative conditions following brain injuries²⁰. SSRIs' protective effects on the brain after stroke may be linked to their ability to increase growth factors in the brain and promote neurogenesis. Specifically, studies have found DAP to protect against neuronal damage caused by glutamate, likely due to effects on mitochondria within these cells¹⁸. The co-administration of sildenafil and dapoxetine is becoming increasingly common in clinical practice for men experiencing both ED and PE, offering a combined therapeutic approach²¹.

Premature ejaculation and erectile dysfunction are separate but often comorbid clinical conditions, and each drug targets different

mechanisms. Sildenafil increases penile blood flow via PDE-5 inhibition, while dapoxetine increases serotonin levels to delay ejaculation²². Although dapoxetine is approved for on-demand treatment of premature ejaculation, its rapid onset and short half-life make it suitable for acute sexual performance modulation²³. Some random controlled trials (RCTs) have shown that combined use of a PDE-5 inhibitor (like sildenafil) with dapoxetine improves clinical outcomes compared to monotherapy in selected patient populations^{24,25}.

Clinically, sildenafil is typically administered on an as-needed basis for erectile dysfunction, while dapoxetine is a short-acting selective serotonin reuptake inhibitor (SSRI) developed specifically for on-demand treatment of premature ejaculation. This pattern of use differs from prolonged exposure, increasing non-prescription access and recreational use of sexual performance - enhancing agents has raised concerns regarding repeated or prolonged intake beyond recommended dosing schedules. In this context, this study was designed to evaluate the behavioural and neurobiochemical consequences of prolonged exposure to a sildenafil-dapoxetine formulation, modelling a 'worst-case' scenario of repeated use and potential misuse. This approach provides toxicological relevance by assessing whether cumulative exposure may influence central nervous system function and behavioural phenotypes.

While both sildenafil and dapoxetine have well-established mechanisms of action related to sexual function, their potential effects on the central nervous system (CNS), particularly in the context of prolonged combined formulation use, are less clear. Sildenafil, although primarily acting peripherally, can cross the blood-brain barrier to a limited extent and influence

neuronal signaling pathways, including those involved in learning and memory²². Dapoxetine, as an SSRI, directly affects serotonin neurotransmission, a critical regulator of mood, cognition, and behavior¹⁸.

The hippocampus, a brain region crucial for spatial learning and memory, is particularly vulnerable to neurochemical imbalances and synaptic dysfunction. Both serotonin and dopamine, neurotransmitters targeted by dapoxetine and potentially influenced by sildenafil, play vital roles in hippocampal-dependent cognitive processes²⁴.

MATERIALS AND METHODS

Animal use and care

In this study, thirty-five (35) Swiss male mice weighing 20–25g were sourced from the laboratory animal house of the Faculty of Basic Medical Sciences, Delta State University (DELSU), Abraka. The rats were housed in animal cages under standard conditions, including a 12/12-hour light/dark cycle and a temperature of 28.1 °C. They had free access to a standard rodent pellet diet (Vital Feeds®, Delta, Nigeria) and water ad libitum. Approval (RBC/FMBC/ DELSU/25/657) for the study was obtained from the Animal Care and Use Research Ethics Committee of the Faculty of Basic Medical Sciences, DELSU.

Drugs and reagents

Scopolamine, Sildenafil, Dapoxetine, Sildenafil-Dapoxetine (Embraga Forte) and Donepezil were obtained from a Pharmacy (Nigeria). Other chemical reagents, such as thiobarbituric acid (TBA) used for the study were of high grade and quality.

Drugs preparation and administration

Freshly prepared sildenafil, dapoxetine, sildenafil-

dapoxetine, scopolamine and donepezil was suspended in 15mL of water (H₂O) and administered at 1mg/kg respectively and vehicle (**distilled water**, 10 mL/kg) by oral gavage for 60 days at two days interval based on preliminary studies. The doses of the above mentioned drugs were based on previous studies, and all treatments were administered between 8 and 10 a.m. daily.

Experimental design

Thirty-five (35) mice were randomly divided into seven (7) groups (n=5) as follows. Group 1: received vehicle (10 mL/kg *oral*), group 2: received scopolamine (SCO, 1mg/kg, *oral*)²⁶, group 3: received sildenafil (SIL, 1mg/kg)²⁷, group 3: received dapoxetine (DAP, 1mg/kg)²⁸, groups 5-6 received sildenafil-dapoxetine, (SIL-DAP) (2.5 and 5.0 mg/kg, *oral*) respectively, while group 7 was treated with donepezil (DON) (1mg/kg, *oral*)²⁹⁻³⁰, alternately for 60 days. Behavioural tests were done at the end of the month, and the results were obtained and recorded.

Behavioural evaluations

Barnes maze³¹

Barnes maze is used to determine how well a mouse learns and recall the position of a designated target, using visual cues placed around the testing area as orientation guides. The number of judgmental errors and latency to escape are recorded.

Rota-rod test³²

On day 31, a test was conducted to assess motor coordination in mice. The mice were placed on a rota-rod, and the time taken for each mouse to fall off during the 120 s test duration was recorded³².

Animal euthanasia and tissue processing

Following behavioural evaluation, three (3) animals per group were euthanized for biochemical assays and homogenized (10 min at 10,000 revolutions per minute, rpm) for postmortem biochemical assays with phosphate buffer (PBS) (10 % w/v, 0.1 M, pH 7.4). Decanted supernatants were stored at -20°C before the assays. Two (2) mice underwent transcatheter perfusion with 4 % PBS formaldehyde for cortico-striatal histomorphology post-mortem analysis. Brain excision and proper fixation in 4 % formalin were carried out simultaneously.

Biochemical assays

Brain oxidative measurement

We measured brain oxidative markers, such as superoxide dismutase (SOD) and malondialdehyde (MDA) activities, which were assayed by using the disappearance of 1hydrogen peroxide, and adrenaline was used to adjudge their levels³³. The lipid peroxidation, malondialdehyde was assayed using Tris-potassium chloride thiobarbituric acid and Griess reagent³⁴.

Histological examination

Haematoxylin and eosin staining techniques were used to evaluate the viability of neuronal cells in the medial prefrontal cortex hippocampus³⁵. The fixed brain tissues were processed to obtain paraffin wax-embedded tissue blocks, trimmed, sectioned, and processed through the stages of fixation, dehydration, clearing, infiltration and staining using hematoxylin and eosin³⁶. Slides were images were captured with a digital camera (Leica ICC50 E, Germany) connected to a computer interface (Magnafire). The number of viable neurons was counted in the striatum using Image-J software (NIH, Bethesda, MD, USA).

Statistical analysis

After normality tests, the data were analyzed using

two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons between the experimental groups. The results were presented as mean \pm SEM using GraphPad Prism software version 8.4.3. All statistical differences were considered significant at $p < 0.05$.

RESULTS

Effects of Sildenafil–Dapoxetine on Spatial Learning in the Barnes Maze

Escape latency in the Barnes maze (Fig. 2) revealed significant differences following 30-day treatment. SCO substantially delayed escape relative to VEH, confirming deficits in spatial learning. SIL and DAP produced increased

latencies relative to VEH as well, indicative of cognitive improvement. Compared with SCO, improvement in spatial memory can be seen in SIL, DAP, and both SIL–DAP doses. Against the DON group, SIL–DAP 2.5 mg/kg increased escape latency, whereas SIL–DAP 5.0 mg/kg showed a mild reduction.

At 60 days, escape latencies were elevated across all SCO-treated groups, indicating worsening learning impairment with continued cholinergic dysfunction. SIL–DAP 2.5 mg/kg showed a more marked increase in latency over time, while the 5.0 mg/kg dose also failed to sustain improvements, suggesting dose-dependent divergence in long-term cognitive outcomes.

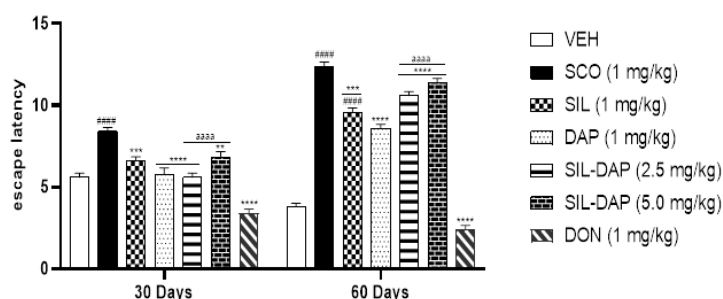


Fig. 1: The Effect of SIL-DAP on spatial working memory in male mice using Barnes maze test. Bars are expressed as mean \pm SEM of grouped mice ($n=5$). #### $p < 0.0001$ Vs VEH group. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ Vs SCO group. ^a $p < 0.05$, ^{aaaa} $p < 0.0001$ Vs DON group (Two-way ANOVA followed by Turkey Post-hoc test).

Prolonged administration of Sildenafil–Dapoxetine Alters Motor Coordination in the Rotarod Test

Grip strength analysis (Fig. 4) showed that SCO and DAP groups presented significant reductions relative to VEH at 30 days, whereas SIL increased grip strength. When compared with SCO, all groups including SIL, DAP, both SIL–DAP doses, and DON displayed significantly enhanced grip strength, induced motor function. Against DON, both SIL–DAP

doses elicited additional increases in strength, further indicating enhanced motor coordination. By 60 days, a decline in grip strength emerged across groups excluding DON group, particularly in SCO-exposed animals, demonstrating progressive impairment during prolonged cholinergic dysfunction. All treatments showed reduced performance relative to 30-day values.

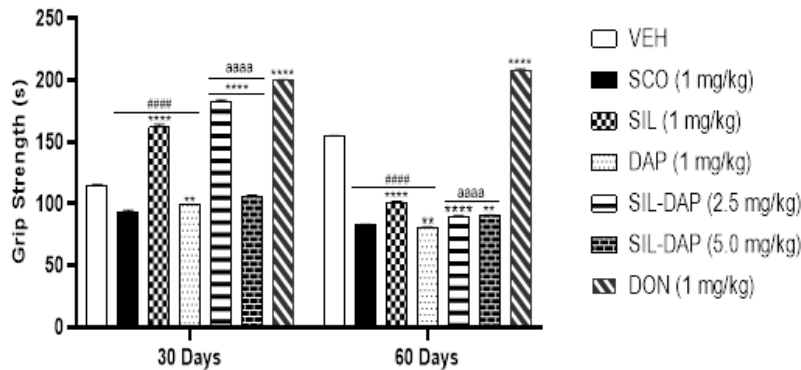


Fig. 2: The Effect of SIL-DAP on motor coordination (Grip Strength) in male mice using Rota Rod. Bars are expressed as mean ± SEM of grouped mice (n=5). ##### p<0.0001 Vs VEH group, **** p<0.0001, ** p<0.01 Vs SCO group, **** p<0.0001 Vs DON group. (Two-way ANOVA followed by Turkey Post-hoc test)

Prolonged Administration of Sildenafil-Dapoxetine Alters Superoxide Dismutase Levels

SOD activity (Fig. 6) was significantly reduced in SCO, SIL, and DAP groups relative to VEH at day 30. Groups administered SIL, DAP, SIL-DAP 2.5 and 5.0 mg/kg, and DON

elevated SOD relative to SCO. At 60 days, further reductions in SOD were observed in SCO, SIL, DAP, SIL-DAP 2.5mg/kg and SIL-DAP 5.0 mg/kg groups. DON significantly retained SOD levels compared with SCO, indicating stronger antioxidant activity at this duration.

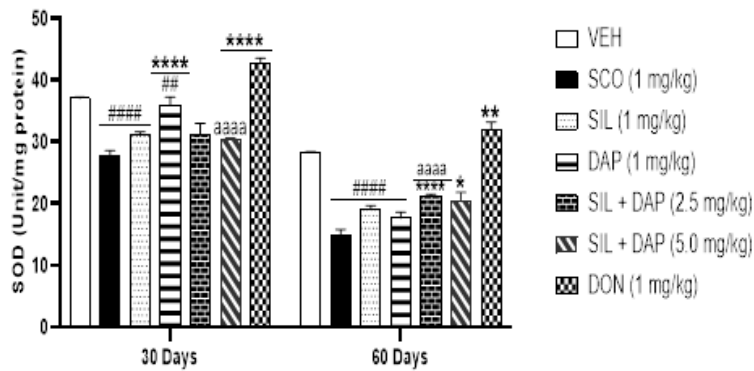


Fig. 3: The Effect of SIL-DAP on superoxide levels in mice brain. Bars are expressed as the mean ± SEM of grouped mice (n=5). ##### p<0.0001, ## p<0.05 Vs VEH group. **** p<0.0001, ** p<0.001, * p<0.01 Vs SCO group. **** p<0.0001 compared to DON group. (Two-way ANOVA followed by Turkey Post-hoc test)

Sildenafil-Dapoxetine Reduces Malondialdehyde Formation

MDA levels (Fig. 9) increased significantly in SCO-treated animals compared with VEH at 30 days. SIL-DAP 2.5 mg/kg and SIL-DAP 5.0 mg/kg significantly reduced MDA relative to

SCO. At 60 days, SCO, SIL, and DAP showed further increases in MDA. SIL, DAP, SIL-DAP 2.5 mg/kg, and DON showed significant reductions relative to SCO. SIL-DAP 5.0 mg/kg was elevated relative to DON, indicating potential oxidative stress at the higher dose.

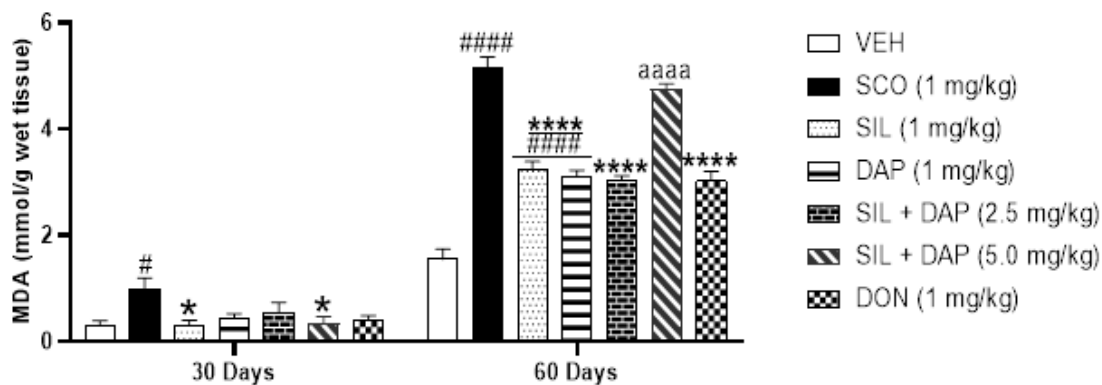


Fig. 4: The Effect of SIL-DAP on malondialdehyde levels in male mice brain. Bars expressed as mean \pm SEM of grouped mice (n=5). #####p<0.0001, #p<0.05 Vs VEH group. ****p<0.0001, *p<0.05, Vs SCO group. aaaa p<0.0001 Vs DON group. (Two-way ANOVA followed by Turkey Post-hoc test)

Histopathological Alterations in the medial prefrontal cortex (mPFC)

Histological examination (Figs. 12) revealed preserved neuronal architecture in VEH and DON groups at both time points. SCO produced marked neuronal necrosis in mPFC at 30 and 60 days. SIL and DAP groups exhibited

emerging degenerative changes by 60 days. SIL-DAP 2.5 mg/kg displayed neuronal shrinkage at 60 days, whereas SIL-DAP 5.0 mg/kg showed overt necrosis, indicating a dose-dependent exacerbation of neurotoxicity with prolonged exposure.

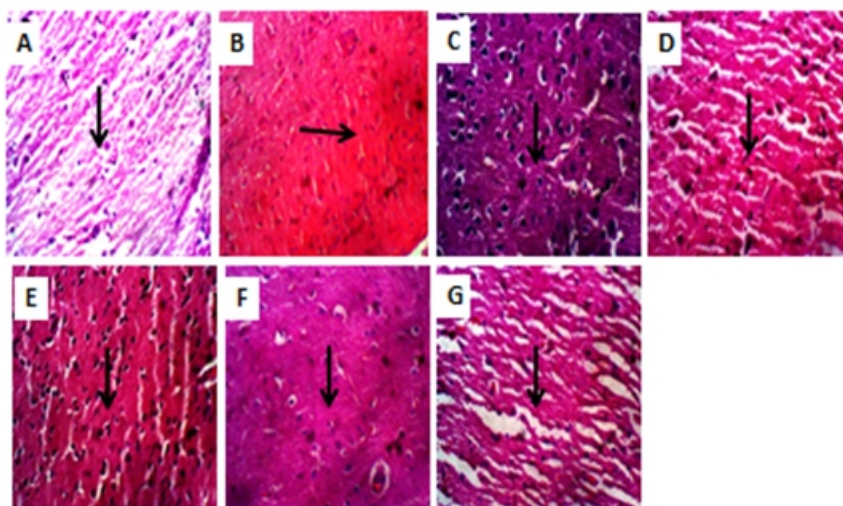


Plate. 1 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the medial prefrontal cortex (mPFC) in mice for 30 consecutive days. A: VEH, B: SCO (1mg/kg), C: SIL (1mg/kg), D: DAP (1mg/kg), E: SIL-DAP 2.5 (1mg/kg), F: SIL-DAP 5.0 (1mg/kg), G: DON (1mg/kg). Sections stained with H and E. Magnification *400, scale =10um. Sections stained with H and E. Magnification *400, scale =10um

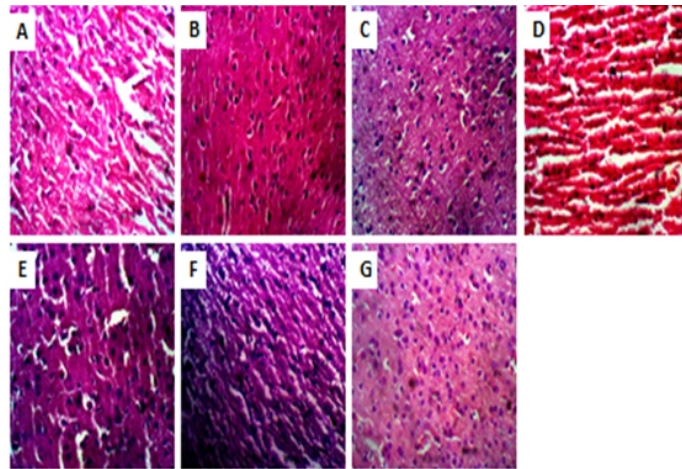


Plate. 2 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the medial prefrontal cortex (mPFC) in mice brain for 60 consecutive days. **A:** VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections stained with H and E. Magnification *400, scale =10um.

Histopathological Alterations in the Hippocampal CA1 Region

Histological examination (Fig. 13) revealed preserved neuronal architecture in VEH and DON groups at both time points. SCO produced marked neuronal necrosis in CA1 at 30 and 60 days. SIL and DAP groups exhibited

emerging degenerative changes by 60 days. SIL-DAP 2.5 mg/kg displayed mild neuronal shrinkage at 60 days, whereas SIL-DAP 5.0 mg/kg showed overt necrosis, indicating a dose-dependent exacerbation of neurotoxicity with prolonged exposure.

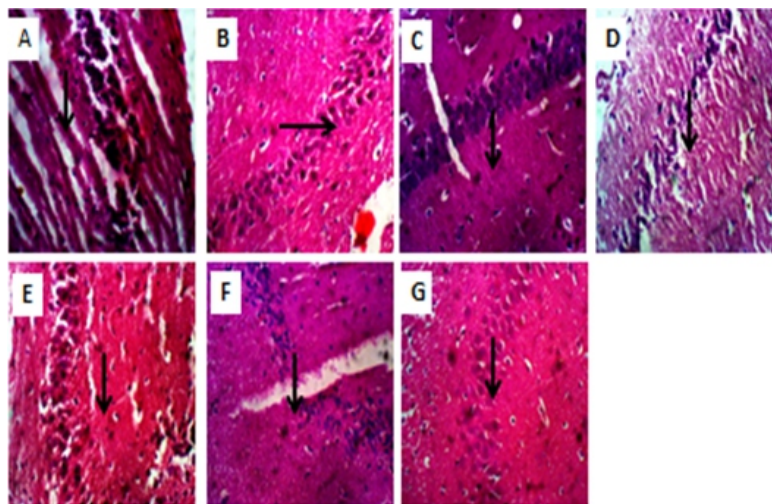


Plate. 3 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA1) in mice for 30 consecutive days. **A:** VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections stained with H and E. Magnification *400, scale =10um.

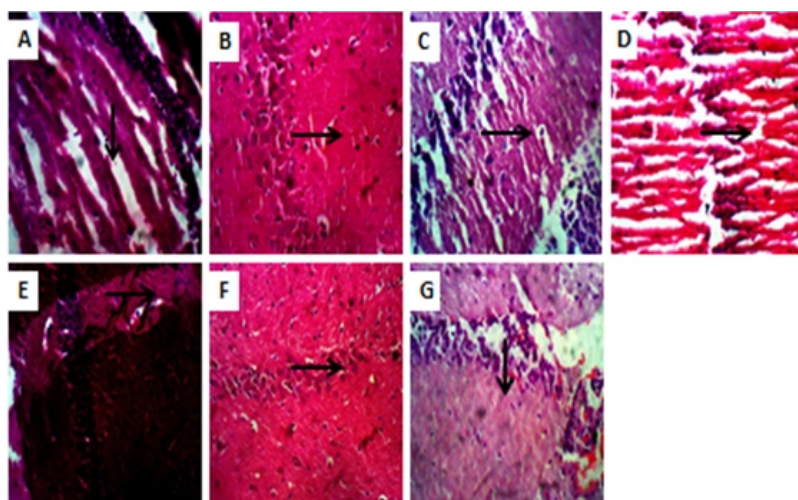


Plate. 4 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA1) in mice brain for 60 consecutive days. **A:** VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections stained with H and E. Magnification *400, scale =10um.

Histopathological Alterations in the Hippocampal CA3 Regions

Histological examination (Fig. 14) revealed preserved neuronal architecture in VEH and DON groups at both time points. SCO produced marked neuronal necrosis in CA3 at 30 and 60 days. SIL and DAP groups exhibited

emerging degenerative changes by 60 days. SIL-DAP 2.5 mg/kg displayed mild neuronal shrinkage at 60 days, whereas SIL-DAP 5.0 mg/kg showed overt necrosis, indicating a dose-dependent exacerbation of neurotoxicity with prolonged exposure.

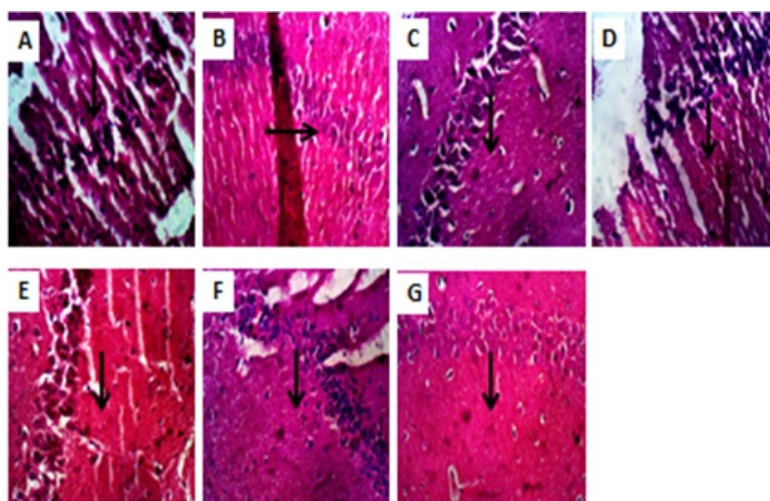


Plate. 5 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA3) in mice for 30 consecutive days. **A:** VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SILDAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections stained with H and E. Magnification *400, scale =10um.

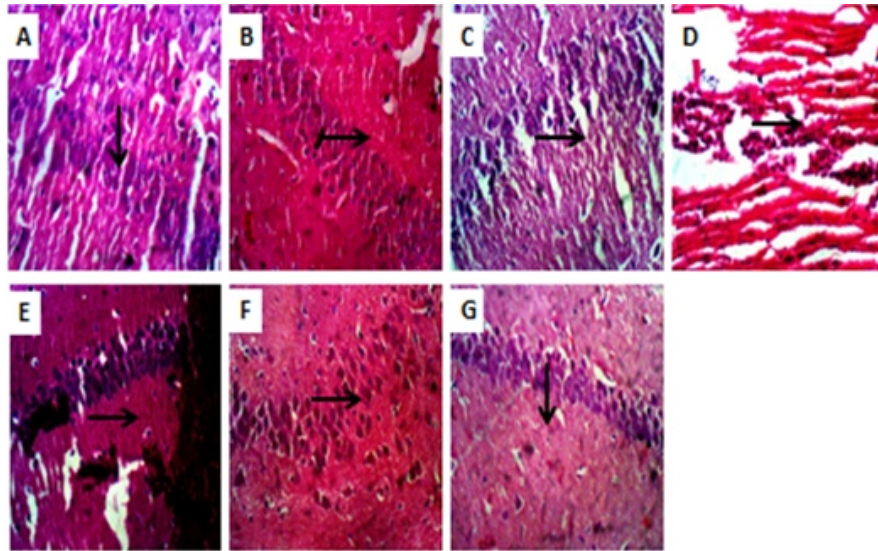


Plate. 6 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA3) in mice brain for 60 consecutive days. **A:** VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SILDAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections stained with H and E. Magnification *400, scale =10um.

DISCUSSION

Memory is both a result of and an influence on learning perception and attention³⁷. The act of remembering consists of attention to an event followed by the representation in the brain of the event. Repetitive practice results in a cumulative effect on memory and enables several activities such as reading and playing games. However, memory is prone to distortions that can have serious consequences in everyday life, which can disrupt normal functioning in specific brain regions³⁸. For example, damage to the hippocampus, a crucial structure for learning and memory processing can interfere with long-term memory storage and retrieval³⁹. Similarly, damage to the amygdala, which plays a vital role in storing, retrieving, and processing emotional memories, can affect emotional memory, emotional responses, and decision-making abilities⁴⁰.

This study investigated the neurobehavioural,

biochemical and histopathological consequences of prolonged administration of a sildenafil–dapoxetine (SIL–DAP) combination therapy on cognitive and biochemical indices in mice. Using a comprehensive assessment of behavioural tasks, oxidative stress markers, and histology, our findings reveal a complex, duration- and dose-dependent profile of SIL–DAP effects. While low-dose treatment (2.5 mg/kg) demonstrated cognitive improvement at early time points, prolonged administration—particularly at 5.0 mg/kg—was consistently associated with oxidative stress, and structural degeneration across prefrontal and hippocampal regions, accompanied by functional deterioration in memory, motor coordination and neurotrophic signalling.

The effect of SIL–DAP on memory performance was also evaluated in mice through the Barnes maze test, which assesses non-spatial working memory⁴¹ by leveraging on the ability of mice to

learn and remember the location of a target zone using a configuration of distal visual cues located around the testing area⁴². Also, the effect of SIL-DAP on motor coordination was evaluated using the Rota Rod test and indices such as grip strength was assessed. The time the mice spend on the turning cylinder reflects its balance, coordination, physical condition, and motor planning skills⁴³. Across the Barnes maze, and Rotarod tasks, SCO-treated animals consistently exhibited profound impairments, confirming disruption of cholinergic neurotransmission. These deficits intensified over 60 days, indicating progressive deterioration of motor coordination and working, spatial memory under persistent cholinergic antagonism. SIL-DAP produced a biphasic behavioural profile. At 30 days, the 5.0 mg/kg and 2.5 mg/kg doses improved spontaneous alternation and reduced escape latency relative to SCO, suggesting partial cognitive compensation. At 60 days, both doses exhibited declining performance in the Barnes maze and Rotarod tasks, reflecting emergent cognitive disruption with prolonged treatment. This pattern suggests that SIL-DAP may initially modulate neurotransmission favourably but eventually induces neurochemical instability that compromises cognition. This progressive impairment parallels the trajectories observed in other psychoactive combinations, where chronic modulation of monoaminergic pathways disrupts cholinergic plasticity and hippocampal-dependent learning.

MDA is a biomarker for oxidative stress and lipid peroxidation⁴⁴, it's a reactive aldehyde formed when polyunsaturated fatty acids in cell membranes are damaged by free radicals⁴⁵. SOD acts as an enzyme that ensures the conversion of oxidative molecules such as superoxide anions into oxygen and hydrogen peroxide⁴⁶. The peculiarity of its actions elucidates O₂- and

hydrogen peroxide, and hence, the likelihood of its central role in the defence mechanism⁴⁷. Biochemical assays revealed that prolonged SCO exposure induced profound oxidative stress, demonstrated by elevated MDA and corticosterone levels, alongside reductions in SOD, CAT, and GSH. These changes are well-documented hallmarks of cholinergic neurotoxicity and impair synaptic signaling by promoting lipid peroxidation, mitochondrial failure, and neuronal apoptosis.

SIL and DAP monotherapies modulated these oxidative profiles but, over time, contributed to significant depletion of antioxidant reserves. Importantly, the SIL-DAP combination intensified these changes, especially at the 5.0 mg/kg dose. MDA elevation alongside reductions in SOD, at 60 days indicates that chronic SIL-DAP exposure generates an increasingly pro-oxidative neural milieu. This indicates a dose dependent relationship, the higher the dose, the more pronounced antioxidant effect.

Histological analysis of the mPFC and hippocampal CA1/CA3, corroborated the biochemical and behavioural findings. SCO induced classical necrotic profiles consistent with cholinergic neurodegeneration across day 30 and 60 respectively. SIL-DAP exhibited clear dose-dependent neurotoxicity at day 60. The 2.5 mg/kg dose produced neuronal shrinkage and cytoplasmic alterations, while the 5.0 mg/kg dose consistently showed overt necrosis across all regions examined at day 60. These patterns indicate that combining a PDE5 inhibitor with an SSRI amplifies neurotoxic vulnerability over time, likely through synergistic effects on mitochondrial burden, excitotoxicity, and impaired synaptic repair mechanisms.

Collectively, these findings demonstrate that while short-term administration of SIL-DAP

may confer behavioural benefit, prolonged exposure induces marked oxidative stress, cholinergic depletion, neurotrophic suppression, and structural degeneration, resulting in progressive cognitive and motor impairments.

The dose-dependent neurotoxic effects observed particularly at 5.0 mg/kg raise significant concerns regarding chronic combined use of sildenafil and dapoxetine. Given their frequent use in human contexts, these results underscore a need for caution and further mechanistic investigation into long-term interactions between serotonergic and nitric oxide–cGMP pathways within prefrontal and hippocampal circuits.

CONCLUSION

The present study provides compelling mechanistic evidence that prolonged co-administration of sildenafil and dapoxetine produces significant neurocognitive impairment through converging disruptions across cholinergic, oxidative, and neurotrophic pathways. Chronic SIL–DAP exposure impaired spatial working memory, spatial learning, and motor coordination, reflecting widespread dysfunction across prefrontal–hippocampal circuits. These behavioural deficits corresponded with suppression of endogenous antioxidant defences, and increased lipid peroxidation indicating a pronounced shift toward oxidative toxicity.

Histomorphology findings provide structural confirmation of these biochemical disturbances, demonstrating neuronal shrinkage, cytoplasmic vacuolation, pyknotic profiles, within the medial prefrontal cortex and hippocampal CA1/CA3 subregions. Together, these alterations represent a mechanistic cascade in which oxidative damage, and

neurotrophic depletion, collectively undermine neuronal viability and synaptic plasticity.

Taken together, these findings indicate that prolonged sildenafil–dapoxetine exposure imposes substantial neurobiological and cognitive risk, particularly when used outside clinical supervision. This study underscores the urgent need for stronger public health messaging and regulatory attention regarding the unmonitored use of sildenafil–dapoxetine combinations, and provides a foundational mechanistic framework for future investigations into their long-term neuropsychiatric consequences.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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<https://dx.doi.org/10.4314/ajtmbr.v8i2.1>

Pre-Examination Stress Among Pre-Clinical Medical Students: A Nigerian Survey

¹Ominde, BS, ²Enaohwo, MT, ²Jeremiah, O, ²Omor, OF, ²Igbigbi, PS.

ABSTRACT

Introduction: The demands of medical education predispose to high stress levels, compromising students' well-being and performance. This study investigated the sources, severity, and effects of pre-examination stress and the coping mechanisms adopted by medical students in a Nigerian University.

Materials and Methods: This e-survey conducted in Delta State, Nigeria adopted a descriptive cross-sectional design. A questionnaire developed by Google Forms was disseminated using WhatsApp to 200 and 300 level medical students in the Faculty of Basic Medical Sciences after ethical authorization. It encompassed questions concerning the causes and effects of pre-exam stress, and the coping mechanisms. S-Anxiety subscale of the State-Trait Anxiety Inventory (STAI) was included to assess the prevalence and severity of stress. Fully completed questionnaires were received from 167 respondents, aged 16-30 years. The Statistical Package for Social Sciences (Version 27.0) compared the frequencies using the Chi-Square test and analysed the differences in STAI scores using the independent t-test and the analysis of variance. Significance was set at $p < 5\%$.

Results: Mean anxiety scores were higher in females, 200 level students and those residing in personal rooms. High anxiety levels were more prevalent in females (46, 49.5%) than males (28, 37.8%) ($p < 0.05$). Prayer and physical exercises were the predominant coping strategies in females and males respectively.

Conclusion: Medical education can adversely affect students' mental health. To mitigate this, universities should design curricula that address students' challenges and provide mental health resources for students.

Key words: Exam stress, mental health, academic performance, coping strategies.

¹.Aga Khan University, 3rd Parklands Avenue, Nairobi Kenya

².Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Corresponding author: Ominde BS

INTRODUCTION

Stress refers to any threat to an individual's biopsychosocial well-being, including reactions to distress that exceeds one's tolerance capacity.^{1,2} It encompasses one's physical, psychological, or emotional feedback to modifications that pose potential danger to one's stability and well-being.^{3,4}

Medicine education, though noble, is highly competitive, requiring outstanding entrance exam performance,⁵ with an aim of producing skilled physicians, the programme's comprehensive curricula can have overwhelming effect on students' psychosocial wellbeing.⁶⁻⁸ Mental health challenges can drastically reduce quality of life.¹ Medical students experience higher levels of stress, anxiety and depression compared to the

general populace, yet institutional support for their psychological well-being are often insufficient.^{4,9} Suicide ranks as the second leading cause of student mortality.⁴

The intense pressure to excel in exams contributes to medical students' anxiety.¹⁰⁻¹² Test anxiety elicits cognitive, physiological and behavioural responses, driven by fear of failure.^{5,13} Excessive anxiety negatively impacts academic performance leading to psychological distress, while moderate levels of anxiety can be motivating.^{3,9}

Exam stress arises from the competitive medical training and the pressure to excel, driven by societal expectations and scholarship requirements.^{2,4} Fear of exams, recurring tests, ineffective study habits, tight schedules, past failures, poor time management, uncertainty about the future, and overwhelming workloads are the main contributors.^{2,4,13,14} Exam stress is furthermore aggravated by social factors like high parental expectations, poor living conditions, financial constraints, insufficient rest and poor nutrition.^{3-5,12}

Exam anxiety elicits a multifaceted response, including changes in behaviour, metabolism, hormone levels, psychology and immunity whose degrees vary depending on factors such as exam type, physical activity, age, past experiences, gender, endurance, individual's temperament, spirituality and cultural background.^{9,12,15} Junior students and commonly females experience more significant impact of exam anxiety characterized by the following; burnouts, memory impairment, anxiety disorders and decreased focus.^{6,9-11,16} Students become isolated and experience impaired cognitive function that subsequently compromises their academic performance and also patient care in future.^{4,7} Common

documented symptoms of exam stress include sweating, headache, palpitations and fatigue.^{2,16} Extreme or high anxiety levels make learning difficult and this may prompt the students to use stimulants in order to relive stress symptoms. Unfortunately, despite knowing the potential side effects of these stimulating substances, students choose to disregard them.¹⁷

Addressing student stress levels is crucial for ensuring quality medical education.^{1,16} Identifying the causes of stress helps in effective prevention of depression and anxiety.^{4,9,15} The intention of this research was to investigate the sources, levels, and effects of pre-examination stress at a Nigerian university and identify the relevant coping strategies.

Materials and methods

This study employed a descriptive cross-sectional e-survey design, targeting second- and third-year medical students at a Nigerian tertiary institution. Approval was secured from the ethics committee of the Faculty (Ref. No: RBC/FBMC/DELSU/24/464).

Participants included adult students (aged ≥ 18 years) enrolled in the Medicine and Surgery program who were preparing for their end-of-semester examinations, scheduled to begin two weeks after the distribution of the questionnaire. Students with diagnosed psychiatric conditions or those currently taking antidepressants or antipsychotic medications were excluded to minimize confounding variables. Students aged below 18 years were also excluded from this study. The investigators explained the purpose and rationale for the study to those who met the selection criteria. Participants who gave their informed consent were included in a WhatsApp group. A questionnaire was developed using Google Forms and distributed via the WhatsApp

platform. It was sent to a total of 127 second-year students (200 level) and 43 third-year (300 level) students, with 170 questionnaires filled and returned, forming the study population. To ensure confidentiality, no identifying information, such as names or matriculation numbers, were included in the questionnaire.

The questionnaire contained five sections: the first section gathered demographic information, while the second section utilized the Spielberger State-Trait Anxiety Inventory (STAI), specifically the state anxiety subscale (S-STAI). The third section assessed the psychological and academic causes of stress, while section four investigated the physical and mental effects of exam stress. The final stage focused on evaluating which coping strategies the students utilized to alleviate stress symptoms.

A validated tool called the S-Anxiety subscale of the S-STAI, confirmed for accurately assessing exam-stress among medical students, was employed to determine the severity and prevalence of exam stress.¹² This self-rated 20-item questionnaire yields scores ranging from 20 to 80, with responses rated on a four-point Likert scale: 1 (not at all), 2 (somewhat), 3 (moderately), and 4 (very much so). Negative items, such as "I feel tense," were scored directly, whereas positive items, such as "I feel calm," were reverse scored. A total score below 40 indicated low anxiety, while scores between 40-59 and 60-80 indicated moderate and high anxiety levels, respectively¹⁸

Data collected were entered into Microsoft Excel and analyzed using SPSS software (Version 27.0, Chicago IL, USA). The prevalence of stress was calculated and presented as percentages, with comparisons based on gender and year of study assessed using the Chi-Square test. Quantitative variables,

such as age and STAI scores, were summarized using means and standard deviations, and their differences evaluated using the unpaired t-tests and analysis of variance. These tests were significant at $P < 0.05$.

RESULTS

Out of the 219 medical students, 170 voluntarily participated in this study. However, 3 students did not fully complete their questionnaires hence, these were excluded from the study. The study evaluated the anxiety levels of 167 medical students, including 125 second-year students (74.9%) and 42 third-year students (25.1%), representing 78.13% and 71.19% of their respective classes. The sample comprised more females (93, 55.7%) than males (74, 44.3%). Participants' ages ranged from 16 to 30 years, with a mean age of 19.56 ± 1.96 years. Notably, males had a higher average age (20.19 ± 2.35 years) than females (19.06 ± 1.41 years), while the mean age of second-year students (19.46 ± 2.13 years) was slightly lower than that of third-year students (19.86 ± 1.30 years), although this difference was not statistically significant ($p = 0.524$). Participants were categorized by age, with the majority falling within the 16-20 age group (126, 75.4%), followed by the 21-25 age group (38, 22.8%), and lastly the 26-30 age group (3, 1.8%). Most participants resided in university hostels (94, 56.3%), while others lived in private hostels (41, 24.6%) or in their own accommodations (32, 19.2%) (Table 1). Mean anxiety scores were significantly higher among females compared to males ($P = 0.035$) and among second-year students compared to third-year students ($P = 0.047$). Both class and sex categories exhibited moderate anxiety levels. Regarding living arrangements, students in their own rooms or apartments reported the highest anxiety scores, while those in university hostels had the lowest ($P = 0.042$) (Table 2). The younger age-groups displayed moderate anxiety levels, while those in the 26-30 age group demonstrated

higher anxiety scores although the variances were not statistically significant ($P=0.390$) (Table 2).

A majority of students experienced moderate anxiety (78, 46.7%), followed by high anxiety (74, 44.3%), with only a few reporting low anxiety levels (15, 9.0%) (Table 3). High levels of anxiety were more prevalent in females (46, 49.5%) compared to males (28, 37.8%). In contrast, moderate and low anxiety levels were more common in males ($p=0.031$). Among second-year students, high anxiety levels were predominant (60, 48%), while moderate and low anxiety were more frequent in third-year students ($P=0.024$). Additionally, anxiety levels varied by area of residence ($P=0.019$), with high anxiety most common among those living in their own accommodations (19, 59.4%) and moderate to low anxiety being more frequent among hostel residents (Table 4).

The main causes of exam stress included unsatisfactory revision time (151, 90.4%), heavy workload (144, 86.2%), irrational thoughts about exam results (131, 78.4%), difficulty recalling information (127, 76%), and disturbed sleep (120, 71.9%). Notably, certain stressors, such as difficulty recalling information, feeling inadequately taught, disrupted sleep, lack of physical activity and finding medical concepts difficult were reported more by females ($P<0.05$) (Table 5). The most common effects of exam stress were fatigue (152, 91.0%), disturbed sleep cycles (135, 80.8%), mood swings (119, 71.3%), and irritability (117, 70.1%). Females reported higher rates of various stress effects, including stomach nervousness, loss of appetite, irritability, mood swings, decreased concentration, sleep disturbances, and headaches ($P<0.05$) (Table 6). The predominant coping strategies included praying (145, 86.8%), engaging with social

media (142, 85.0%), listening to music (135, 80.8%), and sleeping (134, 80.2%). Prayer was more frequently utilized by females, while males were more likely to engage in physical exercise to relieve stress ($P<0.05$) (Table 7). Among respondents, 79 students (47.3%) found these methods occasionally eased their stress, while 54 (32.3%) reported that coping strategies often provided relief (Table 8). Most participants (104, 62.3%) noted little improvement in daily performance, although 52 (31.1%) experienced marked improvement. Nine students (5.4%) reported no effect on their performance from coping methods, and two (1.2%) noted a slight decline. No significant gender differences were found in the effectiveness of coping strategies ($P>0.05$) (Table 9). Table 10 summarizes the prevalence of the different levels of anxiety in various populations.

DISCUSSION

The prevalence of different levels of anxiety varied from frequencies reported in different populations (Table 10).^{6,7,10,12} Rahman *et al.*¹ reported 78.3% of Malaysian preclinical students facing stress while Rajanayagam *et al.*² observed a significant rise in anxiety among Indian students during exams. Competitive medical education, especially the basic sciences like Biochemistry and Anatomy, aggravates stress affecting one's mental health.¹¹ Improving the relationships between lecturers and students and addressing the causes of stress may alleviate high anxiety levels.¹⁹

Females scored significantly higher anxiety scores, congruent with Rehman *et al.*⁷ In contrast, lack of sex differences in anxiety scores was documented by Farajpour and Mashoufi⁹ and Divya *et al.*⁸ Severe anxiety was more predominant in females, while moderate and low anxiety levels were prevalent in males, conforming to the findings by Memon *et al.*¹⁰ Patil & Aithala³ described higher anxiety in males. The

metacognitive beliefs, traditional responsibilities and higher academic expectations make females more prone to anxiety.¹⁰ Females have higher adrenocorticotrophic hormone (ACTH) levels and subsequent elevation in estradiol and cortisol levels, which impact their responses to stress.¹⁸ Furthermore, females tend to over-report symptoms and express more concerns about their workload.¹⁹

Both 200-level and 300-level students showed moderate anxiety, with 200-level students scoring significantly higher. Patil *et al.*¹⁶ found mild to moderate stress in final-year students, while Nagpal *et al.*¹² reported moderate anxiety in first-year students. Stress progressively increases, usually reaching a peak among final-year students¹⁶⁻²⁰. Variations in anxiety levels may result from the exam experience of 300-level students. Although clinical rotations contribute to stress, final-year students often report fewer symptoms but still face anxiety from excess workloads and transitions to no jobs.^{10,20}

The age differences among the 200-level and 300 level students were not statistically significant. Younger students experience higher anxiety levels, which decreases with age.^{6,10,14,15} Older students have developed better coping strategies for exam stress over time.¹³ Students living in private accommodations reported higher anxiety levels than those in university hostels who perhaps were exposed to more support and better social interaction.

The primary stressors in our study included lack of revision time, excessive workload, irrational thoughts about exam results, inability to recall information, and disturbed sleep cycles. Similar issues were recorded in previous research among clinical students at the same university.¹⁵ Other common causes documented by other scholars include lengthy syllabi,¹⁶ poor time

management and exam marathon³ excessive lectures and financial constraints,¹ high parental expectations and sleep disturbances.¹² Females reported higher stress levels from recalling information and inadequate teaching, while males were more stressed by workloads.³ Additionally, females also reported stress due to distance from parents. Rehman *et al.*⁷ found more females experienced pre-exam memory loss, hence requiring more time and effort to comprehend concepts.

Improving time management skills is essential for reducing last-minute cramming, enhancing comprehension, and alleviating exam anxiety.¹² To manage course overload, curricula should be structured into “must know,” “desirable to know,” and “nice to know” categories in an 80:20:10 ratio, with student input and interactive teaching.¹² Reducing teacher-related stress requires clear learning outcomes, effective communication, and adequate resources.¹⁵ Inadequate sleep and poor nutrition due to financial constraints, negatively affect cognitive function and performance. This emphasizes the need for access to healthy and affordable meals.^{15,19}

In the current study, pre-exam anxiety resulted in fatigue, disturbed sleep, mood swings, and irritability. Rizvi *et al.*¹¹ reported comparable issues, particularly among females, with symptoms linked to increased stress hormones which can lower immunity and contribute to weight gain. In our study, 12.9% of female participants reported menstrual disturbances, marginally lower than the 15.19% in Karachi who associated this with hormonal imbalance.¹¹ Moreover, 32.9% of respondents used energy drinks, lower than the 38.94% reported by Rizvi *et al.*¹¹ Khalifah *et al.*¹⁷ noted that 84.9% consumed tea and 70.1% coffee. These can boost mood but may also lead to sleep disruption and fatigue.¹¹

Students in our study managed stress through prayer, social media, music, and sleep. Prayer and meditation promote calmness and self-esteem.^{11,15} Coping strategies by medical students reported in literature include; sports and religious practices,¹ and reliance on familial support.⁴ None of our students reported alcohol use, and 1.2% smoked, compared to 2.6% and 13.4% reported by Khalifah *et al.*¹⁷ who inferred that medical student have lower levels of substance use compared to their peers. Females primarily used prayer, while males preferred to exercise. According to Sonali *et al.*¹⁴ females are more open to counselling. Despite the knowledge of coping mechanisms, very few medical students employ these techniques.¹⁹ In our study, 47.3% reported coping mechanisms sometimes alleviated stress, with 31.1% noting marked improvement. These were higher than the findings of Loya and Jiwane⁵. There is therefore a need for effective stress management, and universities should prioritize recreational facilities.^{1,12}

There is need for early diagnosis of pre-exam stress and accessible counselling to prevent complications.^{2,6,12,15} A structured orientation program can enlighten students about course expectations and support facilities.^{5,6,12} Collaboration among parents, educators, and administrators is essential to improve living environments and alleviate stress.¹² To protect medical students' mental well-being, revising the curriculum is essential.^{5,14,19} This entails re-evaluating lecture timings and teaching methods.¹⁵ Regular assessments and access to question banks can significantly reduce exam-related anxiety.⁵ The current study employed the state anxiety portion of the S-STAI test to specifically assess pre-examination stress. This focused approach shortened the survey and encouraged more accurate and prompt responses from students, thereby enhancing the

reliability of the collected data. Conduction of the study in a single university and adoption of the convenience sampling restricted the sample size, thus, findings can't be extrapolated to the larger medical student population. The qualitative data gathered may have been subjective while responses could have been constrained owing to the utilization of structured questions. Additionally, there is potential for reporting bias, as students may have adjusted their answers to align with perceived expectations or social desirability. We recommend a multi-institutional study to increase the sample size and allow comparisons across diverse populations. Including clinical-year students would provide a more comprehensive understanding of stress factors in medical education. Future research should also explore the relationship between stress levels and academic performance.

Conclusion

Medical education can adversely affect students' mental health. Universities should design curricula that address students' challenges, foster a supportive learning environment, regularly evaluate students' mental health and make mental health resources accessible and readily available.

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<https://dx.doi.org/10.4314/ajtmbr.v8i2.2>

Evaluation Of Computational And Insecticidal Activities Of Oils From *Ocimum Gratissimum* And *Cymbopogon Citratus* Against *Anopheles Gambiae* Mosquito

¹Elelegu, EJ, ¹Dunkwu, CC, ¹Enyi, KC, ²Onuelu, JE, ¹Onyesom, I.

ABSTRACT

Introduction: This study investigated the insecticidal and computational activities of essential oils from *Ocimum gratissimum* (OgEO), *Cymbopogon citratus* (CcEO), their combination (OgEO+CcEO), and permethrin against *Anopheles gambiae* mosquito stages.

Materials and Methods: Permethrin the standard chemical insecticide, demonstrated superior efficacy, with LC₅₀ values of 0.1 ± 0.07 µg/mL (adulticidal), 0.001 ± 0.006 µg/mL (pupicidal), 0.005 ± 0.0003 µg/mL (larvicidal), and 0.01 ± 0.004 µg/mL (ovicidal), significantly lower than other treatments (*p* < 0.05). The OgEO+CcEO combination exhibited synergistic effects, with LC₅₀ values of 4.5 ± 0.2 µg/mL (adulticidal), 5.0 ± 0.4 µg/mL (pupicidal), 1.6 ± 0.3 µg/mL (larvicidal), and 1.3 ± 0.2 µg/mL (ovicidal), outperforming individual oils (*p* < 0.05). OgEO was more effective than CcEO, with LC₅₀ values of 5.7 ± 0.3 µg/mL (adulticidal) and 1.8 ± 0.5 µg/mL (ovicidal) compared to CcEO's 6.3 ± 0.2 µg/mL and 5.1 ± 0.2 µg/mL, respectively. *In silico* study revealed strong binding of thymol (OgEO) to catalase (-5.14 docking score, -61.84 kCal/mol MMGBSA) and γ-murolene (CcEO) to chorion peroxidase (-5.85 docking score, -74.65 kCal/mol MMGBSA), indicating disruption of key mosquito proteins involved in oxidative stress and eggshell formation.

Results: Pharmacokinetic analyses highlighted thymol's high gastrointestinal absorption and blood-brain barrier permeability, suggesting systemic toxicity, while γ-murolene's high lipophilicity supports its suitability for topical or volatile applications. Both oils exhibited significant reduction in mosquito reproduction and enhancing their vector control potential.

Conclusion: Despite permethrin's unmatched efficacy, the OgEO+CcEO combination offers a promising eco-friendly alternative due to its synergy and lower environmental impact.

KEYWORDS: Mosquito-borne diseases, Essential oils, *Ocimum gratissimum*, *Cymbopogon citratus*, *Anopheles gambiae*, Insecticidal activity, Synergistic effects

1. Department of Medical Biochemistry, Faculty Basic Medical Sciences, joanebubepasbush@delsu.edu.ng, ORCID: 0009-0006-0229-6855

2. Department of Medical Biochemistry, Delta State University, Abraka, Nigeria. Email: doctorryri49@delsu.edu.ng, ORCID: 0009-0001-3759-5218

3. Department of Medical Biochemistry, Delta State University, Abraka, Nigeria. enyi-kingsley@delsu.edu.ng, ORCID: 0009-0008-6861-3731

4. Department of Pharmacology, Faculty of Allied health sciences Delta State University Abraka. onuelujackson@gmail.com, ORCID:0009-0004-1992-9829

5. Department of Medical Biochemistry, Delta State University, Abraka, Nigeria. ionyesom@delsu.edu.ng, onyesominin@yahoo.co.uk, Orcid: 0000 0001 7095 7666

Corresponding author: ELELEGU, Ebube Joan. Department of Medical Biochemistry.

Faculty Basic Medical Sciences joanebubepasbush@delsu.edu.ng, ORCID: 0009-0006-0229-6855

INTRODUCTION

Background to the Study

Mosquito-borne diseases remain a significant

global public health challenge, causing substantial morbidity and mortality, particularly in tropical and subtropical regions. Among the most formidable culprits are malaria, dengue fever,

Zika virus, and chikungunya. According to the World Health Organization (WHO), malaria alone claimed about 597,000 lives in 2024, with the majority of deaths occurring in WHO Africa Region, particularly among pregnant women and children under the age of five.^[1] Malaria, caused by *Plasmodium* parasites and transmitted primarily by *Anopheles* mosquitoes, is one of the oldest and deadliest mosquito-borne diseases. The disease causes low birth weight, miscarriages or stillbirths, and infant death in sub-Saharan Africa.^[2] There are around 500 species of *Anopheles* mosquitoes that serve as *Plasmodium* parasite vectors worldwide.^[3] Mosquitoes are efficient vectors of various pathogens, making them responsible for the transmission of deadly diseases affecting millions of people worldwide. Despite extensive efforts to control and eradicate malaria, the disease continues to impose a heavy toll on human health and well-being, particularly in sub-Saharan Africa.^[4]

Protection against mosquito bites is one way of being used to reduce illness prevalence.^[5] Treatment with antimalaria drugs, use of prophylactic drug, insecticidal treated bed-nets, removal of stagnant waters, among others have been employed in the treatment and control of mosquito-borne diseases.^[7] Some of these strategies are expensive, not easily accessible to rural dwellers, produces discomfort and faced with the issue of resistance.^[8] The extensive use of synthetic insecticides in recent times for mosquito control has led to the development of resistance in mosquito populations.^[8] Mosquitoes can rapidly evolve resistance mechanisms, rendering widely-used insecticides ineffective and limiting the options available for disease control.^[9] The WHO has identified insecticide resistance as a major obstacle to malaria control and eradication efforts.^[10] Chemical insecticides are not only

hazardous to human and animal health but they are also toxic to the environment and their bio-accumulation in the environment results in ecosystem imbalance.^[7] In the face of these challenges, there has been a growing interest in exploring natural alternatives for mosquito control. Essential oils derived from various plant species have shown promise as effective and environmentally friendly mosquito control agents.^[11] Presently, insecticidal properties of different plants including scent leaf and lemon grass have been the subject of intense research.^{[6][12]}

Scent leaf (*Ocimum gratissimum*) is a tropical plant with a long history of use in traditional medicine and culinary practices. Studies have indicated the potential larvicidal and adulticidal activities of essential oil extracted from scent leaf against mosquito vectors.^[13] Also, lemon grass (*Cymbopogon citratus*), another plant with diverse applications, has also exhibited mosquitocidal properties. Nguyen *et al.*^[14] conducted a study highlighting the larvicidal activity of *Cymbopogon citratus* essential oil against *Anopheles gambiae*, a major vector of malaria. Hence, this research aims to assess the synergistic mosquitocidal activity of scent leaf and lemon grass oil across the life cycle stages of *Anopheles gambiae* mosquito.

MATERIALS AND METHODS

Materials

The materials used in this study included; fresh leaves of *Ocimum gratissimum* and *Cymbopogon citratus*, Clevenger-type essential oil distillation unit (Borosil Glass Works Ltd., India), heating mantle (MF500), distilled water, white enamel rearing bowls, glass jars with conical gauze nets, mosquito netting cages, pipettes, plastic containers, yeast, Biological Oxygen Demand (BOD) incubator (Heracell 150i MD), 10 % glucose solution, 20 % glucose solution,

compound microscope (Swift SW380T), universal containers, Permethrin among others.

Collection and Authentication of Plants

Ocimum gratissimum (Scent leaf) and *Cymbopogon citratus* (Lemon grass) plants were collected in Abraka, in Ethiope East L.G.A. of Delta State, Nigeria. Abraka (Longitude: 6° 04'E, Latitude: 5° 54'N) has a tropical wet and dry climate, with a lengthy wet season and relatively constant temperatures throughout the year. The plants were identified by Leaf Snap App at the point of collection and authenticated in the Herbarium Unit, Department of Botany, Delta State University, Abraka. Fresh leaves of *Ocimum gratissimum* with Voucher number: DELSUH-196 and *Cymbopogon citratus* with Voucher number: DELSUH -244 were then taken to Department of Pharmacology and Toxicology, Faculty of Pharmacy, Delta State University, Abraka where the study was carried out. The leaves were washed with running tap water to remove dust particles and debris from their surfaces, and thereafter, allowed to air dry for 21 days at room temperature (23-31 ° C) and pulverized for further analysis^[12]

Oil Extraction by Hydro distillation

Hydro distillation was carried out in a Clevenger apparatus (Clevenger-Type Essential oil Distillation Unit, Borosil Glass Works Ltd., India) according to the methods of Sadgrove and Jones^[15]. About 1kg each of *gratissimum* and *citratus* sample was directly immersed in 10 L of distilled water. The solid-liquid mixture was heated until boiling under atmospheric pressure using heating mantle (MF500). The volatile substance present in the plant sample evaporated along with the steam generating column by the water. This azeotropic mixture was then condensed in the condensing column of the Clevenger apparatus (Clevenger-Type Essential oil Distillation Unit, Borosil Glass

Works Ltd., India) and separated by its density difference and immiscibility. The essential oil collected was stored in universal container at room temperature for use.

Mosquitocidal Test

Mosquito rearing, maintenance and identification

Rearing of mosquito in the laboratory is of primary importance in the maintenance of a mosquito colony. These laboratory maintained colonies are by themselves of great relevance as they readily provide sufficient number of mosquitoes, both aquatic and adult stages, for insecticide bioassays^[14].

The materials and methods for rearing of mosquitoes as described by Onyido *et al.*^[16] were adopted for this study. This involved the use of yeast for *Anopheles* larvae which are surface feeders. A rearing bowl (white enamel pan 30 cm x 12.5 cm) was placed outside for about 24-48 hours to obtain about 500 1st instar larvae of the *Anopheles*, a measured quantity of 0.5g of yeast had been found to be suitable. This technique was developed out of necessity for rearing higher numbers of adult mosquitoes. Distilled water was used as it does not require aeration at any stage of the larval growth.

For the adult maintenance, as the pupae emerged from the larvae, they were hand-picked with the aid of pipette into a glass jar covered with a conical gauze net about 16 cm in diameter and about 19 cm high, with an opening at the apex about 2.5 cm in diameter. This cone prevent the adult from drowning in the water of the pupal bowl and the females from laying the eggs into the pupal bowl, but rather in the special egg bowl prepared and provided for them. The cage also was provided with 10 % solution of glucose on which males and females that had not taken blood meals could feed. This container was in the form of a 7.5 cm x 2.5 cm tube inverted in another tube

slightly larger in diameter and 5 cm longer. The smaller one was covered with a piece of lint and the large diameter tube filled with 20 % glucose solution. The lint acted like a wick and remained damp with glucose solution. The wick was washed twice daily, and the glucose solution also renewed twice a week. Like humans, mosquitoes have need for space. As a result some mosquitoes could mate in the laboratory cage while others cannot. One 30 cm³ cage of mosquito nettings or gauze on a wooden frame was well fitted for the maintenance of those species of mosquito which would mate in captivity. For ordinary day-to-day colony maintenance, the ideal number of adult mosquitoes per cage of this size were 500 mosquitoes (approximately 250 males and 250 females). This ensured adequate mating and egg production for perpetuation of the colony. Larger numbers lead to high mortalities and smaller number to inadequate fertilization of the females. Also, the insects were maintained in a biological oxygen demand (BOD) incubator (Heracell 150i MD), under controlled conditions of temperature 27 ± 2 °C, relative air humidity 75 ± 5 , and a 12-hour light and dark photoperiod.

These reared mosquitoes were then identified to species level using taxonomic keys and morphological characteristics. Experienced entomologists from the department of Animal and Environmental Biology, Delta State University, Abraka, employed microscopes to examine key morphological features of the mosquitoes for accurate identification.

Ovicidal activity

Ovicidal efficacy bioassays were carried according to the methods of Obiang *et al.*^[17]. One mL of the essential oils of *Ocimum gratissimum*, *Cymbopogon citratus* and combine form of both (*Ocimum gratissimum* and

Cymbopogon citratus) at concentration of 1, 2, 3, 4, 5 and 10 % prepared with the appropriate volume of distilled water in six plastic cups (115 mm diameter and 80 mm depth). Then, 20 recently-laid eggs were held in the insectary, and the egg mortality was recorded at 0, 60, 120 and 300 sec post-treatment. The experiment was conducted in duplicates.

Larvicidal assay

A larvicidal assay was conducted to evaluate the efficacy of the oils against *An. gambiae* larvae according to the method outlined by Antonio-Nkondjio *et al.*^[18]. This assay aimed to assess the ability of the oils to kill or inhibit the development of *An. gambiae* larvae, thereby disrupting their life cycle and reducing mosquito populations. Experimental studies were carried out using standardized protocols to determine the larvicidal activity of the oils. Various concentrations (1, 2, 3, 4, 5 and 10 %) of the oil were applied to the larvae, and mortality rates were monitored at 0sec, 60 sec, 120 sec and 24 hrs. Permethrin was used as control to compare the efficacy of the oils against untreated larvae. The experiment was conducted in duplicates

Pupicidal activity

Pupicidal activity was assessed according to the protocol provided by Fernandes *et al.*^[19]. Twenty pupae of *Anopheles gambiae*, with a maximum of 24 of life, were placed in a plastic container with 10 mL of the oily solution at different concentrations (1, 2, 3, 4, 5 and 10 %). Pupae mortality/ mosquito emergence were verified after 0sec to 5min, 60 mins, 24 hrs, 48 hrs and 72hrs of exposure. The experiments was performed in a BOD incubator (Heracell 150i MD) as described above. The negative and positive controls were maintained, and the duplicates were assessed, as described previously for the ovicidal test.

Adulticidal activity

The protocols which was used to evaluate the effect of the oils on adult mosquitoes, were adopted from Nunes *et al.*^[20]. The walls of the plastic containers was moistened with the oily solutions at various concentrations (1, 2, 3, 4, 5 and 10 %) and allowed to dry. Twenty *An. gambiae* mosquitoes (5–6 days of emergence) were placed in these containers, and the mortality was checked at 0sec to 5min, 60 mins, 24 hrs, 48 hrs and 72hrs. The experiment was conducted in duplicates. This method replicate the method of indirect application of insecticides on surfaces and allow the evaluation of their residual effect.

In silico study

In the *In silico* study, target proteins crucial for insecticidal activities in mosquitoes were docked with the retrieved compounds of the plants' essential oils. The focus was, therefore, on insecticidal proteins (Aquaporins-3, Catalase, 3-Hydroxykynurenine transaminase, carbonic anhydrase, Arylalkylamine, N-Acetyltransferase, Chorion peroxidase, V-ATPase, and Phosphofructokinase). As well as insect resistance reversal proteins (Cytochron p450 monooxygenases, Glutathione S-transferases).^[21] *In silico* study employed to determine the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the essential oils include OSIRIS tools.

Retrieval and Preparation of Sterilants, insecticide resistance, and Insecticidal activity protein Targets

The three dimensional structures of flight inhibition targets; Ornithine decarboxylase [UniProt ID: A0A1S4H8Q3], Catalase [UniProt ID: Q6RBZ5], Heme oxygenase [UniProt ID: Q7Q288], Vitellogenin 2 [UniProt ID: Q9NAW8], ATP-dependent 6-phosphofructokinase [UniProt ID:

A0A1S4GVQ5], V-type proton ATPase catalytic subunit A [UniProt ID: Q5TTG1], Catalase [UniProt ID: Q6RBZ5], Carbonic anhydrase [UniProt ID: Q6WLH6], 3-hydroxykynurenine transaminase [UniProt ID: Q7PRG3], Aquaporin [UniProt ID: Q7PWV1], Chorion peroxidase [UniProt ID: Q7QH73], and Glutathione S-transferase 1 [UniProt ID: Q93113] were downloaded from AlphaFold and UniProt database and further prepared using Glide's protein preparation wizard. Missing protein residues (atoms and loops) which are essential to protein structures were fixed using loop refinement methods. Using energy minimization process, the protein structures were optimized to exclude steric clashes, refine conformation and improve geometry.

Ligand Retrieval and Preparation

Bioactive compounds of *Ocimum gratissimum* (commonly known as African basil) and *Cymbopogon citratus* (lemongrass) were obtained from PubChem Database and prepared using the LigPrep 2.4 software, which can generate a number of structures from each input structure with different ionization states, tautomers, stereochemistries, and ring conformations to eliminate molecules based on various criteria such as molecular weight or the number and type of functional groups present with correct chiralities for each successfully processed input structure.^[22] The OPLS-2005 force field was employed for optimization, which resulted in the ligand's low-energy conformer.^[23]

Binding Site Prediction and Receptor Grid Generation

SiteMap was used to generate binding site characteristics, enabling visualization in Maestro. It initiated with a search phase identifying potential binding regions on or near the protein surface, termed sites, using a grid of site points. Subsequently, contour maps are produced,

delineating hydrophobic and hydrophilic features, further categorized into donor, acceptor, and metal-binding regions. The evaluation phase assesses each site by computing various properties, integrated into the Maestro project upon completion. Using the best ranked sitemap, receptor grids were generated for each of the proteins using Receptor Grid Generation module embedded in maestro software suite.^[24]

Receptor Based Virtual Screening

To evaluate the docking parameters, all potential compounds were docked into the protein targets using Schrodinger's Grid-Based Ligand Docking (Glide) software.^{[25] [26]} Glide 5.6's Receptor Grid Generation module was used to define the active site for docking ligands. To investigate the binding modes of the compounds against individual targets, two distinct docking techniques were used, standard precision (SP) and extra precision (XP) docking, were carried out.

Prime MM/GBSA Calculations

The Prime/MM-GB/SA technique is used to compute the free energy of binding. This method is used to calculate the free energy of binding for a given collection of ligands to a receptor. The docked postures were reduced using Prime's local optimization function, and the complex energies were estimated using the Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) (2005) force field and the generalized-Born/surface area (GB/SA) continuum solvent model. The binding free energy, G_{bind} , is computed as follows^[26]:

$$\Delta G_{bind} = \Delta E + \Delta G_{solv} + \Delta G_{SA} \quad (1)$$

$$\Delta E = E_{complex} - E_{protein} - E_{ligand} \quad (2)$$

Where $E_{complex}$, $E_{protein}$, and E_{ligand} are the minimized energies of the protein-inhibitor complex, protein, and inhibitor, respectively

$$\Delta G_{solv} = G_{solv}(complex) - G_{solv}(protein) - G_{solv}(ligand)$$

Where $G_{solv}(complex)$, $G_{solv}(protein)$, and $G_{solv}(ligand)$ are the solvation free energies of the complex, protein, and inhibitor, respectively:

$$\Delta G_{SA} = G_{SA}(complex) - G_{SA}(protein) - G_{SA}(ligand)$$

Where $G_{SA}(complex)$, $G_{SA}(protein)$, and $G_{SA}(ligand)$ are the complex, protein, and inhibitor surface area energies, respectively.

Assessment of Pharmacokinetic (drug-likeness and ADMET) and physicochemical properties

Promising hit compounds having good docking score, XP GScore, and MMGBSA dG Bind were selected for ADMET studies in addition to other physicochemical analysis using ADEMETS lab 3.0 for further analysis. ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties are crucial parameters in drug discovery and development. Predicting the ADMET properties of small molecules is essential for optimizing drug design and development strategies, as well as for assessing the safety and efficacy of drug candidates.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0 for Windows (GraphPad Software, San Diego, CA, USA). For the ovicidal, larvicidal, pupicidal and adulticidal assays, the significant differences between the groups were analyzed using one-way ANOVA and Tukey post-hoc test ($p < 0.05$). The LC_{50} was calculated using non-linear regression, considering a 95% significance level.

RESULTS

Mosquitocidal Studies

The Figures (4.1 a-d) present the lethal concentration (LC_{50}) values required to achieve 50% mortality in *Anopheles gambiae* mosquitoes at

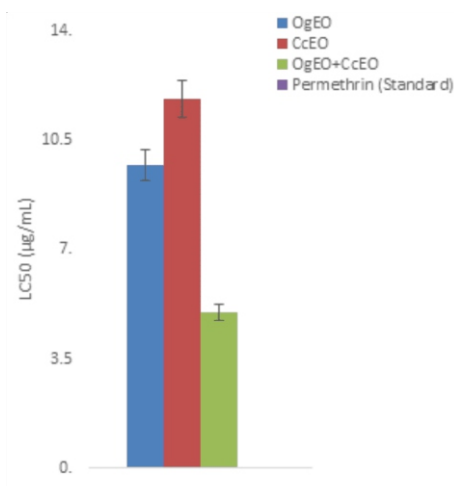
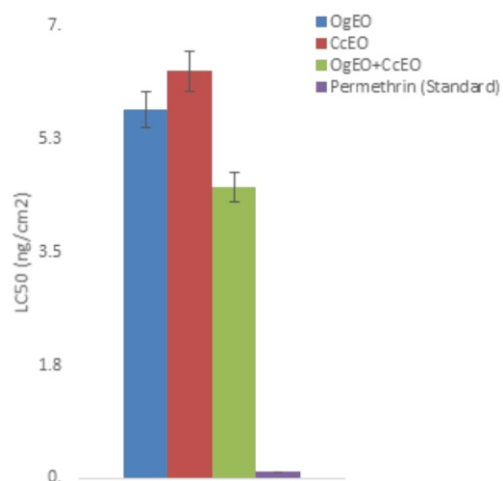
different life stages (ovicidal, larvicidal, pupicidal, and adulticidal) for four treatments: *Ocimum gratissimum* essential oil (*OgEO*), *Cymbopogon citratus* essential oil (*CcEO*), their combination (*OgEO*+*CcEO*), and permethrin (the standard insecticide). The LC_{50} values were reported as mean \pm standard deviation.

OgEO alone is more effective than *CcEO*, with LC_{50} values of 5.7 ± 0.3 for adulticidal, 9.7 ± 4.0 for pupicidal, 4.1 ± 0.2 for larvicidal, and 1.8 ± 0.5 for ovicidal activities, compared with *CcEO*'s 6.3 ± 0.2 , 11.8 ± 0.7 , 5.3 ± 0.6 , and 5.1 ± 0.2 , respectively. *OgEO*'s lower LC_{50} values were statistically significant ($p < 0.05$) compared with *CcEO*, indicating greater potency. The ovicidal activity of both *OgEO* and the combination suggests some statistical overlap, but *OgEO*'s LC_{50} (1.8 ± 0.5) is still significantly lower than *CcEO*'s (5.1 ± 0.2), indicating better ovicidal activity.

CcEO is the least effective among the essential

oils, with the highest LC_{50} values in most stages, and its ovicidal LC_{50} (5.1 ± 0.2) activity is significantly higher than that of permethrin, *OgEO*, and the combination ($p < 0.05$). The pupicidal activities of *OgEO* and *CcEO* suggest that their LC_{50} values (9.7 ± 4.0 and 11.8 ± 0.7 , respectively) may not differ significantly from each other in this stage, although, both were significantly less effective than the combination and permethrin.

Statistically, data showed that the LC_{50} values for each treatment were significantly different from one another within each life stage ($p < 0.05$). The low standard deviations for most LC_{50} values (i.e., 0.07 for permethrin in adulticidal, 0.2 for the combination in larvicidal) suggest high precision in the measurements, reinforcing the reliability of the observed differences. However, the higher standard deviation for *OgEO* in pupicidal activity (9.7 ± 4.0) indicates greater variability, which may warrant further investigation into its consistency in this stage.



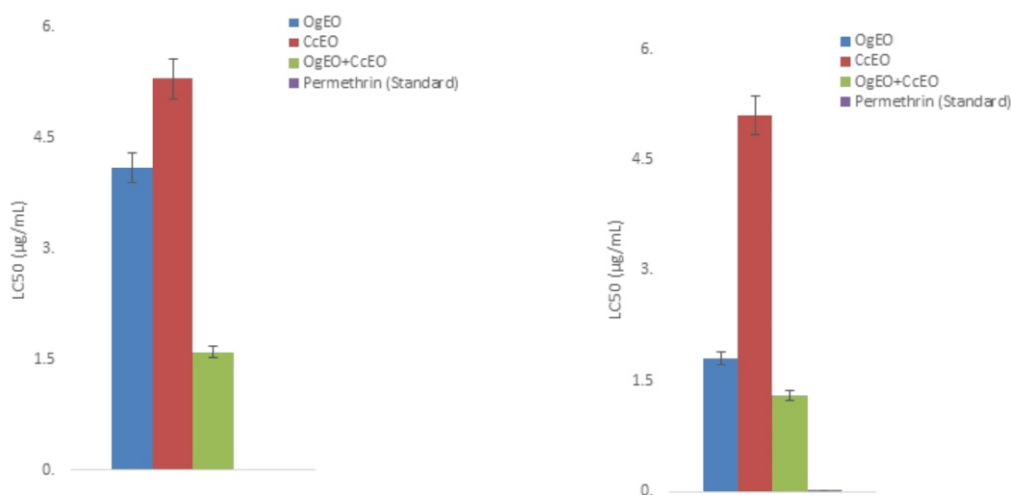


Figure 4.1: Adulticidal (a) and Pupicidal (b) efficacy (LC₅₀) of *Ocimum gratissimum* essential oil (OgEO), *Cymbopogon citratus* essential oil (CcEO), their combination (OgEO+CcEO), and permethrin. Lavicidal (c) and Ovicidal (d) efficacy (LC₅₀) of *Ocimum gratissimum* essential oil (OgEO), *Cymbopogon citratus* essential oil (CcEO), their combination (OgEO+CcEO)

In silico study for target proteins crucial for insecticidal activities

The 17 (camphene, β -caryophyllene, α - and β -pinene, α -humulene, sabinene, β -myrcene, limonene, 1,8-cineole, trans- β -ocimene, linalool, α - and -terpineol, eugenol, α -copaene, β -elemene, p-cymene, thymol, carvacrol) and 12 (6-methylhept-5-en-2-one, camphene, limonene, nonan-4-ol, citronellal, neral, geranial, citral, geranylacetate, β -caryophyllene, γ -muurolene, caryophyllene oxide) compounds, respectively obtained from the essential oils of *Ocimum gratissimum* and *cympopogon citratus* and retrieved from pub database were docked against 9 proteins (aquaporin 3, catalase, 3-hydroxykynurenine transaminase, carbonic anhydrase, aralkylamine, N-acetyltransferase, chorion peroxidase, V-ATPase, phosphofructokinase) essential for *Anopheles gambiae* mosquito survival, and so served as insecticidal targets. The results obtained are displayed in Figures 4.2–4.4.

Figure 4.2 showed the binding data of thymol (PubChem ID: 6989) from *O. gratissimum* with catalase and γ -muurolene (PubChem ID: 12313020) from *C. citratus* with chorion peroxidase (UniProt ID: Q7QH73). These compounds produced the strongest binding among the compounds in *O. gratissimum* and *C. citratus* against the docked targets.

The visual data on two key aspects of binding interactions (bond type and amino acid residue interactions) between thymol and catalase, and then, γ -muurolene and chorion peroxidase are shown in Figure 4.3 a-d.

The 2D interaction complexes between thymol and catalase, and then, γ -muurolene and chorion peroxidase are shown in Figures 4.4.

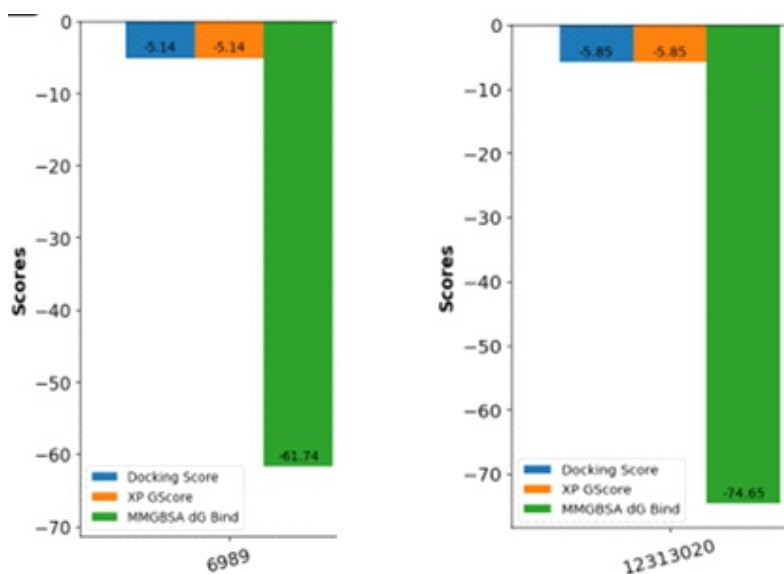


Figure 4.2: Docking scores of thymol (PubChem ID: 6989) against catalase (UniProt ID: Q6RBZ5), (A), and γ-murolene (PubChemID: 12313020) with chorion peroxidase (UniProt ID: Q7QH73), (B).

The docking scores of the best two compounds from *Ocimum gratissimum* (thymol) and *Cymbopogon citratus* (γ-murolene) against two different proteins are presented in Figure 4.5. In A, catalase (UniProt ID: Q6RBZ5), binding with thymol, showed a docking score of -5.14, XP GScore of -5.14 also, with an MMGBSA dG Bind of -61.84 kCal/mol, and an

aggregate score of -1.79. However in B, the stronger interaction was with chorion peroxidase (Q7QH73), where γ-murolene achieved a docking score of -5.85, an XP GScore of -5.85, and an MMGBSA binding energy of -74.65 kCal/mol with a normalized aggregate score of -2.08.

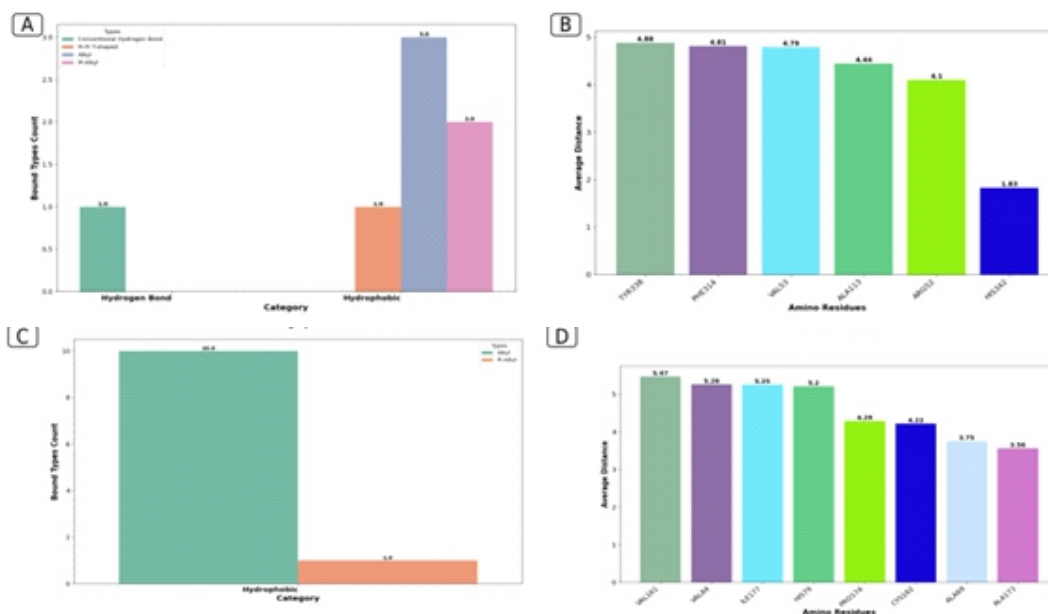


Figure 4.3: The binding interactions between Thymol and Catalase (A and B) and γ -Muurolene and Chorion peroxidase (C and D). On [A], the bond type distribution shows one hydrogen bond and six hydrophobic interactions, specifically consisting of three Pi-Alkyl, two Alkyl, and one Pi-T-shaped bond. On [B], the average distances of these bonds with various amino acid residues are displayed. Tyr 338 and Phe 314 form the longest bonds (~ 4.88 Å and 4.81 Å), while His 342 forms the shortest bond (1.83 Å), indicating a strong interaction. This suggests that hydrophobic interactions dominate the binding of thymol, with hydrogen bonding playing a minor but important role in stabilizing the complex, especially through the

interaction with His 342.

On C, the binding interactions of γ -Muurolene from *Cymbopogon citratus* with Chorion peroxidase (UniProt ID: Q7QH73) are dominated by hydrophobic interactions, with 10 alkyl bonds and 4 pi-alkyl. On D, regarding the amino acid residues involved, Leu 589 had the longest average bond distance at 5.49 Å, followed by Phe 135 (5.08 Å), Phe 534 (5.01 Å), and Ile 629 (4.89 Å). The shortest bond distances are observed with Val 532 (4.14 Å), His 529 (4.33 Å), and Phe 594 (4.35 Å). These distances indicate that Leu 589 plays a key role in binding, with strong hydrophobic interactions throughout the site.



Figure 4.4: 2D Binding interaction complexes between thymol and catalase (A), and then, γ -Muurolene and chorion peroxidase (B).

4.3 Pharmacokinetic and Physicochemical Studies

As shown in Table 4.1, Thymol: exhibits high gastrointestinal absorption and blood-brain barrier permeation. It is moderately lipophilic, does not interact with P-glycoprotein, but inhibits CYP1A2. It shows good drug-likeness with minimal rule violations and easy synthetic accessibility. Gamma-Muurolene: Highly hydrophobic with low gastrointestinal absorption and no blood-brain barrier permeation. It inhibits CYP2C19 and CYP2C9, which suggests possible metabolic interactions. It has moderate drug-likeness but higher synthetic complexity. Ursolic Acid: A large, highly lipophilic molecule with significant polar interaction potential. It shows low gastrointestinal absorption and is not BBB permeant. Despite higher bioavailability score

(0.85), it has multiple rule violations and high synthetic difficulty.

Thymol, with one hydrogen bond acceptor and donor each, has a high MR (48.01) and TPSA (20.23), indicating more potential for polarity. Its iLOGP (2.32) and XLOGP3 (3.3) suggest moderate lipophilicity. Thymol exhibits high GI absorption and is also BBB permeant. It does not act as a Pgp substrate, but inhibits CYP1A2, indicating potential metabolic interactions. Thymol does not inhibit other key CYP enzymes. Its log Kp (-4.87 cm/s) points to low skin permeability. Thymol has no violations of Lipinski's rules, but one violation of Ghose, with a bioavailability score of 0.55 and one lead-likeness violation. The synthetic accessibility score is relatively low at 1.

Gamma-murolene, with no hydrogen bond acceptors or donors, has a relatively high MR (69.04) and no TPSA, indicating strong hydrophobicity. It is characterized by higher lipophilicity, with iLOGP of 3.31 and XLOGP3 of 4.31. Gamma-murolene has low GI absorption and is not BBB permeant. It is neither a Pgp substrate nor an inhibitor of most

CYP enzymes, but does inhibit both CYP2C19 and CYP2C9. Its log K_p (-4.49 cm/s) suggests moderate skin permeability. It has one violation of Lipinski's rule and no violations of Ghose or other rules. With a bioavailability score of 0.55. Gamma-murolene also has two lead-likeness violations and a synthetic accessibility score of 4.35.

Table 4.1: Pharmacokinetic and Physicochemical Studies

Parameter	Thymol	Gamma-Murolene
HBA / HBD	1 / 1	0 / 0
Molecular Refractivity (MR)	48.01	69.04
TPSA	20.23	0
iLOGP / XLOGP3	2.32 / 3.3	3.31 / 4.31
GI Absorption	High	Low
BBB Permeant	Yes	No
Pgp Substrate	No	No
CYP Inhibition	CYP1A2	CYP2C19, CYP2C9
log K _p (cm/s)	-4.87	-4.49
Lipinski Violations	0	1
Ghose Violations	1	0
Other Rule Violations	None	None
Bioavailability Score	0.55	0.55
Lead-likeness Violations	1	2
Synthetic Accessibility	1.00	4.35

Discussion

The results of this study provide significant insights into the insecticidal and computational activities of essential oils derived from *Ocimum gratissimum* (OgEO) and *Cymbopogon citratus* (CcEO), their combination (OgEO+CcEO), and permethrin (control) against *Anopheles gambiae* mosquitoes across various life stages (ovicidal, larvicidal, pupicidal, and adulticidal). Additionally, *In silico* studies elucidate the

molecular interactions of key phytoconstituents, thymol (from *O. gratissimum*) and γ -murolene (from *C. citratus*), with critical mosquito proteins, offering a mechanistic understanding of their insecticidal potential.

The results indicate that permethrin (the control), a synthetic pyrethroid, is the most effective treatment across all life stages of *Anopheles gambiae*, with significantly lower LC₅₀ values (e.g.,

0.1 ± 0.07 µg/mL for adulticidal activity) compared to the essential oils and their combination ($p < 0.05$). This aligns with its well-documented neurotoxic mode of action, which disrupts sodium channels in insect neurons, leading to rapid paralysis and death.^[27] The low standard deviations in permethrin's LC₅₀ values (e.g., 0.07 for adulticidal, 0.0003 for larvicidal) suggest high precision and consistency, reinforcing its reliability as a standard insecticide. However, the environmental persistence and non-target toxicity of pyrethroids, as noted by Schleier and Peterson^[28], highlight the need for eco-friendly alternatives, such as essential oils.

The combination of *Ocimum gratissimum* (OgEO) and *Cymbopogon citratus* (CcEO), demonstrated significantly greater efficacy than either oil alone ($p < 0.05$), particularly in larvicidal (LC₅₀ = 1.6 ± 0.3 µg/mL) and ovicidal (LC₅₀ = 1.3 ± 0.2 µg/mL) activities. This suggests a synergistic interaction between the phytoconstituents of the two oils, likely due to complementary modes of action. For instance, the combination's lower LC₅₀ values compared with individual oils indicate enhanced bioactivity, possibly through multi-target effects on mosquito physiology. This finding corroborates studies by Pavela *et al.*^[29], who reported synergistic effects in essential oil blends, particularly those containing monoterpenes and sesquiterpenes, against *Aedes aegypti* larvae. The synergy observed here could be attributed to the diverse chemical profiles of *Ocimum gratissimum* essential oil (rich in thymol and eugenol) and *Cymbopogon citratus* essential oil (rich in citral and γ-muurolene), which may disrupt multiple biochemical pathways in mosquitoes, such as respiratory, nervous, or enzymatic systems.

Individually, OgEO outperformed CcEO across most life stages, with significantly lower LC₅₀ values (e.g., 1.8 ± 0.5 µg/mL for ovicidal activity vs. 5.1 ± 0.2 µg/mL for CcEO, $p < 0.05$). The higher potency of OgEO may be linked to its

thymol content, a phenolic monoterpene known for its strong insect-repellent and toxic properties as reported by Tabari *et al.*^[30]. CcEO's relatively higher LC₅₀ values, particularly in ovicidal activity, suggest lower efficacy, possibly due to its primary constituents (e.g., citral, γ-muurolene) having less potent interactions with mosquito targets. However, the pupicidal activity of both oils showed higher variability (e.g., OgEO: 9.7 ± 4.0 µg/mL), indicating potential inconsistencies in their efficacy at this stage, which warrants further investigation.

The *In silico* docking studies provided a mechanistic basis for the observed insecticidal activities. Thymol (from OgEO) and γ-muurolene (from CcEO) exhibited strong binding affinities to catalase (UniProt ID: Q6RBZ5) and chorion peroxidase (UniProt ID: Q7QH73), respectively, with docking scores of -5.14 and -5.85, and MMGBSA binding energies of -61.84 and -74.65 *kCal/mol*. These enzymes are critical for mosquito survival, with catalase detoxifying reactive oxygen species and chorion peroxidase contributing to eggshell formation. The strong binding of thymol to catalase, driven by one hydrogen bond (His 342, 1.83 Å) and six hydrophobic interactions (e.g., Tyr 338, Phe 314), suggests inhibition of oxidative stress management, potentially leading to cellular damage in mosquitoes. Similarly, γ-muurolene's interaction with chorion peroxidase, dominated by 14 hydrophobic interactions (e.g., Leu 589, 5.49 Å), may disrupt eggshell synthesis, explaining the oils' ovicidal efficacy.

These findings are consistent with Ugbogu *et al.*^[31], who reported that monoterpenes like thymol target multiple insect proteins, including acetylcholinesterase and cytochrome P₄₅₀ enzymes, disrupting neural and metabolic functions. The dominance of hydrophobic interactions in both compounds' binding profiles aligns with studies by Regnault-Roger *et al.*^[32], who noted that lipophilic terpenoids penetrate insect cuticles more effectively, enhancing toxicity. The

stronger binding of γ -muurolene to chorion peroxidase compared with thymol's interaction with catalase suggests that *CcEO*'s ovicidal activity, although, weaker overall, may involve specific disruption of eggshell formation, complementing *OgEO*'s broader toxicity.

The PK/PD profiles of thymol and γ -muurolene provide insights into their potential as insecticidal agents. Thymol's moderate lipophilicity (iLOGP = 2.32, XLOGP3 = 3.3), high gastrointestinal (GI) absorption, and blood-brain barrier (BBB) permeability suggest good bioavailability and potential systemic effects in insects. Its inhibition of CYP1A2 indicates possible metabolic interactions, which could enhance its persistence in mosquito tissues. In contrast, γ -muurolene's high lipophilicity (iLOGP = 3.31, XLOGP3 = 4.31) and low GI absorption suggest it is better suited for topical or volatile applications, such as repellents. Its inhibition of CYP2C19 and CYP2C9 further supports its metabolic interference potential, which may contribute to toxicity.

Thymol's compliance with Lipinski's rules and low synthetic accessibility score (1) indicate its feasibility for large-scale production, whereas γ -muurolene's higher synthetic accessibility score (4.35) and two lead-likeness violations suggest challenges in formulation. These properties align with findings by Isman^[33], who noted that monoterpenes like thymol are more readily absorbed and metabolized in insects compared with sesquiterpenes like γ -muurolene, which may accumulate in cuticle layers, enhancing contact toxicity.

The superior efficacy of permethrin in this study is consistent with findings by Norris *et al.*^[34], who reported LC₅₀ values of 0.08–0.12 $\mu\text{g}/\text{mL}$ for permethrin against *Anopheles gambiae* adults, closely matching our results ($0.1 \pm 0.07 \mu\text{g}/\text{mL}$). However, the environmental and health concerns associated with pyrethroids, as

discussed by Tang *et al.*^[35], underscore the value of essential oils as safer alternatives. The synergistic effect of *OgEO*+*CcEO* aligns with Benelli *et al.*^[36], who found that blending *Ocimum* and *Cymbopogon* oils reduced LC₅₀ values by 30–50 % against *Aedes* larvae compared with individual oils, supporting the hypothesis of multi-target synergy.

The ovicidal potency of *OgEO* (LC₅₀ = $1.8 \pm 0.5 \mu\text{g}/\text{mL}$) is comparable with results by Govindarajan *et al.*^[37], who reported an LC₅₀ of 1.5 $\mu\text{g}/\text{mL}$ for *O. basilicum* oil against *Anopheles stephensi* eggs, suggesting that *Ocimum* species share potent ovicidal compounds. The higher LC₅₀ values for *CcEO* (e.g., $5.1 \pm 0.2 \mu\text{g}/\text{mL}$ for ovicidal activity) are consistent with Mdoe *et al.*^[38], who noted weaker larvicidal and ovicidal activities for *C. citratus* compared to other essential oils, likely due to lower concentrations of bioactive monoterpenes.

In silico studies by Kamaraj *et al.*^[39] also support our findings, reporting strong binding affinities of thymol (-5.2 kCal/mol) to mosquito detoxifying enzymes, similar to our docking score of -5.14 for catalase. The hydrophobic interaction dominance in γ -muurolene's binding aligns with computational studies by da Silva *et al.*^[40], who noted sesquiterpenes' efficacy against chorion-related proteins in *Culex* mosquitoes.

The significant efficacy of the *OgEO*+*CcEO* combination, particularly in larvicidal and ovicidal stages, suggests its potential as a natural insecticide for integrated vector management. However, the combination's efficacy remains lower than permethrin's, indicating a need for formulation optimization, such as nanoemulsions, to enhance bioavailability and stability, as suggested by Pavoni *et al.*^[41]. Moreover, the *In silico* results highlight thymol and γ -muurolene as key bioactive compounds, but their variable PK/PD profiles suggest tailored applications (e.g., thymol for systemic toxicity, γ -muurolene for contact/volatile effects). Future studies should explore additional protein targets

and validate docking results through *in vitro* enzyme inhibition assays. The higher variability in *OgEO*'s pupicidal activity ($LC_{50} = 9.7 \pm 4.0$ $\mu\text{g}/\text{mL}$) warrants further investigation to optimize its consistency, possibly through standardized extraction methods.

Conclusion

This study demonstrates that permethrin exhibits superior insecticidal efficacy against *Anopheles gambiae* across all life stages compared with *Ocimum gratissimum* (*OgEO*) and *Cymbopogon citratus* (*CcEO*) essential oils and their combination, with significantly lower LC_{50} values ($p < 0.05$). The *OgEO*+*CcEO* combination, however, shows promising synergistic effects, particularly in larvicidal ($LC_{50} = 1.6 \pm 0.3$ $\mu\text{g}/\text{mL}$) and ovicidal ($LC_{50} = 1.3 \pm 0.2$ $\mu\text{g}/\text{mL}$) activities, outperforming individual oils. *In silico* analyses reveal that thymol (from *OgEO*) and γ -muurolene (from *CcEO*) strongly bind to mosquito proteins like catalase and chorion peroxidase, supporting their insecticidal mechanisms.

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<https://dx.doi.org/10.4314/ajtmbr.v8i2.3>

Induced Abortion As A Method Of Contraception By Married Women With Unintended Pregnancy In Delta State, Nigeria: Prevalence, Predictors, And Reproductive Health Burden

¹Oduvbun WO, ²Nwachi A, ³Esemuede O.

ABSTRACT

Introduction: Unsafe abortion is an important cause of maternal death, globally. Nigeria contributes about twenty percent to the global figure. Induced abortion by married women as a method of contraception may be associated with life threatening complications. The study determined the prevalence, predictors, and the reproductive health burden of induced abortion, as a method of contraception, among married women with unintended pregnancy in Delta State.

Materials and Method: This was a cross-sectional study conducted in three public health facilities in Delta State. Participants were recruited through census sampling. Interviewer assisted questionnaire was used to collect data, which was subsequently analysed Statistical Product and Service Solution version 23. The level of statistical analysis was set at $P < 0.05$.

Results: The age range of participants was 18-49 years, with a mean age of 29.52 ± 5.78 years. Prevalence of unintended pregnancy was 58.0%. A third (30.7%) had a history of contraceptive use, while 42.3% a history of termination of pregnancy. The major (38.8%) reason for unintended pregnancy was contraceptive failure. The main (8.8%) complication was abdominal pain. The number of living children and unplanned pregnancy were the predictors for induced abortion ($P < 0.001$; $P < 0.001$), respectively.

Conclusion: This study showed a high prevalence of induced abortion among multiparous married women with unintended pregnancy, in Delta State. However, there was minimal adverse reproductive health outcome among the participants.

1. Department of Obstetrics and Gynaecology, College of Health Sciences, Delta State University, Abraka. <https://orcid.org/0000-2-0828-3215>

2. Department of Obstetrics and Gynaecology, Central Hospital, Warri, Delta State, Nigeria. Nwachi4aya@gmail.com

3. Department of Obstetrics and Gynaecology, Eku Baptist Government Hospital, Delta State, Nigeria. esemuedeomonigbo@yahoo.com

Corresponding author: Williams Obukohwo Oduvbun, Department of Obstetrics and Gynaecology, College of Health Sciences, Delta State University, Abraka. willymeg1@gmail.com +2348033722401.

INTRODUCTION

Induced abortion is a medical or surgical procedure that allows artificial termination of unintended or unwanted pregnancies.¹ On a global scale, 25 million of unsafe abortion occur annually, with estimated 97% taking place in developing countries.² Unsafe abortion is among the leading causes of maternal mortality and morbidity in developing countries.^{3,4}

In Nigeria, and most part of Sub-Saharan Africa, induced abortion is illegal and considered criminal offence, with resultant procurement of abortions by women in clandestine, unhygienic environments, and adverse outcome, such as haemorrhage, pelvic sepsis, genital tract trauma, uterine perforation among others.^{3,5,6} The long-term reproductive health complication include chronic pelvic inflammatory disease, ectopic

pregnancy and infertility.⁷

A clinical study by Oye-Adeniran et al,⁸ in the Western part of Nigeria, showed that 30.2% of women who presented for induced abortion were married women, and the aim of their study was to determine the use of pregnancy termination for birth spacing or limitation of births. This practice questions the adoption and practice of modern or even traditional methods of contraception in the study setting and at the time of study. Our study however, aimed to determine the prevalence, predictors, and the reproductive health burden of induced abortion by married women with unintended pregnancy in Delta State, South-South Nigeria. To the best knowledge of the authors, there are no studies on induced abortion by married women in Delta State. It is hoped that this multicentred-health-facility study, would give some insight into the behavioural pattern of married women with unintended pregnancy, contraceptive usage, and induced abortion in Delta State. Findings from this study may help in shaping policy formulation by the state Government on birth control.

MATERIALS AND METHODS

This was a cross-sectional survey conducted in the following three health facilities in Delta State: The Delta State University Teaching Hospital (DELSUTH), Oghara. This is a tertiary health facility, sited in the semi-urban community of Oghara, in the Ethiope-West Local Government Area; Central Hospital Warri (CHW), is a tertiary Health facility located in the densely populated city of Warri, which is regarded as the commercial State Capital, and located in Warri South Local Government Area; Eku Baptist Government Hospital (EBGH), is a busy rural Secondary Health facility, located in the Ethiope East Local Government Area. While DELSUTH and EBGH are located in the

Delta Central Senatorial zone, CHW is located in Delta South Senatorial zone. The study population were allocated proportionately according to the different monthly post- natal volume of married women between the age of 18 and 49 years. The CHW, had the largest population of respondents, followed by EBGH. The study was conducted in three months, from January 1, 2025 - March 31, 2025.

At each facility, eligible respondents were recruited consecutively in a census sampling technique until the allotted number for each facility was completed.

Inclusion criteria: Married women from the age of 18-49 years, with at least a living child who presented at the post- natal clinic of study facilities.

Exclusion criteria: Single mothers and acutely ill-married women who required emergency service Data was collected using a semi-structured interviewer administered questionnaire on sociodemographic characteristics, reproductive health history and knowledge. Information was obtained on unintended pregnancy and the induced abortion. The population in the study settings widely speak and understand the English Language, and where indicated interpreters were deployed. Information from the study questionnaires was transferred to a computer dataset, which was cleaned up by the lead researcher before data analysis.

Data processing and Analysis.

Data collected was analysed using SPSS version 25.0 software. The socio-demographic characteristics of respondents was summarized using frequency tables and percentages. The awareness of respondents about contraceptives; the use of contraceptives; desire to space and limit birth; proportion of married women with

unintended and induced abortion was, method of induced abortion, and complication from induced abortion was determined by using univariate analysis. Chi-square was used for test of association; a p-value of <0.05 was considered statistically significant. Multivariate binary logistic regression was used to assess the predictors of induced abortion.

RESULTS

A total of 390 eligible women were recruited for the study, out of which the questionnaires for 362(92.8%) was available for analysis.

Information in the remaining 28 proformas was insufficient for inclusion in the analysis.

Table 1 shows the baseline sociodemographic parameters of the study population. Many (53.6%), of the participants were in the 21-30 age group, with a mean age of 29.52 ± 5.78 years. In addition, the majority (95.6%), of the participants were Christians, with secondary level of education (50.6%), traders (48.9%), and had 1 child (35.4%).

Table 1: Sociodemographic parameters of participants

		Frequency (n)	Percentage (%)
<i>Age group (years)</i>	≤ 20	21	5.8
	21-30	194	53.6
	31-40	138	38.1
	≥ 41	9	2.5
	<i>Mean \pm SD</i>	29.52 ± 5.78	
<i>Religion</i>	<i>Christian</i>	349	95.6
	<i>Islam</i>	12	3.3
	<i>Others</i>	4	1.1
<i>Level of Education</i>	<i>No formal education</i>	6	1.6
	<i>Primary</i>	26	7.2
	<i>Secondary</i>	183	50.6
	<i>Post-secondary</i>	147	40.6
<i>Occupation</i>	<i>Unemployed</i>	57	15.7
	<i>Unskilled</i>	32	8.8
	<i>Artisan</i>	35	9.7
	<i>Trader</i>	177	48.9
	<i>Farmer</i>	12	3.3
	<i>Professional</i>	49	13.5
<i>Number of children</i>	1	128	35.4
	2	109	30.1
	3	68	18.8
	4	41	11.3
	5	16	4.5

The prevalence of unintended pregnancy and induced abortion is shown in Table 2. Only about a third of the participants (30.7%) had ever used any contraceptive, with more than half (58.0%) having unintended pregnancies.

Furthermore, 42.3% of the participants had induced abortion, with 21.0% performing it once, while 15(6.9%) participants had a history of three or more induced abortions.

Table 2: Prevalence of unintended pregnancy and induced abortion among participants

		Frequency (n)	Percentage (%)
<i>Contraceptive ever used</i>	<i>Yes</i>	111	30.7
	<i>No</i>	251	69.3
<i>Unintended pregnancies</i>	<i>Yes</i>	210	58.0
	<i>No</i>	152	42.0
<i>Number of Unintended Pregnancies</i>	<i>None</i>	152	42.0
	<i>1</i>	68	18.8
	<i>2</i>	75	20.7
	<i>3</i>	34	9.4
	<i>≥4</i>	33	9.2
<i>History of Induced abortion</i>	<i>Yes</i>	153	42.3
	<i>No</i>	209	57.7
<i>History of Number of induced abortions (n=153; 42.3%)</i>	<i>1</i>	76	21.0
	<i>2</i>	52	14.4
	<i>≥3</i>	25	6.9

Table 3 shows the reasons for unintended pregnancy and actions taken by participants. The commonest reason for unintended pregnancy was Contraceptive failure (38.8%) and of this group of participants, Emergency contraceptives failure was the leading (25.7%) cause. The commonest method of induced abortion was the use of drugs (34.3%). Other

methods of induced abortion by participants, included MVA (8. %) and D&C (11.3%),

In addition, of the 53.6% participants who had induced abortions, 17.4% (63/362) were successful, while 36.2% (31/362) were unsuccessful in the attempts at the termination. Sixteen (4.4%), participants did nothing about the unplanned pregnancies

Table 3: Reasons for unintended pregnancy, method of contraception, actions taken by respondents and method of termination

		Frequency (n)	Percentage (%)
<i>Reasons for unintended pregnancy</i> (n=210; 58.0%)	No contraceptive use	40	19.2
	Contraceptive failure	170	38.8
<i>Methods of contraceptive that failed</i> (n=170; 47.0%)	Implant	11	3.0
	Daily pills	27	7.5
	IUCD	2	0.6
	Natural method	21	5.8
	Injectables	16	4.4
	Emergency contraceptives	93	25.7
<i>Actions taken concerning the unplanned pregnancy</i> (n=210; 58.0%)	Unsuccessful attempt at termination **	63	17.4
	Successful attempt of the unplanned pregnancy **	131	36.2
	<i>Did nothing</i>	16	4.4
<i>Methods of induced abortions</i>	<i>Drugs</i>	124	34.3
	<i>MVA</i>	29	8.0
	<i>D & C</i>	41	11.3

** Total number of induced abortions = 194 (53.6%) participants

Table 4 shows the personnel responsible for the induced abortion, and pattern of post-abortion complications. The majority 27.3% of study participants who carried out induced abortion was by Misoprostol purchased at patent medicine (Chemist) stores, and 16% of

respondents with history of induced abortion suffered post-abortion complications, with the commonest complication being abdominal pain. The complications were mostly (27/362), treated in hospitals/clinic

Table 4: Personnel responsible for termination, pattern of post-abortion complications and treatment of the complication.

		Frequency (n)	Percentage (%)
<i>Who performed the abortion</i> (n=194; 53.6%)	Doctors	9	2.5
	Nurse	34	9.4
	Pharmacist	18	5.0
	Self-administered	99	27.3
	Misoprostol from Chemist stores		
	Trad. Birth Attendants	34	9.4
<i>Place where the termination took place</i> (n=194; 53.6%)	<i>Private Health Facility</i>	47	12.9
	<i>Chemist</i>	69	19.1
	<i>Trad. Birth Attendants</i>	34	9.4
	<i>Others</i>	44	12.2
<i>Post abortion complication</i>	<i>Yes</i>	58	16.0
	<i>No</i>	136	37.6
<i>Type of Complication</i> (n=58; 16.0%)	<i>Abdominal pain</i>	32	8.8
	<i>Excessive Bleeding</i>	26	7.2
<i>Treatment of Post abortion complications</i> (n=58; 16.0%)	<i>Hospital/ clinic</i>	27	7.5
	<i>Chemist</i>	16	4.4
	<i>Self-medication</i>	15	4.1

Table 5 highlighted the association between the sociodemographic and induced abortion. A comparison between the sociodemographic parameters with induced abortion showed that there was a statistically significant association when comparing the age groups, religion, occupation and number of children with the

study outcome ($p < 0.050$). Comparing the age groups, religion, occupation and number of children between those with induced abortion and those without, revealed that more participants in the former group were between 31-40 years (42.8% vs. 32.1%), were Christians (94.8% vs. 96.4%) and traders (51.0% vs. 46.4%).

Table 5: Association between socio-demographic and induced abortion

		Induced abortion N (%)	No abortion N (%)	χ^2	p-value
<i>Age group (years)</i>	<i>≤20</i>	10 (5.2)	11 (6.5)	9.686	*0.021
	<i>21-30</i>	93 (47.9)	101 (60.1)		
	<i>31-40</i>	83 (42.8)	55 (32.7)		
	<i>≥41</i>	8 (4.1)	1 (0.6)		
<i>Religion</i>	<i>Christian</i>	184 (94.8)	162 (96.4)	8.911	*0.012
	<i>Islam</i>	10 (5.2)	2 (1.2)		
	<i>Others</i>	0 (0.0)	4 (2.4)		
<i>Level of Education</i>	<i>None</i>	3 (1.5)	3 (1.8)	0.200	0.978
	<i>Primary</i>	14 (7.2)	12 (7.1)		
	<i>Secondary</i>	100 (51.5)	83 (49.4)		
	<i>Post-secondary</i>	77 (39.7)	70 (41.7)		
<i>Occupation</i>	<i>Unemployed</i>	35 (18.0)	22 (13.1)	7.486	0.187
	<i>Unskilled</i>	13 (6.7)	19 (11.3)		
	<i>Artisan</i>	21 (10.8)	14 (8.3)		
	<i>Trader</i>	99 (51.0)	78 (46.4)		
	<i>Farmer</i>	5 (2.6)	7 (4.2)		
	<i>Professional</i>	21 (10.8)	28 (16.7)		
<i>Number of living children</i>	<i>1</i>	23 (11.9)	105 (62.5)	102.382	*<0.001
	<i>2</i>	77 (39.7)	32 (19.0)		
	<i>3</i>	53 (27.3)	15 (8.9)		
	<i>4</i>	30 (15.5)	11 (6.5)		
	<i>5</i>	11 (5.7)	5 (3.0)		

Table 6 shows that the number of living children and history of unplanned pregnancies were predictors of induced abortion by married women, $P < 0.001$ and $P < 0.005$, respectively.

Table 6: Regression analysis to show the predictors of induced abortion.

Categories		B	S.E.	OR	95% C.I. for OR		p-value
					Lower	Upper	
Age Group	≤ 20			1.000			0.690
	21-30	0.361	0.544	1.435	0.494	4.168	0.507
	31-40	0.123	0.572	1.130	0.369	3.466	0.830
	≥ 41	-0.501	1.253	0.606	0.052	7.067	0.689
Religion	Christian			1.000			0.699
	Islam	-1.105	1.260	0.471	0.032	4.307	0.999
	Others	-1.246	1.733	0.398	0.021	8.974	0.999
Number of living children		-0.729	0.129	0.482	0.375	0.621	* <0.001
History of contraceptive use		0.351	0.279	1.420	0.821	2.455	0.210
History of unplanned pregnancy		1.503	0.265	4.493	2.674	7.551	* <0.001

Discussion.

This study examined the prevalence, predictors, and reproductive health burden of induced abortion by married women with unintended pregnancy, as a method of contraception.

There was a very high (58%) rate of unintended pregnancies, and a prevalence rate of 42.3% of induced abortion by married women in the study. There was 30.7% contraceptive uptake by the participants. Contraceptive failure (38.8%) was the main reason for the unplanned pregnancies. The use of drugs was the popular (34.3%) method of termination, while abdominal pains (8.8%) was the leading complication. The number of living children and history of unplanned pregnancies were predictors of induced abortion by participants.

The high rate of unintended pregnancy in this study higher than rates in similar studies in the western part of Nigeria.⁹ The rate was also

higher than the 41.4%,¹⁰ in a study in Ethiopia. The differences may be related to the heterogeneity in contraceptive awareness and uptake in the various settings. Unplanned pregnancies are frequently associated with induced abortions.^{11,12}

Contraceptive failures were the leading cause of unplanned pregnancy in this study. The contraceptive failure rate observed in this was considerably higher than that reported in a survey conducted across 8 Nigerian states,¹³ where 16% of women using modern contraceptive methods, mainly daily oral pills, reported unintended pregnancies. In contrast, emergency contraceptive use was the predominant contributor to contraceptive failure in the present study. While, Emergency contraceptive pills may be considered a useful backup method of pregnancy prevention, its use as a primary method of birth control may result in unplanned pregnancies as shown in this study.

The prevalence rate of induced abortion in this study was considerably higher than the rate by Ojo, et al, and Okonofua, et al.^{9, 14}. In the study by Okonofua, the participants were made up of both single and married women, while Ojo, et al used the same denominator in this study, used a combination of quantitative and qualitative methods. The differences in prevalence rates may be a reflection of the study designs, settings and social behaviour spanning different timelines.

The patronage of patent medicine dealers in this study for the conduct of pregnancy termination by a high number of participants was an unusual observation. This finding contrasted with the 82% documented by Ojo, et al,⁹ in the western part of Nigeria, and 45.6%,¹⁵ in India, in which majority of induced abortions were carried out in private health facilities. The widespread distribution of chemist stores in part of Nigeria where this study was conducted and, across the counter sale of Misoprostol for medical termination of pregnancy may have been responsible for this observation, and likely explain the difference between the result of this study and that by Ojo et al.⁹ Nigeria, like many other countries in the Sub-Saharan region of Africa have restrictive abortion laws.^{3, 16} Because of the legal consequences of pregnancy termination in public health facilities in Nigeria and other parts of Sub-Saharan African countries, most women in need of abortion services go about it in clandestine manner, including patronising Chemist stores for drugs.³ The use of Misoprostol obtained from medicine stores appears to be one of the ways to circumvent the restrictive abortion laws. The complications of abdominal pains and bleeding observed among the participants who had induced abortion is similar to previous studies.^{9, 14, 17} Treatment of complications were mostly in private or public health facilities. None

of the participants suffered any serious adverse outcome from the induced abortion. While the study did not include the gestational age at which the pregnancies were terminated, the possibilities of early pregnancy in most of the participants could not be excluded.

There was a significant association between the older (≥ 31 years) married women and induced abortion. A previous study,⁹ documented that married women who are 40 years and beyond had three-fold likely to terminate their pregnancy, compared to the younger age groups. It could be argued that the need to limit family size, the need to reduce social -economic burden in raising a large family, among others, may be an explanation for induced abortions in the older women, who are also of higher parity. Association between increasing parity and induced abortion in this study was also demonstrated in other studies in Nigeria, Kenya, and in India.^{9, 18, 19, 20}

Unplanned pregnancies, especially, among women who are multiparous were predictive of induced abortion in this study. For several women with unintended pregnancies, there may be other undisclosed reasons underpinning their decisions to terminate another pregnancy that was not desired.

Conclusion

This study showed a high prevalence of induced abortion among multiparous married women with unintended pregnancy, in Delta State. However, there was minimal adverse reproductive health outcome among the participants that had induced abortion. The need for more effective education of women during prenatal and post-natal clinic on contraceptive effectiveness and provision is suggested by this study.

Study limitations

This study has some limitations, which included the small sample size; the exclusion of 28 (7.2%) questionnaires may have affected the completeness of the data that was analyzed. Considering the illegal status of abortion in Nigeria, it is impossible to exclude some level of under-disclosure of induced abortion among participants. It is hoped that the confidentiality and the anonymity assurance, would have addressed the fear of participants' prosecution. Another study limitation was the fact that the study took place in two out of the three senatorial zones in Delta State, thus making the generalisation of study findings difficult. These two zones are largely populated by the Urhobos, Itsekiris, and the Ijaw ethnic groups. Married women in the Delta North senatorial zone, inhabited by the Ibo speaking ethnic group, were not included in this study. Further study studies may be required, that is more inclusive, that should include qualitative components that may capture some socio-cultural nuances that could contribute to induced abortion by married in Delta State.

Acknowledgement

On behalf of the authors, I want to thank all the staff of the study facilities and express my appreciation to the study participants for their co-operation during the conduct of the student.

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Duration-Dependent Post-Trauma Sleep Deprivation Differentially Modulates PTSD-Like Anxiety and Oxidative Markers in Mice

¹Ben-Azu B, ¹Omogbiya AI, ¹Ayereoghene SM, ²Usin SG, ²Oyovwi MO

Abstract

Introduction: Post-traumatic stress disorder (PTSD) is characterized by intrusive memories and persistent hyperarousal, with sleep playing a critical role in traumatic memory consolidation. While normal sleep supports memory refinement, post-trauma sleep disruption may shift outcomes toward fear retention or extinction. This study examined the duration-specific effects of post-trauma sleep deprivation (SD) on PTSD-like behaviors and oxidative neurobiological alterations.

Materials and Methods: Male Swiss mice (n = 10 per group) were subjected to the single prolonged stress (SPS) protocol, restraint, forced swimming, and ether exposure followed by varied SD regimens: no SD, daily SD for 2, 4, or 8 hours over 7 days, or a single 24-hour deprivation. Anxiety-related behavior was assessed using the light–dark box test. Oxidative stress markers, including superoxide dismutase (SOD), glutathione-S-transferase (GST), and sulphite oxidase (SO), were quantified in the hippocampus and prefrontal cortex.

Results: Brief SD (2–4 h daily) mitigated SPS-induced anxiety-like responses, increased GST activity, and normalized SO hyperactivity, suggesting interference with aversive memory consolidation and enhanced antioxidant defense. In contrast, prolonged daily SD (8 h) intensified anxiety and depleted antioxidant capacity, whereas a single 24-hour SD produced partial behavioral recovery with variable oxidative outcomes.

Conclusion: Post-trauma SD exerts duration-dependent, biphasic effects on PTSD-relevant behaviors and redox homeostasis. Limited SD promotes adaptive modulation by disrupting traumatic memory stabilization and enhancing antioxidant resilience, whereas extended deprivation aggravates oxidative imbalance and anxiety-like symptomatology.

Keywords: Post-traumatic stress disorder, Single prolonged stress, Sleep deprivation, Anxiety-like behaviour, Oxidative stress, Traumatic memory processing

¹DELSU Joint Canada-Israel Neuroscience and Biopsychiatry Laboratory, Department of Pharmacology, Faculty of Allied Health Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.

²Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University of Science and Technology, Ozofo, Delta State, Nigeria.

***Corresponding author:** Email: pharmben4ever@yahoo.com; bben-azu@delsu.edu.ng; Tell: +2348030881152
ORCID: <https://orcid.org/0000-0003-3569-3575>

INTRODUCTION

Characterized by intrusive recollections, chronic hyperarousal, and the avoidance of trauma-linked stimuli, post-traumatic stress disorder (PTSD) is a severe psychiatric condition

triggered by exposure to extreme stressors such as combat or violence^{1,2}. While the global annual prevalence is approximately 3.9%, these rates are significantly higher in populations with frequent trauma exposure, including refugees and military

personnel³. Established evidence suggests that the development of PTSD is closely tied to the maladaptive processing of traumatic memories, a process regulated by the complex interplay between sleep and the timing of trauma^{4,5}.

The stabilization and refinement of neural memory traces are fundamentally dependent on sleep architecture. Both rapid eye movement (REM) and non-REM sleep stages are critical for the consolidation and modification of memories^{6,7}. REM sleep, in particular, facilitates emotional regulation through coordinated activity between the hippocampus and the amygdala^{8,9}. However, in the context of PTSD, REM sleep may play a dualistic role: it can either promote the extinction of fear or reinforce traumatic memories, leading to persistent flashbacks^{10–12}. The influence of sleep on trauma processing is closely regulated by the hypothalamic–pituitary–adrenal (HPA) axis. Acute glucocorticoid surges may transiently enhance memory consolidation; however, chronic HPA dysregulation disrupts synaptic plasticity and impedes fear extinction through sustained amygdala excitation and prefrontal inhibition^{13,14}.

Circadian misalignment, such as sleep deprivation (SD), profoundly influences the encoding and consolidation of traumatic memories. Its impact depends on both duration and temporal proximity to the traumatic event^{15,16}. Acute SD immediately following trauma may disrupt fear consolidation by altering synaptic plasticity in the amygdala, hippocampus, and prefrontal cortex^{17,18}. Conversely, prolonged or repeated SD intensifies oxidative damage, leading to impaired neurochemical homeostasis, behavioural regulation and delayed fear extinction^{19–21}. Despite extensive studies on PTSD models such as single prolonged stress (SPS), which is a multimodal triple-hit model which mimics

pathobiological features of PTSD^{17,22}, the role of post-trauma SD duration in shaping behavioural and neurobiological outcomes in the SPS model remains unknown. Hence, this study explores how varying intervals of post-trauma SD affect PTSD-like behaviour and associated brain changes in mice subjected to SPS. We evaluated anxiety-like phenotypes and measured markers of oxidative stress, including superoxide dismutase (SOD), glutathione-S-transferase (GST), and the molybdenum enzyme sulphite oxidase within the prefrontal cortex and hippocampus. We hypothesized that brief SD would exert neuroprotective effects by disrupting traumatic memory consolidation, whereas extended SD would exacerbate symptoms via oxidative imbalance. These findings aim to refine the “Sleep to Remember or Forget” hypothesis and contribute to the development of non-pharmacological early interventions for PTSD.

MATERIALS AND METHODS

Animal Use and Care

A total of 70 adult male Swiss albino mice (6–8 weeks old, 20–35 g) were used, randomly assigned to seven groups ($n = 10$ each). Sample size was determined a priori using effect sizes from previous PTSD studies²³. Animals were obtained from the Animal House of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. Animals were housed in standard plastic cages within a temperature-controlled environment (22 ± 3 °C) under a 12-hour light/dark cycle. They were provided unrestricted access to a balanced rodent diet and clean water. Mice were acclimated for at least two weeks prior to the start of the experiments. All experimental procedures were conducted in accordance with the ethical guidelines of the Faculty of Basic Medical Sciences, Delta State University, and approved by its Research Ethics Committee (RBC/FBMS/DELSU/25/935). Effect sizes reported in comparable prior studies were used to

inform sample size determination. The sample size for this study was determined based on previously reported effect sizes from previous studies^{23,24}.

Chemicals and Reagents

Trichloroacetic acid (TCA), thiobarbituric acid (TBA), Tris buffer, and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydrogen phosphate and other analytical-grade reagents were supplied by LOBA Chemie (India). All reagents were freshly prepared using distilled water and maintained at pH 7.4 unless otherwise stated

Experimental Design: SPS-Induced PTSD Model

The single prolonged stress (SPS) procedure was conducted between 9:00 a.m. and 2:00 p.m. following established protocols with minor modifications to incorporate sleep deprivation (SD) conditions²³⁻²⁶. Each mouse underwent a sequence of three stressors: Restraint stress - immobilisation for 2 hours in a ventilated restrainer; Forced swimming - 20 minutes in a transparent cylinder (50 cm height, 24 cm diameter) filled two-thirds with water maintained at 24 °C; Ether exposure - brief inhalation until loss of consciousness. After swimming, mice were dried and given a 15-minute recovery period before ether exposure. Following the SPS procedure, animals were subjected to sleep deprivation.

Sleep Deprivation (SD) Modelling and Animal Grouping

After completing the SPS procedure, the mice were randomly divided into seven groups of ten animals each. The first group served as the control and received no treatment. The second group was exposed only to the SPS procedure. The third group was used as a sleep deprivation

control and experienced eight hours of sleep deprivation daily without SPS exposure. The remaining four groups were subjected to both SPS and different durations of sleep deprivation. These durations included two, four, or eight hours per day and night for seven consecutive days, as well as one group that experienced a single 24-hour period of sleep deprivation immediately after SPS exposure. Sleep deprivation began immediately following SPS. It was carried out using a modified grid-over-water technique, in which mice were placed on a metal grid positioned one centimetre above water in a plastic cage. The grid bars were spaced two centimetres apart, preventing the animals from entering sustained sleep but allowing them to move, eat, and drink freely. This setup effectively maintained wakefulness throughout the scheduled deprivation periods. At the end of the experimental phase, all animals underwent behavioural assessments designed to measure PTSD-like symptoms, including anxiety-related behaviours, exploratory activity, social interaction, and cognitive performance.

Behavioural Tests

Assessment of Anxiety-Like Behaviour

Anxiety-like behaviour was evaluated on the sixth day of the experiment using the light-dark box (LDB) tests. The LDB apparatus measured 21 × 42 × 25 cm and contained a light compartment (two-thirds of the box) and a dark compartment (one-third), separated by a small opening (3 × 5 cm). Each mouse was placed in the light compartment at the start of the test, and the number of transitions and time spent in each compartment were recorded for five minutes. After each trial, the apparatus was cleaned with 70% ethanol to remove residual odours and prevent scent-based bias.

Tissue Collection and Biochemical Analyses

Twenty-four hours after the final behavioural test

(day 8), mice were deeply anaesthetised with ketamine hydrochloride (100 mg/kg, intraperitoneally) and euthanised. Blood samples were collected via cardiac puncture into sterile 2 mL vials for corticosterone analysis. The brain was immediately removed and placed on an ice-cooled tray (4 °C). The prefrontal cortex and hippocampus, which are key regions implicated in PTSD due to their involvement in fear regulation, emotional control, and memory processing, were carefully dissected^{27,28}. Brain tissues were homogenised in 1 mL of 0.1 M phosphate buffer (pH 7.4) and centrifuged under the same conditions. The resulting supernatants were collected into 1.5 mL vials and stored for biochemical analyses.

Assay for Oxidative Markers

Brain oxidative markers, including superoxide dismutase (SOD) and glutathione-S-transferase (GST). The activity of SOD was expressed as units per mg protein, with one unit defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50%. The activity was expressed in Unit (U)/mg²⁹. GPx activity was measured by mixing brain homogenate with phosphate buffer (0.1 M, pH 7.4), EDTA, sodium azide, glutathione reductase, GSH, NADPH, and H₂O₂. The rate of NADPH oxidation was monitored spectrophotometrically at 340 nm, and GST activity was expressed as nmol NADPH oxidized/min/mg protein²⁹.

Determination of Molybdenum Enzyme Activities

The activity of molybdenum-dependent enzyme, namely sulphite oxidase, was measured in the hippocampal and prefrontal cortex homogenates using spectrophotometric methods²⁹. These enzymes are important indicators of oxidative metabolism and cellular stress. Sulphite oxidase activity was quantified by

measuring the oxidation of sodium sulphite to sulphate, with absorbance read at 600 nm after forming a barium sulphate precipitate using barium chloride. The enzyme assays were carried out in 0.1 M phosphate buffer (pH 7.4) at 37 °C. Protein concentrations were determined using the Lowry method, and all measurements were performed in triplicate to ensure reliability and reproducibility.

Statistical Analysis

Data were analyzed using one-way or two-way ANOVA as appropriate, followed by Bonferroni's post hoc tests. Factors for two-way ANOVA included treatment (SPS/SD) and SD duration. Data normality was verified using the Shapiro–Wilk test, and outliers were defined as values >2 SD from the group mean. Results are presented as mean ± SEM, with significance set at $p < 0.05$.

RESULTS

Effect of Sleep Deprivation in Modulating Anxiety-Like Behaviours in a Duration-Dependent Manner in SPS-induced PTSD

Latency to enter the dark zone (Fig. 1a), a measure of avoidance behaviour, was significantly reduced by SPS ($p < 0.01$) compared to the saline control. However, SPS mice that underwent SD for 2 h ($p < 0.0001$), 4 h ($p < 0.01$), and 8 h ($p < 0.05$) had increased latency to dark compared to SPS, indicating amelioration of anxiety-like behaviour. Similarly, in the light-dark box (LDB) (Fig. 1b), SPS reduced time in the light compartment ($p < 0.0001$) but increased dark zone duration. The SPS with SD intervention for 2 h displayed an intensified avoidance behaviour ($p < 0.0001$), while those with 24 h of SD intervention after SPS had reversed ($p < 0.01$), suggesting that 24 h of SD immediately after traumatic events may reduce anxiety-related behaviour.

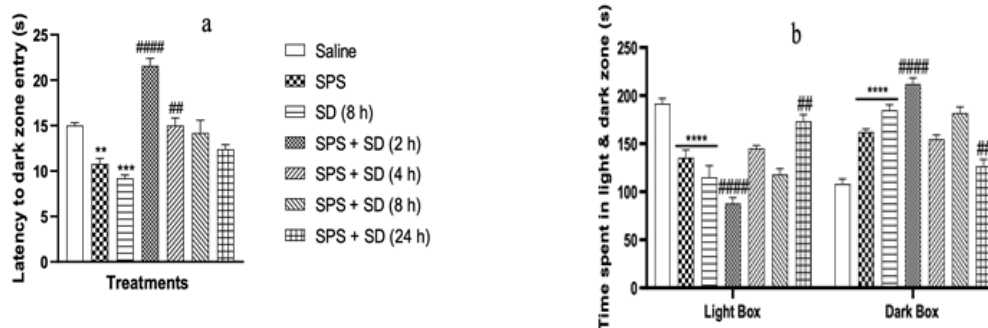


Fig. 1: Sleep Deprivation Modulates Anxiety-Like Behaviours in a Duration-Dependent Manner: (a) Latency to dark in light & dark box (LDB), (b) Time spent in light and dark zone. Bar are expressed as mean \pm SEM ($n = 10$), **** $p < 0.0001$ vs Saline; ## $p < 0.01$, $p < 0.001$, #### $p < 0.0001$ vs SPS; where SPS = Single Prolong Stress, SD = Sleep Deprivation.

Effects of Sleep Deprivation on Anti-oxidative Levels in the Prefrontal Cortex and Hippocampus of Mice Exposed to SPS-induced PTSD

Fig. 2a shows that SPS significantly reduced GST activity in both the prefrontal cortex and hippocampus ($p < 0.0001$), denoting impaired phase II detoxification. SD (8 h) alone produced similar reductions ($p < 0.0001$) relative to the saline group. Notably, short-term SD (2–24 h) in SPS mice restored GST activity in the prefrontal

cortex ($p < 0.001 - 0.0001$), suggesting compensatory antioxidant activation, except SPS + SD (8 h), which further suppressed GST ($p < 0.0001$). Regarding SOD activity, SPS decreased SOD activity in the prefrontal cortex and hippocampus ($p < 0.05$, $p < 0.0001$; Fig. 2b), whereas SD (8 h) reduced SOD activity only in the hippocampus ($p < 0.05$). Combined SPS + SD (8 h, 24 h) further lowered SOD activity ($p < 0.05$, $p < 0.001$), reflecting aggravated oxidative stress.

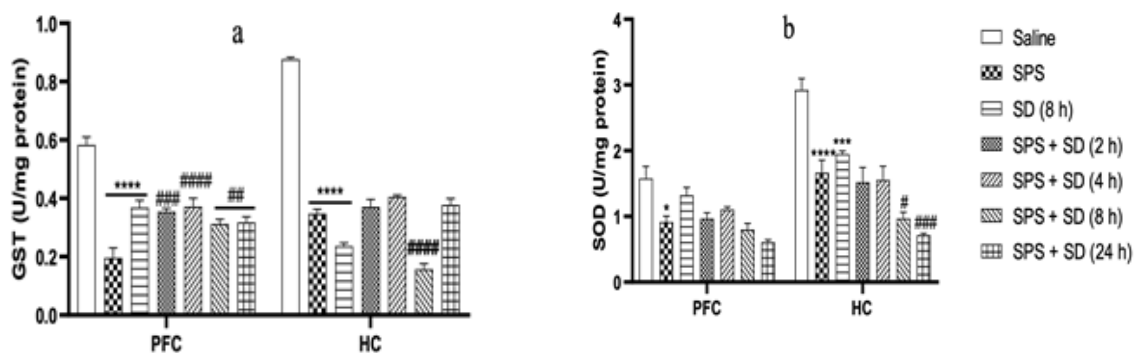


Fig. 2: Effects of Sleep Deprivation on Anti-oxidative Levels in the Prefrontal Cortex and Hippocampus of Mice Exposed to SPS-induced PTSD: (a) Glutathione-S-transferase (GST) and (b) Superoxide dismutase (SOD). Bar are expressed as mean \pm SEM ($n = 10$), **** $p < 0.0001$ vs Saline; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ vs SPS; where SPS = Single Prolong Stress, SD = Sleep Deprivation.

Effects of Sleep Deprivation on Molybdenum Enzyme Activities in the Prefrontal Cortex and Hippocampus of SPS-induced PTSD Mice.

The effects of SPS and durations depended SD on molybdenum enzyme activity, sulphite oxidase in the prefrontal cortex and hippocampus are shown in Fig. 8c, SPS caused a significant rise in sulphite oxidase activity ($p <$

0.001) in the prefrontal cortex, while SD alone (8 h) produced a modest increase ($p < 0.05$). Short-term SD (2 h, 4 h) significantly reduced sulphite oxidase relative to the SPS ($p < 0.01$, $p < 0.001$), suggesting partial restoration of redox balance. Short-term SD (24 h) further elevated SO ($p < 0.05$), indicating enhanced oxidative enzyme expression and ROS generation (Fig. 3).

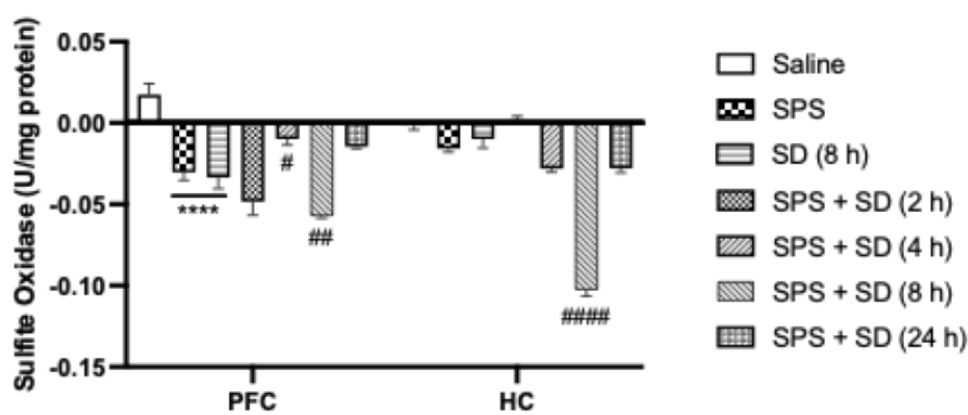


Fig. 3: Effects of Sleep Deprivation on Sulphite Oxidase in the Prefrontal Cortex and Hippocampus of SPS-induced PTSD Mice. Bars are expressed as mean \pm SEM ($n = 10$), **** $p < 0.0001$ vs saline; # $p < 0.05$, ## $p < 0.01$, #### $p < 0.0001$ vs SPS; where SPS = Single Prolonged Stress, SD = Sleep Deprivation.

Discussion

The present study investigated how varying durations of post-trauma SD modulate anxiety-like behaviour and oxidative balance in mice exposed to SPS, an established model of PTSD. Using the LDB test and oxidative biomarkers, GST, SOD, and sulphite oxidase, we demonstrate that SD exerts duration-dependent, bidirectional effects on behavioural and biochemical indices of stress adaptation.

Exposure to SPS alone produced the expected behavioural signature of heightened anxiety and avoidance, evidenced by reduced latency to enter the dark compartment and prolonged time spent therein. These findings align with prior studies reporting that SPS evokes persistent hyperarousal and impaired risk appraisal

through dysregulation of the amygdala–prefrontal–hippocampal circuitry^{30,31}. When SD was introduced after trauma, its effects varied with duration. Brief SD periods (2–4 h daily) mitigated anxiety-like behaviours, increasing latency to dark entry and reducing avoidance tendencies. Conversely, extended wakefulness (8 h per day) worsened anxiety indices, while a single 24 h SD episode immediately after SPS partially normalized behaviour. This pattern suggests that both the timing and length of sleep disruption critically shape post-traumatic behavioural outcomes.

These behavioural patterns can be interpreted through the framework of sleep-dependent memory processing. Short, acute SD soon after

trauma may transiently interrupt consolidation of aversive memories, thereby dampening conditioned fear responses^{17,18}. In contrast, chronic or poorly timed deprivation disrupts emotional regulation by exacerbating stress hormone release and impairing prefrontal inhibition of amygdala reactivity³². The partial anxiolytic effect observed with a single 24 h SD period may reflect adaptive recalibration of limbic circuits or compensatory neurotransmitter modulation that supports emotional recovery following acute stress exposure.

Oxidative stress parameters further support this biphasic interpretation. SPS markedly suppressed GST and SOD activities in the prefrontal cortex and hippocampus, consistent with depleted antioxidant capacity and elevated redox strain. Notably, short-term SD restored GST levels, suggesting activation of compensatory antioxidant mechanisms. In contrast, prolonged or repeated SD accentuated SOD depletion, indicating oxidative exhaustion. These findings suggest that brief SD may induce a preconditioning effect that strengthens redox resilience, whereas extended deprivation amplifies oxidative injury. Changes in sulphite oxidase (SO) activity reinforce this duality: reduced SO following short SD implies redox stabilization, while increased SO after prolonged SD or SPS reflects escalated reactive oxygen species (ROS) generation. Since SO is a key molybdenum-dependent enzyme involved in oxidative metabolism, its upregulation under stress likely represents both a marker and driver of cellular oxidative load^{29,33}.

Collectively, these results indicate that the influence of post-trauma SD on PTSD-like outcomes is duration-dependent and mechanistically linked to redox homeostasis. Brief, controlled SD may disrupt maladaptive fear consolidation and enhance antioxidant

defence, whereas sustained wakefulness intensifies oxidative dysfunction and anxiety-like behaviour. These findings refine the “Sleep to Remember or Forget” framework by emphasizing the temporal window during which sleep manipulation can shift neural and biochemical recovery trajectories after trauma. Future work employing polysomnography and region-specific molecular assays will be valuable in delineating the precise neurochemical pathways through which SD duration governs adaptive versus pathological outcomes.

In conclusion, this study demonstrates that the behavioural and biochemical consequences of post-trauma sleep deprivation are distinctly duration-dependent in mice subjected to single prolonged stress. Short-term deprivation (2–4 h) after trauma alleviated anxiety-like behaviours and partially restored antioxidant enzyme activities, suggesting adaptive modulation of fear consolidation and redox balance. In contrast, extended or repeated deprivation exacerbated oxidative stress and behavioural disturbances, reflecting metabolic exhaustion and impaired emotional regulation. Alterations in glutathione-S-transferase, superoxide dismutase, and sulphite oxidase activities indicate that oxidative mechanisms are central to these effects. Together, the findings highlight that controlled timing and duration of sleep loss can differentially influence post-traumatic adaptation, offering new insight into the potential of sleep-based interventions as adjunct strategies for preventing or mitigating PTSD-like outcomes.

Compliance with Ethical Standards

All experiments received approval and were conducted in accordance with the guidelines of the Faculty of Basic Medical Sciences, Delta State University, Animals Ethics Committee (REC/FBMS/DELSU/23/187) and the National Institutes of Health Guide for the Care

and Use of Laboratory Animals (Publication number: 85-23, revised 1985).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

Authors declare that they have no conflict of interest

Acknowledgements

The Authors are grateful to the technical staff of the Department of Pharmacology, Faculty of Basic Medical Sciences, Delta State University, for their technical assistance during the study.

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<https://dx.doi.org/10.4314/ajtmbr.v8i2.8>

Assessment of Cytotoxicity and Growth Inhibitory Effects of Methanol Extract of *Ageratum conyzoides* Linn.

¹Ikoya, S, ²Apitikori-Owumi, JE, ³Nwoguzue, BC, ⁴Agboola, OE, ¹Ekakitie, LI, ⁵Odeghe, OJ, ⁶Ofoke, IH, ⁷Oviri, MO

ABSTRACT

Introduction: Evaluating cellular cytotoxicity is a crucial step in the development of specific anticancer therapies. As a medicinal plant, *Ageratum conyzoides* is well-known for its abundance of bioactive constituents, anti-inflammatory and anti-bacterial properties. In this study, its effect was evaluated to confirm if its methanol extract exhibits cytotoxic and growth-inhibitory properties, with potential relevance to oncology.

Materials and Methods: Extraction of 1000 g of dried leaf material with methanol yielded 100 g of crude extract (10 %), indicating a high content of methanol-soluble bioactive compounds. The cytotoxic potential of the extract was assessed using *Ranicep ranninus* tadpoles as a preliminary model.

Results: The study's findings demonstrated a clear concentration-dependent increase in mortality, with significant effects observed at 160 and 320 µg/mL and a moderate LC₅₀ value of 100.76 µg/mL, confirming the extract's capacity to impair cell viability. Additionally, the extract exhibited significant inhibitory effects on the radicle growth of *Sorghum bicolor*, with both dose- and time-dependent responses. Higher concentrations (8-16 µg/mL) and prolonged exposure periods resulted in marked suppression of radicle elongation, as reflected by percentage inhibition and IC₅₀ values.

Conclusion: These findings suggest that the extract can interfere with cell division and proliferation, processes analogous to those driving uncontrolled growth in cancer cells. Collectively, this is an indication that *A. conyzoides* methanol extract possesses compounds with both cytotoxic and antiproliferative activity. These effects in non-cancerous models suggest preliminary potential as natural anticancer sources agents.

¹.Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria. ufuomasuccess247@gmail.com; ORCID: <https://orcid.org/0009-0002-9710-4027>

².Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University, P.M.B. 1, Abraka, Nigeria.

³.Department of Physiology, Faculty of Basic Medical Sciences, Delta state University, P.M.B. 1, Abraka, Nigeria

⁴.Institute for Drug Research and Development, Bogoro Research Centre, Afe Babalola University, Ado-Ekiti, Nigeria

⁵.Department of Educational Management, Delta State University, P.M.B. 1, Abraka, Nigeria.

⁶.Department of Biochemistry, Faculty of Science, Madonna University, Nigeria

⁷.Department of Biochemistry, Faculty of Science, Michael and Cecilia Ibru University, Delta State, Nigeria.

Corresponding author: Success Ikoya; Email: ufuomasuccess247@gmail.com; Phone Number: +2347018671743

INTRODUCTION

In biological sciences, cytotoxicity refers to the capacity of a substance to damage cells or induce cell death and is a fundamental concept in toxicology, pharmacology, and biomedical research.¹ Cytotoxicity assessment is routinely performed to evaluate the effects of chemical substances, pharmaceutical agents, or environmental pollutants on cell survival and

viability.² Such agents may induce cell death through mechanisms such as necrosis or apoptosis, underscoring the need to ensure that potential therapeutic compounds are effective while remaining safe for patients. Because substances vary greatly in their cytotoxic potential, systematic evaluation is essential before clinical or environmental application.³ Indeed, many chemotherapeutic drugs currently used in

cancer treatment exert their effects through well-established cytotoxic mechanisms.⁴⁻⁶ Growth inhibition, by contrast, describes the suppression or reduction of cell, tissue, or organismal growth following exposure to a given substance.⁷ Growth inhibition assays are widely applied in biological and pharmacological research to assess the activity of chemical compounds, plant extracts, or drugs against targets such as cancer cells, microorganisms, or developing plant tissues.^{8,9}

Medicinal plants remain a major source of novel therapeutic agents,¹⁰⁻¹¹ with ongoing research focused on identifying bioactive lead compounds and evaluating the efficacy of whole-plant preparations.¹² Numerous studies demonstrate that medicinal plants contain compounds with antibacterial, anti-inflammatory, antioxidant, and anticancer activities,¹³⁻¹⁵ and several naturally derived molecules have played key roles in modern anticancer drug development.¹⁶ Consequently, scientific evaluation of plants used in traditional medicine is critical to establish safety standards and minimise risks associated with toxic herbal products.¹⁷ Historically, plants have served as reservoirs of unique secondary metabolites capable of treating a wide range of diseases,^{18,19} and many contemporary pharmaceuticals are plant-derived.²⁰ Rigorous assessment of traditionally used plants is therefore necessary to identify compounds that are both safe and effective, while reducing potential toxicity.¹⁵

Ageratum conyzoides is a medicinal plant widely used in traditional healthcare systems worldwide, with applications varying by region. In Nigeria, it is known as *Imi esu* among the Yoruba people.²¹⁻²³ In India, it is used as a bactericidal, anti-dysenteric, and anti-lithic agent, while in parts of Asia, South America, and Africa, aqueous extracts are commonly

employed for antibacterial purposes.²⁴ In Central Africa, the plant is used to treat pneumonia and to promote wound and burn healing,²⁵ as well as for managing fever, rheumatism, headaches, and colic. Phytochemical studies reveal that *A. conyzoides* contains diverse secondary metabolites, including terpenoids, flavonoids, chromenes, alkaloids, coumarins, and lignans.²⁶ Its essential oil is rich in biologically active compounds such as quercetin derivatives, chromenes, phytosterols, and saponins,²⁷ highlighting its pharmacological potential. This study therefore evaluated the cytotoxic and growth-inhibitory effects of the methanol extract of *Ageratum conyzoides*.

MATERIALS AND METHODS

Collection and authentication of plant materials

Fresh leaves of *A. conyzoides* were obtained from the surroundings of campuses II (5.79093 ° N, 6.09847 ° E) and III (5.78072 ° N, 6.10169 ° E) of Delta State University, Abraka, Nigeria (Figure 1). Preliminary identification and authentication of the plant were carried out by Akinnibosun Henry Adewale at the Department of Plant Biology and Biotechnology, Herbarium Unit, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. A voucher specimen was deposited in the herbarium with the reference number UBH-A344 (*Ageratum conyzoides* Linn). The freshly collected leaves were thoroughly rinsed under running tap water to eliminate dirt and debris, after which they were air-dried at ambient laboratory temperature (23–29 °C) in the Department of Medical Biochemistry laboratory, Delta State University, Abraka. Drying was continued for 21 days until a constant weight was achieved, following the methods described by Usin and Ugwu²⁸ and Odeghe *et al.*²⁹ The dried leaves were then ground into a fine powder and stored in airtight containers at 4 °C prior to extraction.

Preparation and extraction of plant materials

The harvested plant material was washed and allowed to air-dry at ambient temperature for 14 days. Thereafter, the dried sample was milled into a fine powder using an electric grinding blender. A measured quantity of the powdered leaves (1000 g) was subjected to methanol extraction using a Soxhlet apparatus, following the method previously described by Usin and Ugwu.²⁸ The resulting extract was concentrated on an electrothermal constant water bath to obtain a greenish semi-solid residue, which was subsequently preserved in a refrigerator at 4 °C for subsequent analyses.

Sources of tadpoles (*Raniceps ranninus*)

Tadpoles were collected from toad breeding sites in small water bodies within Campus II (5.79093 ° N, 6.09847 ° E) of Delta State University, Abraka, Nigeria. Species identification was carried out by the Department of Animal and Environmental Biology, Faculty of Sciences, Delta State University, Abraka. Following identification, tadpoles aged 5 - 6 days were excluded from the study.

Evaluation of cytotoxic activity of *A. conyzoides* fractions on tadpole (*Raniceps ranninus*)

The cytotoxic activity of *A. conyzoides* fractions was assessed using a procedure adapted from Ayinde *et al.*³⁰ with minor modifications. Ten tadpoles were introduced into 250 mL beakers containing 15 mL of water obtained from the original tadpole habitat, which was then diluted with distilled water to a volume of 49 mL. The final volume was adjusted to 50 mL by adding 0.5, 1, 2, 4, or 8 µL of the fractions prepared in 5 % dimethyl sulfoxide (DMSO) in water, corresponding to final concentrations of 20, 40, 80, 160, and 320 µg/mL, respectively. Control groups were treated accordingly, and tadpole

mortality was monitored over a period of at least 24 hours.

Group 1: Tadpoles + 5 % DMSO in distilled water.

Group 2: Tadpoles + 20 µg/mL of methanol extract

Group 3: Tadpoles + 40 µg/mL of methanol extract

Group 4: Tadpoles + 80 µg/mL of methanol extract

Group 5: Tadpoles + 160 µg/mL of methanol extract

Group 6: Tadpoles + 320 µg/mL of methanol extract

The experiment was carried out in triplicate.

Sources of guinea corn (*Sorghum bicolor*)

Guinea corn was obtained from the Abraka Main Market and was cleansed with absolute ethanol. The viability of the seeds was determined by their ability to remain submerged in water.³¹ The seeds that remained submerged in water were selected and dried for use in this study.

Evaluation of growth inhibitory activity of *A. conyzoides* fractions on guinea corn radicle length

Precisely 10 mL of the fractions at concentrations of 1, 2, 4, 8, and 16 µg/mL, prepared in 5 % DMSO in water, were dispensed into 9 cm Petri dishes lined with cotton wool and Whatman No. 1 filter paper. Seed viability was assessed by pre-soaking twenty seeds in 50 mL of distilled water, after which they were evenly distributed on each Petri dish and incubated in a dark cabinet. The lengths (cm) of the emerging radicles were measured at 24, 48, 72, and 96 hours. Control seeds received 10 mL of 5 % dimethyl sulfoxide in distilled water without any extract, in accordance with the method described by Ayinde *et al.*³⁰

Group 1: Guinea corn seeds + 5 % DMSO in distilled water.

Group 2: Guinea corn seeds + 1 µg/mL of methanol extract

Group 3: Guinea corn seeds + 2 µg/mL of methanol extract

Group 4: Guinea corn seeds + 4 µg/mL of methanol extract

Group 5: Guinea corn seeds + 8 µg/mL of methanol extract

Group 6: Guinea corn seeds + 16 µg/mL of methanol extract

RESULTS

Percentage yield methanol extract of *A. conyzoides* leaf

The percentage yield of the methanol extract of *Ageratum conyzoides* leaf is presented in Table 1. It shows that the extraction of 1000 g of plant material with methanol produced 100 g of crude extract, giving a percentage yield of 10 %. This indicates that methanol was an effective solvent for extracting soluble bioactive constituents from the plant material, suggesting a relatively high abundance of methanol-soluble compounds in the sample.

Cytotoxic effect of the methanol extract of *A. conyzoides* on *Ranicep ranninus* (tadpole)

The results from Tables 2 and 3 show that the methanol extract exerted a clear, concentration-dependent cytotoxic effect on *Ranicep ranninus* tadpoles. Both cytotoxic response and percentage mortality increased steadily with rising extract concentration, with statistically significant effects observed at 160 and 320 µg/mL when compared with the control. The absence of mortality in the D.H₂O control confirms that the observed effects were due to the extract. The LC₅₀ value of 100.76 µg/mL indicates moderate cytotoxic potency of the methanol extract.

Growth inhibitory effect of the methanol

extract of *A. conyzoides* of *S. bicolor* (guinea corn) radicle.

The results in Tables 4 and 5 indicate that the methanol extract of *Ageratum conyzoides* affected the radicle growth of *Sorghum bicolor* in a concentration- and time-dependent manner. While the control showed normal progressive radicle elongation, exposure to the extract led to significant growth inhibition at several concentrations, particularly at higher doses (8 - 16 µg/mL) and longer exposure periods. The percentage inhibition data further confirm this trend, with positive inhibition values increasing over time and corresponding IC₅₀ values demonstrating measurable phytotoxic activity.

DISCUSSION

Cancer is fundamentally characterised by uncontrolled cell proliferation, resistance to cell death, and sustained growth signalling.³² Therefore, substances capable of inducing cytotoxicity and inhibiting growth in biological models are of considerable interest as potential anticancer agents.³³ The extraction yield of 10 % obtained using methanol suggests that the plant leaf is rich in methanol-soluble secondary metabolites. Methanol is known to extract compounds such as flavonoids, alkaloids, terpenoids, and phenolic compounds, many of which have been widely reported to possess anticancer properties.^{34,35} The appreciable yield observed in this study indicates a high likelihood that the extract contains sufficient quantities of these bioactive compounds capable of exerting biological effects, including cytotoxicity against rapidly dividing cells. The cytotoxicity assay using *Ranicep ranninus* tadpoles revealed a clear concentration-dependent toxic effect of the methanol extract, with significant mortality at higher concentrations and an LC₅₀ value of 100.76 µg/mL. Although tadpoles are not cancer cells, this model serves as a preliminary biological system to evaluate general cytotoxic potential.³⁶

Furthermore, the growth inhibitory effect of the methanol extract on *Sorghum bicolor* radicle elongation reinforces its relevance to cancer growth suppression. Guinea corn radicle growth depends on active cell division, elongation, and differentiation, processes that are also exaggerated in cancer cells.^{37,38} The significant reduction in radicle length at higher extract concentrations and longer exposure times indicates that the extract interferes with cellular proliferation.³⁹ In cancer treatment, inhibiting cell cycle progression and suppressing proliferative capacity are key therapeutic goals. The observed concentration- and time-dependent growth inhibition, therefore, suggests that the extract may contain anti-proliferative agents capable of limiting abnormal cell growth. The percentage inhibition and IC₅₀ values further support this interpretation, as increasing inhibition over time reflects sustained suppression of growth-related processes. In cancer models, such sustained inhibitory effects are desirable, as they imply the ability of a compound to restrain tumour expansion rather than producing only transient effects continuously. The presence of both growth-stimulatory effects at very low concentrations and inhibitory effects at higher concentrations is also consistent with the behaviour of many anticancer agents, which may exhibit hormetic responses depending on dose.

CONCLUSION

The present study suggests that the methanol extract of *Ageratum conyzoides* leaf could exhibit cytotoxic and growth-inhibitory properties, which are relevant in the field of oncology. Even though these findings are based on non-cancer biological models, they provide preliminary evidence that the extract might contain bioactive compounds capable of inducing cell death and

suppressing proliferation, which are the two critical targets in cancer treatment. However, further studies using established cancer cell lines, apoptosis assays, and molecular mechanism analyses are necessary to validate the anticancer potential of this extract and to identify the specific compounds responsible for these effects.

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<https://dx.doi.org/10.4314/ajtmbr.v8i2.11>

Evaluating the Impact of Computer-Assisted Cognitive Remediation on Recovery Outcomes in Schizophrenia: A Quasi-Experimental Study from Northwest Nigeria

¹Yakubu AA*, ²Bashar SM, ¹Aveka AI, ³Murinyi MM, ²Khalid S, ¹Yakasai BA

ABSTRACT

Introduction: Cognitive deficits are a core feature of schizophrenia, profoundly impacting functional recovery. Computer-assisted cognitive remediation (CACR) has shown promise in improving cognitive and functional outcomes globally, but its effectiveness in low-resource settings like Nigeria remains under-investigated. This study aimed to evaluate the impact of CACR on recovery outcomes among patients with schizophrenia in Northwest Nigeria.

Materials and Methods: A quasi-experimental study was conducted with 500 participants attending the psychiatric clinic of Ahmadu Bello University Teaching Hospital, Shika-Zaria. Participants were allocated to either a 12-week CACR programme (n=250) using the *BrainHQ* platform or treatment as usual (TAU, n=250). Recovery was assessed using the Recovery Assessment Scale (RAS). Data were analysed using independent t-tests, Chi-square tests, and multivariate analysis of covariance (MANCOVA).

Results: Post-intervention, the CACR group showed a significant improvement in total RAS scores (mean=78.3, SD=9.1) compared to the TAU group (mean=62.4, SD=10.5) ($p<0.001$). All RAS subscale scores (Personal Confidence, Willingness to Ask for Help, Goal Orientation, Reliance on Others, No Domination by Symptoms) were also significantly higher in the intervention group (all $p<0.001$).

Conclusion: CACR very significantly led to improved recovery outcomes among patients with schizophrenia in this Nigerian setting. The findings support the integration of CACR into routine psychiatric rehabilitation in resource-limited contexts to enhance functional recovery.

Keywords: Cognitive Remediation, Computer-Assisted, Recovery, Schizophrenia

¹Department of Psychiatry, Ahmadu Bello University, Zaria, Nigeria

²Department of Psychiatry, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

³Department of Child and Adolescent Mental Health Services, Lincolnshire Partnership NHS Foundation Trust (LPFT), Galaxy Suite, Boston Archway Centre, United Kingdom

Corresponding author: Dr. Abdullahi Abdulwahab Yakubu, Department of Psychiatry, Ahmadu Bello University, Zaria, Nigeria Email: ayabdullahi@abu.edu.ng; Tel.: +2348032804588

INTRODUCTION

Schizophrenia is a severe and chronic mental disorder affecting approximately 20 million people worldwide, with a significant burden in sub-Saharan Africa.¹ Beyond the hallmark positive and negative symptoms, cognitive impairment; encompassing deficits in attention, memory, executive function, and social

cognition, is now recognised as a core feature of the illness.² These cognitive deficits are stronger predictors of poor functional recovery, including impaired independent living, unemployment, and social isolation, than the symptomatic manifestations themselves.³

The concept of recovery in schizophrenia has

evolved from a purely clinical remission of symptoms to a more personal, multidimensional process of rebuilding a meaningful life and a positive sense of self.⁴ Consequently, interventions that target the functional barriers to this process, particularly cognitive deficits, are paramount. Cognitive remediation therapy (CRT) is a behavioural training intervention designed to improve cognitive processes with the ultimate goal of enhancing psychosocial functioning.⁵ Computer-assisted cognitive remediation (CACR) is a form of CRT delivered via standardised, adaptive software programmes that target core cognitive domains such as attention, memory, and executive function through repeated exercises and feedback. The advent of computer-assisted cognitive remediation (CACR) has standardised delivery, increased scalability, and allowed for more engaging, adaptive training protocols.⁶

Globally, meta-analyses have consistently demonstrated that CACR confers moderate to large improvements in both cognitive performance and functional outcomes.^{7,8} However, the vast majority of this evidence originates from high-income countries (HICs). In low- and middle-income countries (LMICs), including those in Africa, the implementation and evaluation of such technologically driven interventions face unique challenges, including limited resources, infrastructure, and trained personnel, alongside different cultural perceptions of illness and recovery.⁹ In Nigeria, Africa's most populous nation, mental health services are severely under-resourced, with a treatment gap for severe mental disorders exceeding 90%.¹⁰ While pharmacological treatment remains the mainstay, psychosocial interventions are scarcely available.¹¹ Preliminary studies from other parts of Nigeria have hinted at the acceptability and potential efficacy of cognitive interventions,¹² but a large-scale, rigorous evaluation is lacking.

This study, therefore, sought to evaluate the impact of a structured CACR programme on recovery outcomes among a large cohort of patients with schizophrenia in Northwest Nigeria. It aims to contribute critical evidence on the translatability and effectiveness of evidence-based digital therapeutic tools in a low-resource, African setting, where such innovations could potentially transform rehabilitation services.

MATERIAL AND METHODS

Study Design

A quasi-experimental study design with a non-equivalent control group was employed. The control group was considered non-equivalent because participants were not randomly assigned to intervention or control conditions; instead, allocation was based on clinic attendance days in order to minimise contamination between groups. As a result, baseline equivalence could not be fully guaranteed, necessitating statistical adjustment for baseline differences during analysis using MANCOVA.

Study Setting

The study was conducted at the psychiatric outpatient clinic of the Ahmadu Bello University Teaching Hospital (ABUTH), Shika-Zaria, a major tertiary referral centre serving Northwest Nigeria.

Study Participants

The study participants were adult schizophrenia patients attending the psychiatric outpatient clinic of Ahmadu Bello University Teaching Hospital (ABUTH), Shika-Zaria, Northwest Nigeria. A total of 500 participants diagnosed with schizophrenia were enrolled.

Sample Size Determination

The sample size was calculated using the formula for comparing two means;¹³ $n = [2 \times (Z_{\alpha/2} + Z_{\beta})^2 \times \sigma^2] / d^2$, where $Z_{\alpha/2} = 1.96$ (for $\alpha=0.05$), $Z_{\beta} =$

1.28 (for 90% power), σ = estimated pooled standard deviation, and d = effect size.¹³ With an effect size (d) of 0.4 (moderate, based on prior meta-analyses),⁷ a power of 90%, and an alpha level of 0.05, yielding a minimum of 199 per group. Anticipating a 20% attrition rate, the sample was inflated to 250 per group (500 total).

Sampling Technique

Consecutive sampling was used to recruit participants until the required sample size was achieved. Participants were allocated to the intervention (CACR) or control (Treatment As Usual - TAU) group based on their clinic attendance days to minimise contamination.

Inclusion Criteria

The inclusion criteria were; (i) Diagnosis of schizophrenia (F20) confirmed using the Mini-International Neuropsychiatric Interview (MINI) version 7.0.2;¹⁴ (ii) Aged ≥ 18 years; (iii) Clinical stability (no hospitalisation or major medication change in preceding 8 weeks); (iv) On stable antipsychotic medication for ≥ 4 weeks; (v) Provided written informed consent.

Exclusion Criteria

The exclusion criteria were; (i) Co-morbid major neurological disorder or intellectual disability; (ii) Severe sensory impairment precluding computer use; (iii) Active substance dependence (excluding caffeine and tobacco); (iv) Participation in another structured psychosocial intervention.

Study Instruments and Measures

Socio-demographic and Clinical Proforma: Collected information on age, gender, education, occupation, duration of illness, and medication type.

Mini-International Neuropsychiatric Interview (MINI) 7.0.2: Used for diagnostic confirmation.

It has been validated in Nigeria and shows good concordance with clinical diagnoses.¹⁵

Recovery Assessment Scale (RAS): The primary outcome measure. This 24-item self-report scale assesses personal recovery across five subscales: Personal Confidence and Hope, Willingness to Ask for Help, Goal and Success Orientation, Reliance on Others, and Not Being Dominated by Symptoms. Items are rated on a 5-point Likert scale (1=Strongly Disagree to 5=Strongly Agree). The total score ranges from 24 to 120, with higher scores indicating greater recovery. The RAS has demonstrated good psychometric properties and has been used and validated in Nigerian populations with mental illness, showing high internal consistency (Cronbach's alpha > 0.85).^{16,17}

Study Procedure

Ethical approval was obtained from the ABUTH Health Research Ethics Committee. After screening and consent, baseline assessments were conducted. The intervention group received the CACR programme in addition to TAU, while the control group received only TAU (regular psychiatric consultations and pharmacotherapy). The CACR intervention was administered over 12 weeks, with three 45-minute sessions per week, supervised by a trained research assistant. The programme used the *BrainHQ* online platform (Posit Science), which features adaptive exercises targeting speed of processing, attention, memory, and executive function. Post-intervention assessments were conducted within one week of programme completion for both groups.

Ethical Considerations

The study procedures were reviewed and approved by the Health Research Ethics Committee of Ahmadu Bello University Teaching Hospital, Shika-Zaria (Ref: ABUTH/HREC/B63/2025). Informed consent was obtained from all participants prior to their

inclusion in the study. Confidentiality and anonymity of participants was maintained throughout the research process.

Statistical Analysis

Data were analysed using IBM SPSS Statistics version 26. Descriptive statistics (mean, standard deviation, frequencies, and percentages) were used to summarise data. Baseline group differences were assessed using independent samples t-tests for continuous variables and Chi-square tests for categorical variables. The primary analysis used Multivariate Analysis of Covariance (MANCOVA) to compare post-intervention RAS total and subscale scores between the CACR and TAU groups, controlling for any significant baseline differences and baseline RAS scores. Effect sizes were reported as partial eta squared (η^2). Statistical significance was set at $p < 0.05$.

RESULTS

A total of 500 participants with schizophrenia were enrolled, with 250 allocated to the Computer-Assisted Cognitive Remediation (CACR) group and 250 to the Treatment As Usual (TAU) group. The baseline socio-demographic and clinical characteristics of both groups are presented in Table 1. The mean age of participants was approximately 35 years (CACR: 34.8 ± 8.7 ; TAU: 35.2 ± 9.1). The majority of participants were male, comprising 137 (54.8%) in the CACR group and 132 (52.8%) in the TAU group. The mean duration of illness was around 9 years for both groups. There were no statistically significant differences between the groups in terms of age, gender, years of education, duration of illness, or the proportion prescribed atypical antipsychotics (all $p > 0.05$). However, a statistically significant difference was observed in the baseline Recovery Assessment Scale (RAS) total score, with the TAU group scoring slightly higher (mean=63.5, SD=10.8) than the

CACR group (mean=61.8, SD=11.2) ($p=0.049$).

The primary analysis, a Multivariate Analysis of Covariance (MANCOVA) controlling for the baseline RAS total score, revealed a statistically significant overall effect of the intervention group on the combined post-intervention RAS scores (Pillai's Trace = 0.342, $F(5, 493) = 51.28$, $p < 0.001$).

The detailed post-intervention outcomes are shown in Table 2. Following the 12-week intervention, the adjusted mean RAS total score for the CACR group was 78.3 (SE=0.6), which was significantly higher than the adjusted mean of 62.4 (SE=0.6) for the TAU group ($F(1, 497)=202.74$, $p < 0.001$).

Analysis of the individual RAS subscales showed a consistent pattern of superior performance in the CACR group. For the *Personal Confidence and Hope* subscale, the CACR group's adjusted mean was 19.8 (SE=0.2) compared to 15.2 (SE=0.2) for the TAU group ($p < 0.001$). On the *Willingness to Ask for Help* subscale, the CACR group scored an adjusted mean of 15.5 (SE=0.2) versus 12.1 (SE=0.2) for the control group ($p < 0.001$). Similarly, for *Goal and Success Orientation*, the adjusted mean was 16.9 (SE=0.2) in the CACR group and 13.4 (SE=0.2) in the TAU group ($p < 0.001$).

The scores for the *Reliance on Others* subscale were 14.2 (SE=0.2) for the intervention group and 11.6 (SE=0.2) for the control group ($p < 0.001$). Finally, on the *Not Dominated by Symptoms* subscale, the CACR group achieved an adjusted mean of 11.9 (SE=0.1), which was higher than the TAU group's mean of 10.1 (SE=0.1) ($p < 0.001$). All comparisons were statistically significant at the $p < 0.001$ level.

Table 1: Baseline Socio-demographic and Clinical Characteristics of Participants

Characteristic	CACR Group (n = 250)	TAU Group (n = 250)	p-value
Age, Mean (SD)	34.8 (8.7)	35.2 (9.1)	0.621
Gender, Male, n (%)	137 (54.8)	132 (52.8)	0.658
Education (Years), Mean (SD)	11.4 (3.8)	11.1 (4.0)	0.387
Duration of Illness (Years), Mean (SD)	9.2 (5.6)	8.9 (5.8)	0.556
On Atypical Antipsychotic, n (%)	198 (79.2)	201 (80.4)	0.744
Baseline RAS Total, Mean (SD)	61.8 (11.2)	63.5 (10.8)	0.049

Table 2: Post-Intervention Recovery Assessment Scale (RAS) Scores: Comparison Between Groups (Adjusted Means from MANCOVA)

RAS Scale	CACR Group Mean (SE)	Adjusted TAU Group Mean (SE)	Adjusted F-value (1, 497)	p-value	Partial η^2
Total Score	78.3 (0.6)	62.4 (0.6)	202.74	< 0.001	0.289
Personal Confidence	19.8 (0.2)	15.2 (0.2)	165.33	< 0.001	0.249
Willingness to Ask for Help	15.5 (0.2)	12.1 (0.2)	142.19	< 0.001	0.222
Goal Orientation	16.9 (0.2)	13.4 (0.2)	151.67	< 0.001	0.234
Reliance on Others	14.2 (0.2)	11.6 (0.2)	98.45	< 0.001	0.165
Not Dominated by Symptoms	11.9 (0.1)	10.1 (0.1)	112.58	< 0.001	0.185

Note: SE = Standard Error

DISCUSSION

This quasi-experimental study of 500 patients with schizophrenia in Northwest Nigeria provides robust evidence that a 12-week Computer-Assisted Cognitive Remediation programme very significantly improves self-reported recovery outcomes. The intervention group demonstrated large, statistically superior gains on the overall RAS and across all its constituent domains; personal confidence, help-seeking, goal orientation, social reliance, and symptom management, compared to those receiving treatment as usual.

Our findings align with the global evidence base. International meta-analyses consistently report that cognitive remediation leads to significant improvements in functional outcomes, with effect sizes comparable to the large one ($\eta^2=0.289$) found in this study.^{7,8} The results suggest that the core principles of neuroplasticity and skills training underpinning CACR are effective across diverse cultural and economic contexts. The use of the *BrainHQ* platform, with its adaptive difficulty and engaging interface, likely contributed to the high adherence and observed benefits, echoing successful implementations in HICs.⁶

Within the African context, this study significantly advances the field. While previous Nigerian research has focused on cognitive assessments or brief, non-computerised training,^{12,19} this is one of the largest and most methodologically rigorous trials of a standardised CACR protocol in the region. A smaller pilot study from South Africa also found positive cognitive effects from computerised training,²⁰ but our study explicitly links the intervention to the holistic construct of personal recovery, which is increasingly prioritised in mental health service frameworks.⁴ The significant improvement in *Willingness to Ask for Help* and *Reliance on Others* is particularly noteworthy, as it suggests CACR may foster the social connectivity crucial for recovery in communalistic African societies, potentially countering the stigma and isolation often experienced by this patient group.²¹

The findings must be interpreted considering the study's limitations. The quasi-experimental design, while pragmatic in a busy clinical setting, carries a risk of selection bias, though baseline characteristics were largely similar. The reliance on a self-reported primary outcome (RAS) is a strength for capturing the subjective experience of recovery but could be complemented by objective functional measures (e.g., vocational status) and performance-based cognitive tests in future research. Furthermore, the long-term sustainability of gains beyond the 12-week period remains to be investigated.

Notwithstanding these limitations, the implications are substantial. In a resource-constrained setting like Nigeria, where specialist therapists are scarce, CACR offers a scalable, cost-effective adjunct to pharmacotherapy. It can be administered by trained non-specialist personnel under supervision, a task-shifting model endorsed by the World Health

Organization for LMICs.²² The significant improvements in recovery-oriented outcomes advocate for the integration of CACR into the standard rehabilitation package at tertiary psychiatric centres in Nigeria and similar settings.

CONCLUSION

This study demonstrates that computer-assisted cognitive remediation is a highly effective intervention for enhancing personal recovery among patients with schizophrenia in Northwest Nigeria. It bridges a critical evidence gap, showing that a technologically mediated intervention proven in high-income countries can be successfully implemented and yield substantial benefits in a low-resource African context. We recommend that mental health service planners in Nigeria and similar regions consider the structured integration of CACR programmes to improve functional outcomes and quality of life for individuals living with schizophrenia.

AVAILABILITY OF RESEARCH DATA

Data are available upon reasonable request from the corresponding author.

FUNDING

No funding was received for this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

We thank the study participants, research assistants, and the leadership and staff of the Department of Psychiatry, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, for their support and cooperation throughout the study.

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Yakubu AA, Bashar SM, Aweka AI, Murinyi MM, Khalid S, Yakasai BA . Evaluating the Impact of Computer-Assisted Cognitive Remediation on Recovery Outcomes in Schizophrenia: A Quasi-Experimental Study from Northwest Nigeria *Afr. J. Trop. Med. & Biomed. Res.* 2025; 8(2) 78-85
<https://dx.doi.org/10.4314/ajtmbr.v8i2.12>

Biochemical Impact Of Occupational Cement Dust Exposure On Block Industry Workers In Rivers State, Nigeria

¹John, GC ²Anosike IK

ABSTRACT

Introduction: Occupational exposure to cement dust is a recognized health hazard linked to systemic complications. This study evaluated the impact of chronic cement dust exposure on the biochemical parameters of cement block moulders in Khana Local Government Area (LGA), Rivers State, Nigeria, to assess potential renal and electrolytic alterations.

Materials and Methods: cross-sectional study was conducted involving 70 male participants, categorized into cement block moulders (subjects, n=35) and non-exposed individuals (controls, n=35). Five millilitres (5ml) of venous blood were collected from each participant into heparinized bottles. Samples were analyzed for urea, creatinine, sodium, and potassium using standard spectrophotometric methods. Statistical analysis was performed using Mean and Standard Deviation, with significance set at $P < 0.05$.

Results: The study revealed a statistically significant increase ($P < 0.05$) in serum creatinine and potassium levels among the cement block moulders compared to the control group. Conversely, no significant differences ($P > 0.05$) were observed in serum sodium and urea levels between the two groups.

Conclusion: Chronic exposure to cement dust is associated with significant elevations in serum creatinine and potassium, indicating early signs of altered renal filtration and electrolyte imbalance. While urea and sodium levels remained stable, the elevation in specific markers suggests that block moulders are at an increased risk of developing renal impairment. Regular health screenings and the consistent use of personal protective equipment (PPE) are recommended to mitigate these occupational risks

Keywords: Cement dust, Creatinine, Potassium, Renal function, Occupational health, Khana LGA.

¹ Department of Applied Chemical Science Laboratory Technology, Faculty of Science Laboratory Technology, University of Benin

² Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Benin

Corresponding author: John, G. N (godson.john@uniben.edu) Corresponding Author ORCID No: 0009-0007-6952-0820

Introduction

Cement dust is generated during the production, packaging, loading, and offloading of cement. Everyone in the industry—from directors and managers to staff, customers, and bricklayers—is exposed to the inhalation of this dust. Furthermore, individuals living in the vicinity of cement plants or those passing by are also at risk.

Consistent exposure to cement dust over long periods can cause systemic toxicity due to the accumulation of toxic constituents⁹. The primary chemical components of cement include calcium, silica, alumina, and iron. Calcium is derived from limestone or chalk, while silica, alumina, and iron are sourced from sand and clay¹. Different organs, tissues, and cells are affected in varying ways and

degrees when exposed to these toxic elements. The results for creatinine and potassium in this study align with findings by ², which showed a significant increase in the creatinine levels of exposed subjects compared to the control group, but no significant increase in sodium levels. This research aims to assess the effects of cement dust on specific biochemical parameters among cement block moulders in the Khana Local Government Area of Rivers State, Nigeria. Several studies have demonstrated links between cement dust exposure and the chronic impairment of lung function and respiratory symptoms. Cement dust irritates the skin, the mucous membranes of the eyes, and the respiratory system. Its deposition in the respiratory tract triggers an alkaline reaction, leading to increased pH values that irritate exposed membranes³. Occupational exposure has also been associated with an increased risk of liver abnormalities, pulmonary disorders, and carcinogenesis. Decreased antioxidant capacity and increased plasma lipid peroxidation have been proposed as potential causal mechanisms for these diseases⁴.

Additionally, evidence suggests that cement dust exposure acts as an independent risk factor—separate from tobacco, alcohol, and asbestos—for laryngeal carcinoma⁵. Cement dust contains heavy metals such as nickel, cobalt, lead, and chromium, which are pollutants hazardous to the biotic environment, adversely impacting vegetation, human and animal health, and entire ecosystems⁶. Inhalable dust concentrations in production plants, especially during cleaning tasks, are considerably higher than those found at construction sites⁷. People living in high-exposure zones are severely affected by respiratory problems and gastrointestinal diseases⁸. Studies indicate that these adverse respiratory effects—including increased frequency of symptoms and decreased ventilatory function—cannot be explained by age, BMI, or smoking, and are thus

likely caused by cement dust. Symptoms such as chest pain, cough, and eye irritation are prevalent in villages near cement plants. A relative risk ratio assessment indicates that exposed subjects are 7.5 to 22.5 times more likely to develop these diseases compared to unexposed individuals¹⁰.

Materials and Methods

Sample Collection

This study was carried out in Khana Local government area, Rivers State Port Harcourt. 70 (seventy) adult men were involved in this study, 50 (fifty) were cement block moulders (subject) and (twenty) 20 were non-cement block moulders.

Ethical Considerations

The study followed the ethical principles guiding the use of human participants in research. Ethical approval was sought from University of Port Harcourt Ethical Committee. All procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. (World Medical Association declaration of Helsinki, 2000). Informed consent were obtained from all the research participants. With respect to confidentiality, no identifiers like name of respondents were required.

Serum Preparation

5ml of blood specimen drawn from 50 cement block moulders and 20 drawn from non-cement block moulders and were put into oxalate bottle, and then the serum was then separated from the blood by centrifugation of 3000rpm at 15 minute and was analysed.

Principle: Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured in spectrophotometer. The solution in each of the tubes was mixed and incubated at 37^oc for 10 minutes.

I mixed the solution in each of the test tube and incubated at 37^oc for 15 minutes after which I zeroed the spectrophotometer using distilled water at a wavelength of 546nm. Then I read absorbance of standard and test and then recorded my result.

Potassium Determination

Potassium was assayed using the sodium tetraphenylboron method. In this reaction, potassium ions react with sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The resulting turbidity is directly proportional to the potassium concentration. After incubation for 3 minutes at room temperature, the absorbance was measured at 500 nm against a reagent blank using a spectrophotometer.

Creatinine Determination

Serum creatinine was assayed by the Jaffé method, as previously described by ¹¹. The

principle involves the reaction of creatinine with picric acid in an alkaline medium to form a red-colored complex, which is then measured spectrophotometrically

Data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. Data were expressed as mean and standard error of mean. Student's t-test was adopted for comparison. *P* values of < 0.05 were considered statistically significant

Results

Reject Ho at $P < 0.05$ i.e. $t_{cal} > t_{critical}$

There is a significant difference in creatinine subject when compare with control.

Ho: – No significant difference between the subject and control of urea and sodium.

H₁: There is significant difference between the subject and control of creatinine and potassium respectively.

Table 1: Biochemical parameters of creatinine and urea

Parameter/ Group	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/l)
Subject	126.46 \pm 2.49	3.99 \pm 1.10
Control	89.22 \pm 10.44	4.12 \pm 0.79
P-value	$P < 0.05$	$P > 0.05$

The mean and standard deviation of Biochemical parameters of creatinine and urea concentration in the tested subject are 126.46 \pm 2.49 and 3.99 \pm 1.10 all in ($\mu\text{mol/L}$) while control are 89.22 \pm 10.44 and 4.12 \pm 0.79 all in (mmol/l) are shown above in table 1.

Table 2 Biochemical parameters of potassium and sodium

Parameter/ Group	Potassium (mmol/l)	Sodium (mmol/l)
Subject	3.79 \pm 0.64	143.49 \pm 6.54
Control	4.53 \pm 0.53	143.90 \pm 5.37
P-value	$P < 0.05$	$P > 0.05$

The mean and standard deviation of Biochemical parameters of potassium and sodium concentration in the tested subject are 3.79 \pm 0.64 and 143 \pm 6.54 all in (mmol/l) while control are 4.53 \pm 0.53 and 143.90 \pm 5.37 all in (mmol/l) are shown above in table 2.

DISCUSSION

The observed significant increase in serum creatinine and potassium ($P < 0.05$) among cement block moulders indicates a profound compromise in renal functional integrity. These results align with findings by ², which also reported significant creatinine elevation. The nephrotoxicity observed is likely driven by the inhalation and systemic absorption of heavy metals found in cement, specifically Lead (Pb) and Hexavalent Chromium (Cr VI). These metals are filtered by the glomerulus and subsequently reabsorbed by the proximal convoluted tubules. Once inside the tubular cells, they trigger the production of Reactive Oxygen Species (ROS), leading to oxidative stress and lipid peroxidation of the renal cellular membranes. This biochemical assault results in Renal Tubular Necrosis, effectively reducing the kidney's ability to filter waste. The concurrent rise in creatinine and potassium is a classic hallmark of a declining Glomerular Filtration Rate (GFR): Creatinine: As a byproduct of muscle metabolism usually filtered freely by the kidney, its accumulation in the blood is a direct "proxy" for reduced filtration capacity. Potassium (Hyperkalemia): The kidneys are responsible for excreting 90% of dietary potassium. When the tubular cells are damaged—specifically the distal tubule and collecting ducts—the sodium-potassium exchange pump (Na^+/K^+ -ATPase) fails. Consequently, potassium is not secreted into the urine but is instead retained in the blood. The rejection of the H_1 hypothesis for sodium and urea suggests a "differential sensitivity" in renal markers. Urea levels are highly influenced by non-renal factors such as dietary protein intake and hydration status, making it less specific than creatinine. Similarly, the body employs aggressive homeostatic mechanisms (such as aldosterone regulation) to maintain sodium levels within a narrow range, even during the early stages of renal distress. This indicates that

creatinine and potassium are more sensitive early-warning biomarkers for cement-induced nephrotoxicity in this population. The renal impairment seen here likely represents only one facet of a broader multi-organ toxidrome. The heavy metals identified in cement dust, particularly lead, are known to inhibit delta-aminolevulinic acid dehydratase (ALAD), an enzyme critical for heme synthesis. This suggests that while these workers are currently presenting with renal distress, they are also at high risk for occupational anemia and hepatotoxicity, as evidenced by literature linking chronic exposure to elevated liver enzymes (ALT/AST).

CONCLUSION

In conclusion, this study confirms that occupational exposure to cement dust among block moulders in Khana LGA leads to significant alterations in critical renal biomarkers, specifically creatinine and potassium. The significant elevation of these parameters ($P < 0.05$) in comparison to the control group indicates early-stage renal impairment, likely resulting from the systemic absorption of toxic cement constituents. While sodium and urea levels remained relatively stable ($P < 0.05$), the overall biochemical profile suggests that chronic inhalation and dermal contact with cement dust pose a substantial threat to the long-term health of these workers. To prevent irreversible organ damage, it is imperative that regulatory bodies and health educators implement mandatory annual workshops. These programs must focus on the consistent use of Personal Protective Equipment (PPE), improved dust-suppression techniques, and regular medical surveillance. Ultimately, mitigating these health risks requires a shift in workplace culture where safety protocols are as fundamental to the block-moulding process as the production itself.

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<https://dx.doi.org/10.4314/ajtmbr.v8i2.13>