African Journal of Tropical Medicine and Biomedical Research (AJTMBR)

The Journal is the Official Publication of the College of Health Sciences, Delta State University, Abraka, Nigeria.

ISSN: 2141-6397

Vol. 8, No. 2, December 2025

Focus and Scope

The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

Editorial Notices

The journal will be published biannually in the months of March and September. Annual subscription fee in Nigeria is two thousand naira (N2,000) per volume (2issues); One-thousand-naira single copy (N1000). The annual subscription rate for other parts of the world is as follows: United Kingdom £60 (post free). West Africa $60 (post free). The rest of the World and the United States of America $120 (post free). A charge of $60 is made for reprints inclusive of postage. Cheques should made payable to the African Journal of Tropical Medicine and Biomedical Research and addressed to the Editor-in-Chief.

Journal Contact

All correspondence, including manuscripts for publication (in triplicate) should be addressed to:

Professor P.S. Igbigbi
The Editor-in-Chief,
Department of Anatomy,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Delta State University, Abraka,
Delta State, Nigeria.

Or:
Professor Lawrence Omo-Aghoja
Editor
Department of Obstetrics and Gynecology,
Faculty of Clinical Medicine,
Delta State University, Abraka, Nigeria.
Email: journalajtmbr@yahoo.com
Cc: all email to
eguono_2000@yahoo.com
Tel: 08039377043

All authors are advised to submit an electronic copy in CD-ROM along with a hard copy of their manuscript, as this will spare remarkable time in the reviewing and typesetting processes.

In the alternative, authors can submit their articles and covering letter by email attachments. A covering letter (signed by all authors) accompanying the manuscript should certify that the article has not been previously published and is not being considered for publication elsewhere.

Information for Authors

All manuscript are peer-reviewed and accepted with the understanding that the work has not been published or being considered for publication elsewhere. Indeed, the authors would be requested

to sign a copyright form transferring the ownership of the paper to the African Journal of Tropical Medicine and Biomedical Research. All articles must include the correct names and addresses of author(s) including e-mail addresses and telephone numbers. Articles will be subjected to a thorough peer review process before any decision is made to publish or not. Authors should note that the African Journal of Tropical Medicine and Biomedical Research is not under any obligation to publish articles submitted, as decision to publish will be based on recommendations of reviewers and the editorial advisory board.

Manuscripts

Articles submitted for publication should be typed double-spaced with 2.5cm margins with accompanying CD-ROM in Microsoft Word format for easy and quick peer review and typesetting. Each of the following sections should begin in a new page: title page, abstract, introduction, materials and methods, results, discussion, acknowledgment (s), references, tables, legends to figures and illustrations. The manuscript should include:

Title Page

The title page should include the following information: 1. the title and sub-title; 2. the name(s) of the author(s); 3. the affiliation(s) of the author(s); 4. name and address of the corresponding author and 5. three to six key words for indexing and retrieval purposes.

Abstract

The abstract should be structured and not more than 250 words. It should carry the following headings: Introduction, Materials and Methods, Results and Conclusion.

Original Research- The journal welcomes articles reporting on original research, including both quantitative and qualitative studies. Full-length articles should generally not exceed 3000 words, excluding abstract, tables, figures, and references. The subject matter should be organised under appropriate headings and sub-headings as itemized above.

Review Articles- Comprehensive review articles on all aspects of tropical medicine and biomedical sciences will also be considered for publication in the journal. Reviews should provide a thorough overview of the topic and should incorporate the most current research. The length of review articles must not exceed 3,000 words and the organisational headings and sub-headings used are at the author's discretion.

Short Reports - Brief descriptions of preliminary research findings or interesting case studies will be considered for publication as short reports. The length of the abstract and article should be restricted to 150 and 2,000 words respectively and organisation of short reports are left to the author's discretion.

Commentaries or Editorials- Commentaries or editorials on any aspect of tropical medicine and biomedical sciences in Africa will be considered for publication in the journal. Opinion pieces need not reference previous research, but rather reflect the opinions of the author(s). The length should not exceed 2,000 words.

Tables and Figures

All tables and figures should be submitted on separate sheets of paper and should be clearly labelled. Coloured tables and figures may be reprinted in black and white. Authors should especially take care that all tables are clear and understandable by themselves, independent of

the text. A reader should be able to read only the tables and easily grasp all information without the text.

Acknowledgments

Acknowledgments should be included on a separate sheet of paper and should not exceed 100words. Funding sources should be noted here.

References

References should be in the Vancouver style and numbered consecutively in the order in which they are mentioned in the text. Titles of journals should be abbreviated according to the Index Medicus style. Authors must cross-check and make sure that all information provided in the reference list is complete and correctly written. Reference numbers should be inserted above the line on each occasion a reference is cited in the text, e.g., … as 1-3 reported in other studies. Numbered references should appear at the end of the article and should include the names and initials of all authors. The format of references should be as published by the International Committee of Medical Journal Editors in the British Medical Journal 1988, volume 296, pages 401-405. The following are sample references for an article published in a journal and for a book: Ahmed Y, Mwaba P, Chintu C, Grange JM, Ustianowski A, Zumla A. A study of maternal mortality at the University Teaching Hospital, Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal death. Int J Tuberc Lung Dis 1999; 3: 675-680. Whitby LG, Smith AF, Beckett GJ. Enzyme Tests in Diagnosis. In: Lecture Notes on Clinical Chemistry. Whitby LG, Smith AF & Beckett GJth (eds). 4 editions. Blackwell Scientific Publications. 1988. 103-127.

Units of Measurement

All measurements should be expressed in SI (Systeme International) Units.

Galley proofs

Corrections of galley proofs should be strictly restricted to Printer's error only. Orders for offprints should be made when the corrected proofs are being returned by the authors. Articles accepted for publication remain the property of the journal and can only be reproduced elsewhere in line with section 5 of the copyright agreement.

"For those journals with their own websites, and a very high degree of in-house technical competency, it is possible to get code snippets from the Creative Commons website for the license type chosen, which can be embedded in your online content, but please note that that is definitely not a requirement, just a **"nice to have".**

Table of Contents

Original articles

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

[1]Omogbiya A, [*1]Orovwigho O, [1]Moke EG, [1]Oboh M, [1]Emudainohwo JOT, [2]Awhin 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.

[1]Department of Pharmacology, Faculty of Allied Health Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria.
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria
[3]Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.
[4]Department 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 mammals [2]. 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 factors [3]. 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 function [10]. 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 problems [11]. 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 some [11-12]. Research suggests that cyclic GMP (cGMP) plays a crucial role in memory formation [13]. Consequently, inhibiting phosphodiesterase type 5 (PDE5), an enzyme that breaks down cGMP, presents a promising approach to improve the initial stages of memory consolidation [14]. Studies on healthy young men have demonstrated that sildenafil, a PDE5 inhibitor, can lead to noticeable changes in brain activity patterns associated with improved focus [15]. Furthermore, animal studies using mice have shown that sildenafil enhances both the acquisition and retention of memories in tasks like maze navigation and inhibitory learning [16]. 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 reflexes [17]. Research indicates DAP's ability to modulate potassium channels, which influences neurotransmitter release, thus explaining its therapeutic benefits [18]. While SSRIs are generally prescribed for depression [19], research has shown potential benefits in neurodegenerative conditions following brain injuries [20]. 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 cells [18]. 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 approach [21].

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 ejaculation[22]. 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 modulation[23]. 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 memory [22]. Dapoxetine, as an SSRI, directly affects serotonin neurotransmission, a critical regulator of mood, cognition, and behavior [18].

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 processes [24].

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 ºC. 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 maze[31]

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 test [32]

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 recorded [32].

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 b uffer (PBS) (10 % w/v, 0.1 M, pH 7.4). Decanted supernatants were stored at -20◦C before the assays. Two (2) mice underwent transcardial 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 levels [33]. The lipid peroxidation, malondialdehyde was assayed using Tris-potassium chloride thiobarbituric acid and Griess reagent [34].

Histological examination

Haematoxylin and eosin staining techniques were used to evaluate the viability of neuronal cells in the medial prefrontal cortex hippocampus [35]. 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 eosin [36]. 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 ± 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.

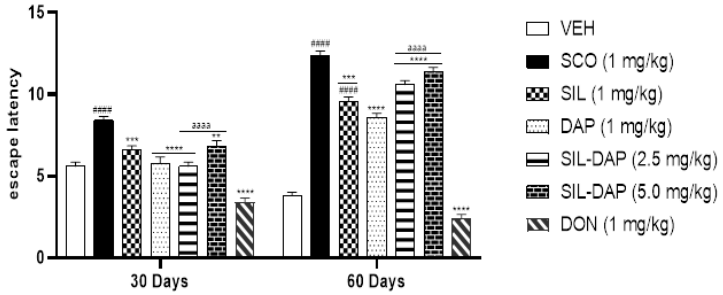

Fig. 1: The Effect of SIL-DAP on spatial working memory in male mice using Barnes maze test. Bars are expressed as mean ± SEM of grouped mice (n=5). $^{\#\#\#\#}p<0.0001$ Vs VEH group. $^{****}p<0.0001$, $^{***}p<0.001$, $^{**}p<0.01$ Vs SCO group. $^{a}p<0.05$, $^{aaaa}p<0.0001$ Vs DON group (Two-way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

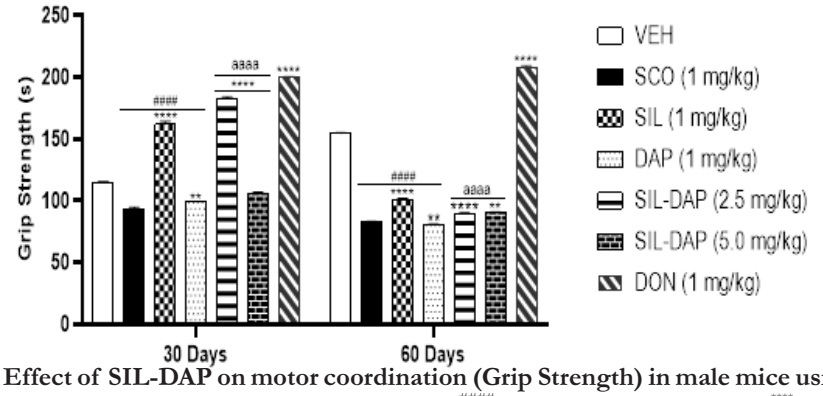

Fig. 2: The Effect of SIL-DAP on motor coordination (Grip Strength) in male mice using Rota Rod. Bars are expressed as mean ± SEM of grouped mice (n=5). $^{\#\#\#\#}p<0.0001$ Vs VEH group, $^{****}p<0.0001$, $^{**}p<0.01$ Vs SCO group, $^{aaaa}p<0.0001$ Vs DON group. (Two-way ANOVA followed by Turkey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

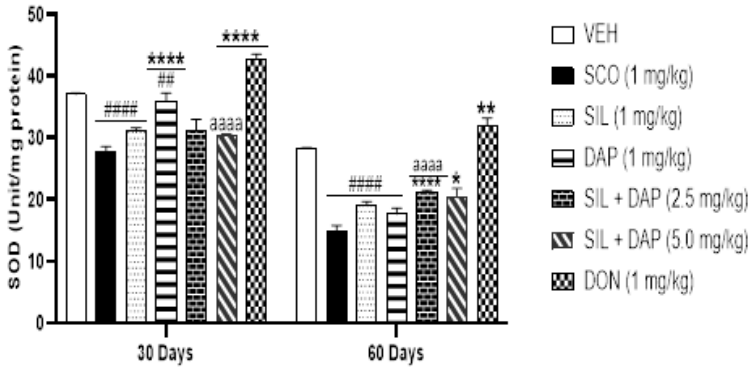

Fig. 3: The Effect of SIL-DAP on superoxide levels in mice brain. Bars are expressed as the mean ± SEM of grouped mice (n=5). $^{\#\#\#\#}p<0.0001$, $^{\#\#}p<0.05$ Vs VEH group. $^{****}p<0.0001$, $^{**}p<0.001$, $^{*}p<0.01$ Vs SCO group. $^{aaaa}p<0.0001$compared to DON group. (Two-way ANOVA followed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

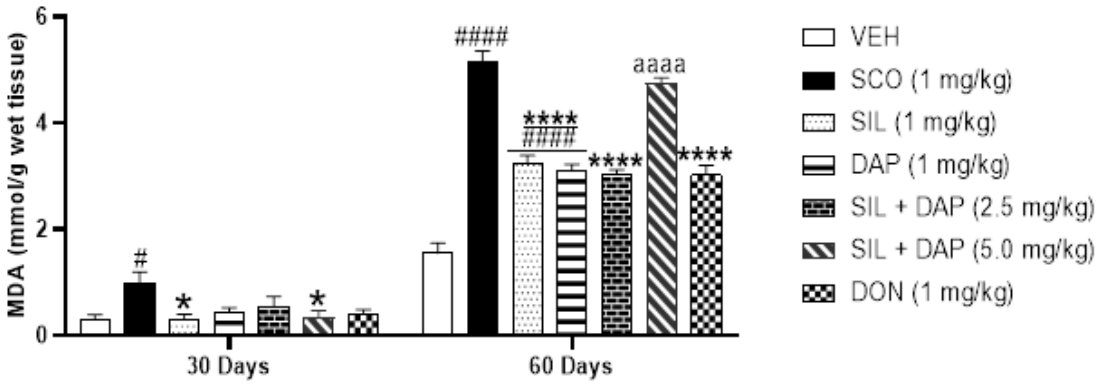

Fig. 4: The Effect of SIL-DAP on malondialdehyde levels in male mice brain. Bars expressed as mean ± SEM of grouped mice (n=5). $^{\#\#\#\#}p<0.0001$, $^{\#}p<0.05$ Vs VEH group. $^{****}p<0.0001$, $^{*}p<0.05$, Vs SCO group. $^{aaaa}p<0.0001$ Vs DON group. (Two-way ANOVA followed by Turkey Post-hoc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

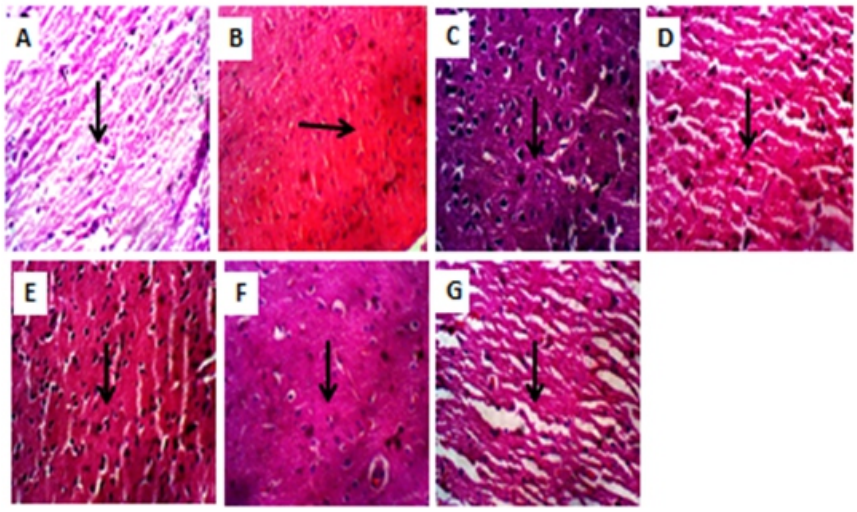

Plate. 1 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the medial prefrontal cortex (mPFC) in mice for 30 consecutive days. A: VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON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

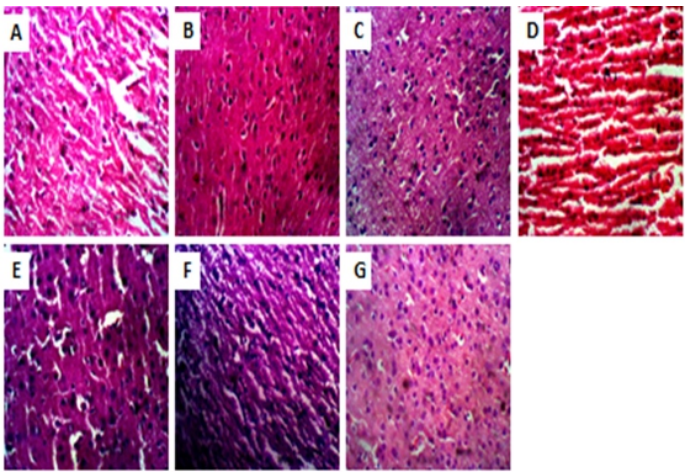

Plate. 2 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the medial prefrontal cortex (mPFC) in mice brain for 60 consecutive days. **A:** VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **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.

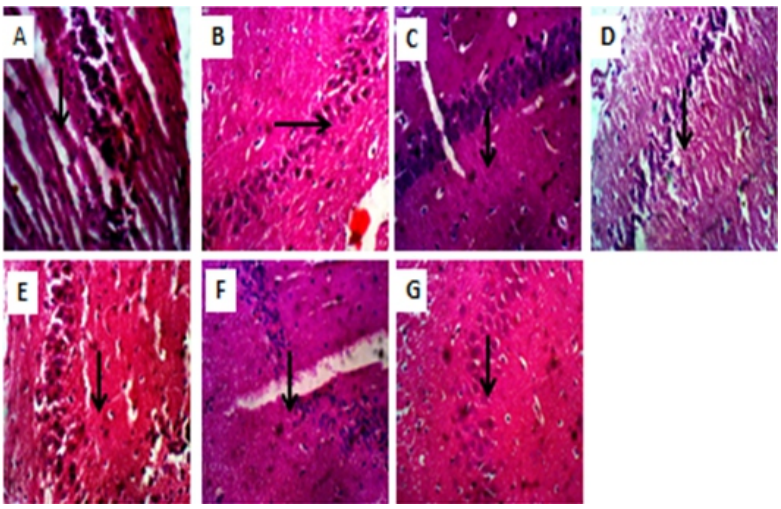

Plate. 3 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA1) in mice for 30 consecutive days. A: VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections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

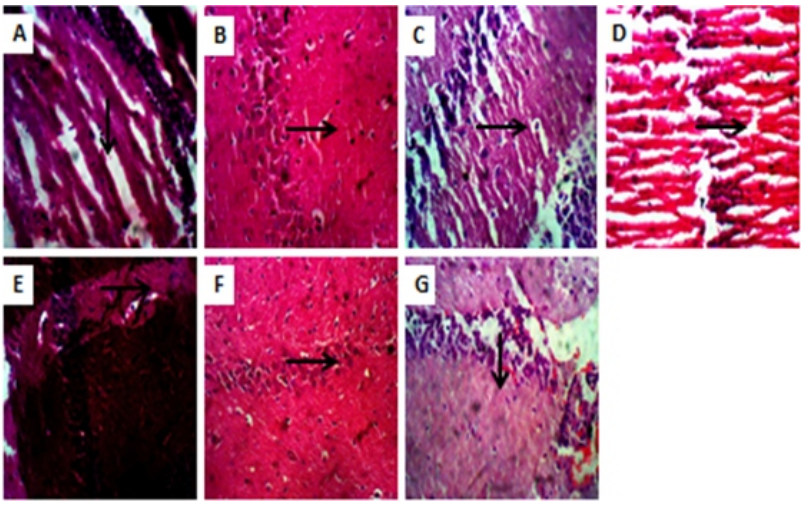

Plate. 4 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA1) in mice brain for 60 consecutive days. A: VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SIL-DAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg).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

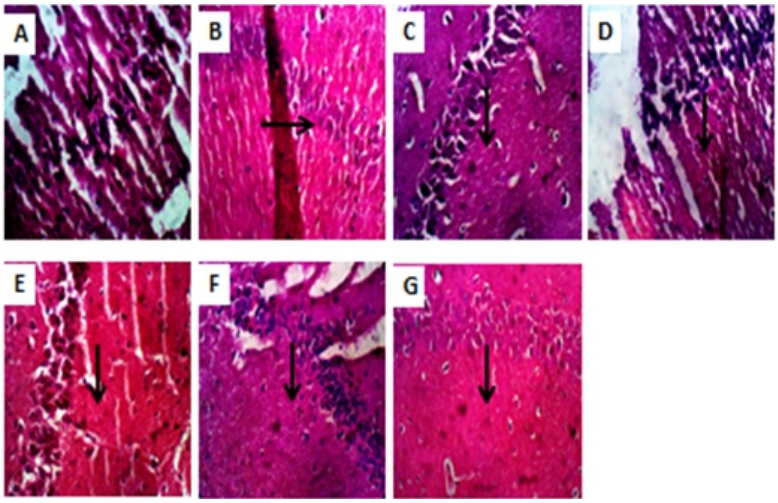

Plate. 5 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA3) in mice for 30 consecutive days. A: VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SILDAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg). Sections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

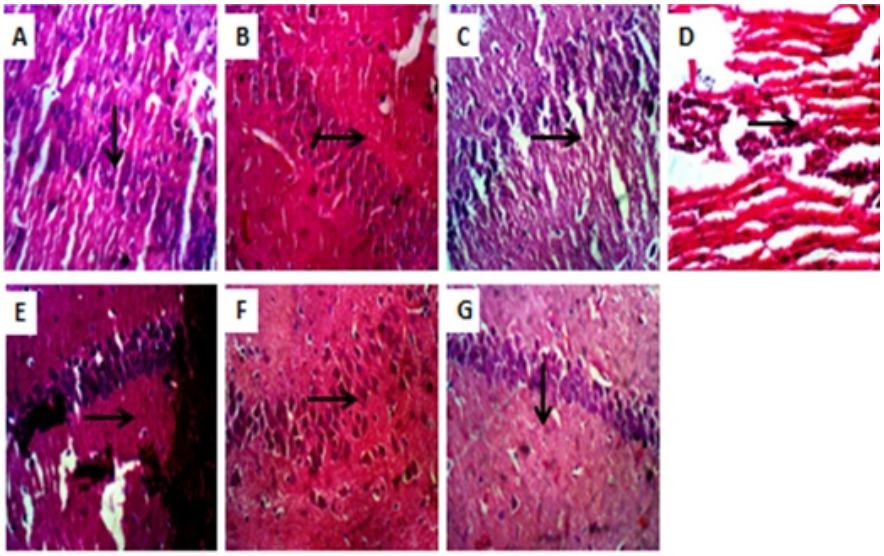

Plate. 6 Photomicrograph of the effect of Sildenafil-Dapoxetine (SIL-DAP) administrations on the hippocampus (CA3) in mice brain for 60 consecutive days. A: VEH, **B:** SCO (1mg/kg), **C:** SIL (1mg/kg), **D:** DAP (1mg/kg), **E:** SILDAP 2.5 (1mg/kg), **F:** SIL-DAP 5.0 (1mg/kg), **G:** DON (1mg/kg).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

DISCUSSION

Memory is both a result of and an influence on learning perception and attention [37]. 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 regions [38]. For example, damage to the hippocampus, a crucial structure for learning and memory processing can interfere with long-term memory storage and retrieval [39]. 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 abilities [40].

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 memory [41] 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 area [42]. 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 skills [43]. 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 peroxidation [44], it's a reactive aldehyde formed when polyunsaturated fatty acids in cell membranes are damaged by free radicals [45]. SOD acts as an enzyme that ensures the conversion of oxidative molecules such as superoxide anions into oxygen and hydrogen peroxide [46]. The peculiarity of its actions elucidates O2- and hydrogen peroxide, and hence, the likelihood of its central role in the defence mechanism [47]. 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.

REFERENCES

1. Herbet G, Duffau H. Revisiting the functional anatomy of the human brain: toward a meta-networking theory of cerebral functions. *Physiol Rev*. 2020;100 (3):1181–1228.
2. Singh S. Analysis of the function and role of brain neurotransmitters in individual activities. *Sriwijaya J Neurol*. 2023;1(2):43–7.
3. Morella IM, Brambilla R, Morè L. Emerging roles of brain metabolism in cognitive impairment and neuropsychiatric disorders. *Neurosci Biobehav Rev*. 2022;142:104892.
4. Rezayof A, Ghasemzadeh Z, Sahafi OH. Addictive drugs modify neurogenesis, synaptogenesis and synaptic plasticity to impair memory formation through neurotransmitter imbalances and signaling dysfunction. *Neurochem Int*. 2023;105572.
5. Guan S, Li Y, Xin Y, Wang D, Lu P, Han F, Xu H. Deciphering the dual role of N-methyl-D-aspartate receptor in postoperative cognitive dysfunction: a comprehensive review. *Eur J Pharmacol*. 2024;176520.
6. Jiang L, Dong R, Xu M, Liu Y, Xu J, Ma Z, Xia

T, Gu X. Inhibition of the integrated stress response reverses oxidative stress damage-induced postoperative cognitive dysfunction. *Front Cell Neurosci*. 2022; 16:992869.

7. Manchinu MF, Pala M, Palmas MF, Diana MA, Maschio A, Etzi M, et al. Region-specific changes in gene expression are associated with cognitive deficits in the alpha-synuclein-induced model of Parkinson's disease: a transcriptomic profiling study. *Exp Neurol*. 2024; 372:114651.
8. Zhang L, Huang T, He H, Xu F, Yang C, Lu L, et al. Unraveling the molecular mechanisms of ACE2-mediated post-COVID-19 cognitive dysfunction through systems genetics approach. *Exp Neurol*. 2024;381:114921.
9. Zhou Q, Zheng Z, Chen C, Liu B, Ma Z, Xia T, Gu X. Activation of ACE2/Ang-(1–7)/Mas axis improves cognitive dysfunction induced by isoflurane in mice via inhibiting oxidative stress. *J Anesth Transl Med*. 2024;3(3):123–31.
10. Pu ca u C, Zanfirescu A, Negre S, eremet OC. Exploring the multifaceted potential of sildenafil in medicine. *Medicina (Kaunas)*. 2023;59(12):2190.
11. Hajilou F. Review of different applications of sildenafil with the focus on its role in Alzheimer's disease. [Journal name missing]; 2023.
12. Sanders O. Sildenafil for the treatment of Alzheimer's disease: a systematic review. *J Alzheimers Dis Rep*. 2020;4(1):91–106.
13. Nelissen E, Argyrousi EK, Van Goethem NP, Zhao F, Hines CD, Swaminath G, et al. Soluble guanylate cyclase stimulator vericiguat enhances long-term memory in rats without altering cerebral blood volume. *Biomedicines*. 2021;9(8):1047.
14. Firdosh N, Yadav BS, Rahman S, Kumar H. PDE-5 restores cognitive and metabolic function in patients with Alzheimer's disease (AD). [Journal name missing]; 2024.
15. Zuccarello E, Acquarone E, Calcagno E, Argyrousi EK, Deng SX, Landry DW, et al. Development of novel phosphodiesterase 5 inhibitors for the therapy of Alzheimer's disease. *Biochem Pharmacol*. 2020;176:113818.
16. Constantin MF, de la Villarmois EA, Bravo JL, Marcotti A, Ghersi M, Castro F, et al. Sildenafil promotes dual memory effects in young rats: involvement of dopamine reuptake. *Psychopharmacology (Berl)*. 2025;1–12.
17. Qiu J, Wang B, You X, Wu L, Hong Z, Zhou W, Zhou Q. Neurobiology of premature ejaculation: serotonergic mechanisms and emerging therapeutic insights. *Andrologia*. 2025;1:2482249.
18. Abdel-Hameed SS, El-Daly M, Ahmed ASF, Bekhit AA, Heeba GH. Dapoxetine prevents neuronal damage and improves functional outcomes in a model of ischemic stroke through modulation of inflammation and oxidative stress. *Naunyn Schmiedebergs Arch Pharmacol*. 2024;397(1):253–66.
19. Boschloo L, Hieronymus F, Lisinski A, Cuijpers P, Eriksson E. The complex clinical response to selective serotonin reuptake inhibitors in depression: a network perspective. *Transl Psychiatry*. 2023;13(1):19.
20. Xu F, Zhang G, Yin J, Zhang Q, Ge MY, Peng L, Li Y. Fluoxetine mitigating late-stage cognition and neurobehavior impairment induced by cerebral ischemia reperfusion injury through inhibiting ERS-mediated neurons apoptosis in the hippocampus. *Behav Brain Res*. 2019;370:111952.
21. Saleh R, Majzoub A, Abu El-Hamd M. An update on the treatment of premature ejaculation: a systematic review. *Arab J Urol*. 2021;19(3):281–302.
22. Shafer LC. 17 Pharmacotherapy of Sexual Disorders and Sexual Dysfunction.

Massachusetts General Hospital Psychopharmacology and Neurothera-peutics-E-BOOK. 2024 Sep 2:200.

23. Vieiralves RR, Favorito LA, editors. Dapoxetine and premature ejaculation. International braz j urol. 2023 Aug 14;49(4):511-4.
24. Nawras M, Beran A, Yazdi V, Hecht M, Lewis C. Phosphodiesterase inhibitor and selective serotonin reuptake inhibitor combination therapy versus monotherapy for the treatment of major depressive disorder: a systematic review and meta-analysis. International Clinical Psychopharmacology. 2023 Jul 1;38(4):261-8
25. Cazzaniga W, Capogrosso P, Boeri L, Montorsi F, Salonia A. Pharmacology of Male Sexual Function. InUrologic Principles and Practice 2020 Jan 2 (pp. 159-174). Cham: Springer International Publishing.
26. Yadang FS, Nguezeye Y, Kom CW, Betote PH, Mamat A, Tchokouaha LR, Taiwé GS, Agbor GA, Bum EN. Scopolamine induced memory impairment in mice: neuroprotective effects of Carissa edulis (Forssk.) Valh (Apocynaceae) aqueous extract. International Journal of Alzheimer's Disease. 2020; 2020(1):6372059.
27. Wang, Z. X., Lian, W. W., He, J., He, X. L., Wang, Y. M., Pan, C. H., et al. 2022. Cornuside ameliorates cognitive impairments in scopolamine induced AD mice: Involvement of neurotransmitter and oxidative stress. *Journal of Ethnopharmacology*, *293*, 115252.
28. Rafi H, Farhan M. Dapoxetine: An innovative approach in therapeutic management in animal model of depression. Pakistan Journal of Pharmaceutical Sciences. 2015;2(1):15-22.
29. Amenotie AJ, Ben-Azu B, Esuku DT, Chijioke BS, Abo E, Ozah EO, Lawrence EO, Efejene OI, Onyeukwu OB, Alabi BA, Ajayi AM. Sabinene Inhibits Lipopolysaccharide-Induced Memory Decline by Enhancing Cholinergic Function, Decreasing Molybdenum Enzymes, and Suppressing Oxidative Stress and Neuroinflammation. Neurotoxicity Research. 2025 Jun;43(3):26.
30. Eduviere AT, Umukoro S, Adeoluwa OA, Omogbiya IA, Aluko OM. Possible mechanisms involved in attenuation of lipopolysaccharide-induced memory deficits by methyl jasmonate in mice. Neurochemical research. 2016 Dec;41(12):3239-49.
31. O'Leary TP, Brown RE. Optimization of apparatus design and behavioral measures for the assessment of visuo-spatial learning and memory of mice on the Barnes maze. Learning & memory. 2013 Feb 1;20(2):85-96.
32. Patel R, Jain NS. Stimulation of central histaminergic transmission attenuates diazepam-induced motor disturbance on rota-rod and beam walking tests in mice. Behavioural Pharmacology. 2024 Sep 1;35(6):351-65.
33. Hajri L, Ghodbane S, Othman H, Sakly M, Abdelmelek H, et al. Ameliorative Effects of Pearl Millet (Pennisetum glaucum L.) Against Hydrogen Peroxide Induced Cognitive Impairment and Oxidative Stress in Rats. Journal of Medicinal Food. 2024 Dec 1;27(12):1210-8.
34. Ajibade TO, Ohore OM, Esan OO, Adeoye BO, Ake AS, Adetona MO, et al. Silymarin attenuates cobalt chloride-induced redox imbalance and cardio-renal dysfunctions in rats. Drug and Chemical Toxicology. 2025 May 7:1-1.
35. Mandour DA, Bendary MA, Alsemeh AE. Histological and imunohistochemical alterations of hippocampus and prefrontal cortex in a rat model of Alzheimer like-

disease with a preferential role of the flavonoid "hesperidin". Journal of Molecular Histology. 2021 Oct;52(5):1043-65.
36. Hasan MI, Das SK, Chowdhury EH. A better and copacetic protocol for histopathological slide preparation using H&E stain: A review. J. Fish. 2021;2:57-67.
37. Llinares C, Higuera-Trujillo JL, Serra J. Cold and warm coloured classrooms: effects on students' attention and memory measured through psychological and neurophysiological responses. *Build Environ*. 2021;196:107726.
38. Theodoratou M, Kougioumtzis GA, Yotsidi V, Sofologi M, Katsarou D, Megari K. Neuropsychological consequences of massive trauma: implications and clinical interventions. *Medicina (Kaunas)*. 2023;59 (12):2128.
39. Wu A, Zhang J. Neuroinflammation, memory, and depression: new approaches to hippocampal neurogenesis. *J Neuroinflammation*. 2023;20(1):283.
40. Guo H, Zheng L, Xu H, Pang Q, Ren Z, et al. Neurobiological links between stress, brain injury, and disease. *Oxid Med Cell Longev*. 2022;2022 (1):8111022.
41. Bertolli A, Halhouli O, Liu-Martínez Y, Blaine B, Thangavel R, Zhang Q, et al. Renovating the Barnes maze for mouse models of dementia with STARR FIELD: a 4-day protocol that probes learning rate, retention and cognitive flexibility. *bioRxiv*. 2024;2024–11.
42. Peris LR, Scheuber MI, Shan H, Braun M, Schwab ME. Barnes maze test for spatial memory: a new, sensitive scoring system for mouse search strategies. *Behav Brain Res*. 2024;458:114730.
43. Ziętal K, Blecharz-Klin K, Joniec-Maciejak I, Piechal A, Pyrzanowska J, Machaj E, et al. Neuroprotective effects of betanin in a mouse model of Parkinson's disease: behavioural and neurotransmitter pathway insights. *Int J Mol Sci*. 2025;26(19):9726.
44. Cordiano R, Di Gioacchino M, Mangifesta R, Panzera C, Gangemi S, Minciullo PL. Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: an update. *Molecules*. 2023;28(16):5979.
45. Mas-Bargues C, Escriva C, Dromant M, Borras C, Vina J. Lipid peroxidation as measured by chromatographic determination of malondialdehyde: human plasma reference values in health and disease. *Arch Biochem Biophys*. 2021;709:108941.
46. Islam MN, Rauf A, Fahad FI, Emran TB, Mitra S, Olatunde A, et al. Superoxide dismutase: an updated review on its health benefits and industrial applications. *Crit Rev Food Sci Nutr*. 2022;62(26):7282–300.
47. Anwar S, Sarwar T, Khan AA, Rahmani AH. Therapeutic applications and mechanisms of superoxide dismutase (SOD) in different pathogenesis. *Biomolecules*. 2025;15(8):1130.

Omogbiya A, Orovwigho O, Moke EG, Oboh M, Emudainohwo JOT, Awhin PE, Saviour GU. Differential Alterations In Behavioural Phenotypes, Brain Biochemical Profiles And Histomorphology In Mice After Administration Of Sildenafil-dapoxetine Formulation. Afr. J. Trop. Med. & Biomed. Res. 2025; 8(2) 12-26
https://dx.doi.org/10.4314/ajtmbr.v8i2.1