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INFORMATION

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A COMPARATIVE STUDY ON THE PROTECTIVE EFFECT OF ASCORBIC ACID (VITAMIN C) ON *CANNABIS SATIVA*-INDUCED OXIDATIVE STRESS IN MALE AND FEMALE WISTAR RATS.

Running title: Effects of Vitamin C and *Cannabis sativa* on oxidative stress in Wistar Rats

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ABSTRACT

Consumption and legalization of *Cannabis sativa* (CS) are increasing at a rapid rate due to its medicinal and recreational importance. However, several studies have revealed that CS stimulates oxidative stress (OS) which could affect the antioxidant defense system of the body. This research examined and compared the protective role of vitamin C (Vit C) on *Cannabis sativa* (CS)-induced oxidative stress in male (M) and female (F)Wistar rats. Post-acclimation (14 days), M and F animals were separately allocated to four groups. Males (1M, 2M, 3M, and 4M) and females (1F, 2F, 3F, and 4F) groups were administered orally, 1.0 mL of distilled water (control), CS (4.0 mg/kg), Vit C (4.0 mg/kg) and CS (4.0 mg/kg) + Vit C (4.0 mg/kg) respectively, for 21 days. Glutathione peroxide (GPx), Superoxide dismutase (SOD), Catalase, Glutathione reductase (GSH), Total antioxidant capacity (TAC), lactate dehydrogenase (LDH), and Malondialdehyde (MDA) were quantified following standard protocols. The study showed no significant differences in measured parameters between the groups treated with Vit C (4mg/kg) as comparable to the controls in both M and F rats. However, the groups treated with CS (4 mg/kg) exhibited significant ($p<0.05$) reduction in GPx, SOD, Catalase, GSH, and TAC while elevating LDH and MDA, compared to control and other treated groups in both M and F rats. In contrast, the groups treated with CS (4mg/kg)+Vit C (4mg/kg) significantly ($p<0.05$) increase GPx, SOD, catalase, GSH, and TAC with significant ($p<0.05$) decrease in LDH and MDA, compared to CS (4 mg/kg), and no significant differences in the controls and Vit C (4mg/kg) treated groups in both M and F rats. In conclusion, Vit C appeared to attenuate CS-associated oxidative stress. The observed effects were more pronounced in males compared to females, indicating a sex-dependent response. The results suggest that Vit C may be used as supplement to prevent oxidative stress which could be induced by CS. Further study is needed to show if similar effects could be observed in human subjects.

Keywords: *Cannabis sativa*; Oxidative stress; Vitamin C; Sex dependent; Wistar rats

INTRODUCTION

Cannabis sativa (CS) has been utilized for medicinal purposes since ancient times due to its abundant phytochemical content [1], hence the quest for preventing its oxidative effects in the body. This substance is widely used as an illicit drug globally [2]. Cells' normal redox state can be disrupted, leading to the production of peroxides and free radicals, damaging all cell components, including proteins, lipids, and DNA [3]. CS, botanically referred to as *Cannabis sativa* (CS), is a multipurpose flowering plant that has been cultivated for centuries for a variety of applications [4]. It has attracted considerable interest due to its complex chemical profile, which comprises more than 100 biologically active compounds known as cannabinoids, in addition to terpenes, flavonoids, and other phytochemicals [5]. Tetrahydrocannabinol (THC) is the main psychoactive constituent responsible for the characteristic euphoric effects commonly associated with cannabis consumption [6]. It is cultivated for multiple purposes, including medicinal, recreational, industrial, and spiritual uses. In clinical settings, it has gained recognition for its potential in managing symptoms related to various medical conditions such as chronic pain, nausea, epilepsy, and certain mental health disorders [7]. Consequently, medical cannabis has been legalized in several regions, permitting its prescription or recommendation by healthcare practitioners for approved indications [8]. Recreational use of cannabis is also widespread globally, primarily due to its psychoactive effects [9]. Several studies have revealed the adverse effects of CS consumption/smoking in the body [10-21]. Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants [22]. The ratio can be influenced by elevated levels of reactive oxygen species (ROS) or a decrease in antioxidant defense mechanisms [23]. OS can also arise from an imbalance in the body's oxidizing system, primarily composed of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) [24]. Antioxidant systems are essential in neutralizing free radicals that can have numerous harmful effects [25]. However, experimental and clinical studies have demonstrated that *Cannabis sativa* may induce oxidative stress in both humans and animal models [26-28].

Vitamin C (ascorbic acid) is widely recognized for its strong antioxidant capacity [29]. In addition, vitamin C supports the regeneration of other antioxidants, including vitamin E, thereby strengthening the body’s defense against oxidative stress [29]. In plants, including cannabis, vitamin C functions as an effective antioxidant by counteracting reactive oxygen species (ROS) and safeguarding cellular components from oxidative injury [30]. Within our research limit, we have not come across any study which has examined and compared the

protective role of Vit C on *Cannabis sativa* (CS)-induced oxidative stress in male (M) and female (F) Wistar rats. This research work therefore aimed to bridge this lacuna so as to identify the potential sex differences and examine oxidative biomarkers (Glutathione peroxide (GPx), superoxide dismutase (SOD), catalase, glutathione reductase (GSH), total antioxidant capacity (TAC), lactate dehydrogenase (LDH), and malondialdehyde (MDA)), to assess the influence of Vit C on CS-induced OS, considering its gender-dependent modulation. The oxidative stress parameters were considered because they provide valuable information about the anti-oxidative defense system of the body. The objective of this study was to investigate the effects of co-administration of Vit C and CS on oxidative stress in male and female Wistar rats as subjects by considering gender-dependent modulation. The hypotheses of our research work were Vit C would not attenuate CS-induced oxidative stress in male and female Wistar rats (null); and Vit C would attenuate CS-induced oxidative stress in male and female Wistar rats (alternative).

METHODOLOGY

Sample collection:

Cannabis sativa (CS) leaves were donated by National Drug Law Enforcement Agency (NDLEA), Nigeria, for research purpose only.

Extraction of Cannabis sativa leaves [27]:

Extraction of *Cannabis sativa* (CS) was done with Soxhlet apparatus by soaking 400 grams of CS in 98% ethanol for 48 hours. It was filtered and the filtrate was poured into a round-boom conical flask it was fixed with a rotary evaporator. The filtrate was then evaporated and cooled. The dried yield of the extract was 45g (weight of the extract obtained after drying).

Experimental animals:

Twenty male rats with mean weight of 180g ± 1.89g and twenty female rats with mean weight of 165g ± 1.23g used in the present study were obtained from Temilade Animal Venture, Ogbomoso, Oyo State, Nigeria. The animals were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. The experimental protocol was approved by Ethical Committee of the University of Ilesa, Ilesa, Osun State, Nigeria with approval number (UNILESA-2025/EARC/012).

Experimental protocol:

After 2 weeks of acclimatisation, the animals (male (M) and female (F)) were separately and randomly assigned into four groups of five animals each for male and female. Males (1M, 2M, 3M, and 4M) and females (1F, 2F, 3F, and 4F) groups were administered orally, 1.0 mL of distilled water (control), CS (4.0 mg/kg), Vit C

(4.0 mg/kg) and CS (4.0 mg/kg)+Vit C (4.0 mg/kg) respectively, once daily via oral gavage between 8:00 am to 10:00 am for twenty-one (21) days. The animals had access to food and water ad-libitum. The animals were sacrificed after day 21.

Preparation of serum:

The male and female rats were sacrificed under ketamine anesthesia and blood was collected by cardiac puncture into sterile sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×*g* for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England). The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

Drug and assay kits:

The liquid form of Vit C was purchased from One Step Pharmaceutical Company, Ilorin, Kwara State, Nigeria. Lactate dehydrogenase (LDH) activity was assayed spectrophotometrically (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA) following the kit manufacturer's procedures (product code BXC0243; Fortress Diagnostics, UK). The determination of serum superoxide dismutase (SOD) concentration was done with SOD colorimetric assay kit (Fortress Diagnostics Ltd., Antrim, UK; Product code: BXC0531), following the manufacturer's protocols. The determination of serum glutathione peroxidase (GPx) activity was done with GPx colorimetric assay kit (BioVision Inc., Milpitas, CA, USA), following the manufacturers protocols. Based on the manufacturer's protocol, total antioxidant capacity (TAC) measurement in the serum was done with a spectrophotometric microplate reader (Spectramax Plus, Molecular Devices, Sunnyvale, CA, USA) using OxiSelect TAC assay kit that uses the single electron transfer mechanism (Cell Biolabs, Inc. San Diego, CA. cat no: STA-360). The continuous catalase activity was determined through spectrophotometric reading [31]. Reduced glutathione (GSH) was measured according to the method of [32]. The assay method of [33], modified by [34] was adopted for Malondialdehyde (MDA).

Statistical analysis:

Results were expressed as the mean ± standard error of mean (S.E.M). Data was analyzed using a two-way Analysis of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with the aid of graph pad, version 9.0. Differences with values of $P<0.05$ were considered statistically significant.

RESULTS

The results obtained are presented in Table 1. There was no significant ($p>0.05$) difference in measured parameters between the groups treated with Vit C (4mg/kg) as compared to the

controls in both M and F rats. However, the groups treated with CS (4 mg/kg) exhibited significant ($p<0.05$) reduction in GPx, SOD, Catalase, GSH, and TAC while elevating LDH and MDA, compared to the controls and Vit C (4mg/kg) treated groups in both M and F rats. In contrast, the groups treated with CS (4mg/kg)+Vit C (4mg/kg) significantly ($p<0.05$) increase GPx, SOD, Catalase, GSH, and TAC, with significant ($p<0.05$) decrease in LDH and MDA, compared to CS (4 mg/kg), and with no significant differences in the controls and Vit C (4mg/kg) treated groups in both M and F rats.

Table 1: Showing oxidative stress markers of rats (male (M) and female (F)) for control and treated (4.0 mg/kg bw CS, 4.0 mg/kg bw Vit C, and 4.0 mg/kg bw CS +4.0 mg/kg bw Vit C) groups.

Treated groups	GPx (mmol/l)	SOD (U/L)	Catalase, (u/l)	GSH (u/l)	TAC (umol/l)	LDH (u/l)	MDA (umol/l)
Control (M)	8.10 ±0.78	158.63±6.43	47.72±0.23	132.12±4.56	95.21±1.72	754.23±20.14	28.56±0.50
Control (F)	3.07 ±0.26	120.50±6.98	28.05±0.51	110.08±4.70	87.40±1.50	626.13±23.38	23.40±0.52
4mg/kg CS (M)	4.45 ±0.65*	92.34±3.65*	34.32±1.02*	100.67±4.01*	80.56±1.52*	895.23±12.98*	35.23±9.65*
4mg/kg CS (F)	2.28±0.09*	74.34±7.01*	24.94±0.42*	94.28±1.12*	73.39±2.11*	694.76±23.01*	26.00±0.32*
4mg/kg Vit C (M)	8.10 ±0.78	158.63±6.43	47.72±0.23	132.12±4.56	95.21±1.72	754.23±20.14	28.56±0.50
4mg/kg Vit C (F)	3.07 ±0.26	120.50±6.98	28.05±0.51	110.08±4.70	87.40±1.50	626.13±23.38	23.40±0.52
4mg/kg CS + 4mg/kg Vit C(M)	7.10 ±0.71#	151.21±5.43#	43.26±0.18#	128.65±3.78#	94.34±1.68#	728.10±23.13#	27.29±0.53#
4mg/kg CS+4mg/kg Vit C (F)	3.02 ±0.26#	118.91±6.05#	27.11±0.46#	106.12±5.10#	80.65±1.21#	622.45±23.21#	20.67±0.83#

Values are expressed as mean ± SEM; *P< 0.05 vs control and other treatment groups (M and F); #P< 0.05 vs CS-treated groups (M and F)

DISCUSSION

Oxidative stress is the result of an imbalance between the systemic expression of reactive oxygen species (ROS) and a biological system's ability to rapidly detoxify the reactive intermediates or repair the harm they cause [35]. Peroxides and free radicals are created when a cell's natural redox state is destabilized. These molecules damage proteins, lipids, DNA, and other components of the cell and can have harmful consequences [36]. Oxidative stress brought on by oxidative metabolism damages DNA strands along with their bases [37]. Reactive oxygen species such superoxide radical (O^{2-}), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) are the primary causes of indirect base damage [38]. Previous studies have established that vit C acts as an antioxidant [19, 39]. Catalase is a major antioxidant enzyme that facilitates the breakdown of hydrogen peroxide into water and molecular oxygen through a two-step reaction [40]. Similarly, glutathione (GSH) has gained considerable attention as a biomarker of

oxidative stress due to its essential role in xenobiotic detoxification and protection against oxidative damage. The availability of glutathione in its reduced form (GSH) is critical for maintaining cellular health [41]. Reduced GSH levels have been associated with aging and the pathogenesis of several diseases, including AIDS, rheumatoid arthritis, muscular dystrophy, amyotrophic lateral sclerosis, Alzheimer's disease, alcoholic liver disease, cataract formation, respiratory distress syndrome, progeria, and Werner syndrome, as reported in both human and animal studies [42]. Our results revealed that oxidative parameters (catalase, SOD, GSH, glutathione peroxidase (GPx), and total antioxidant capacity (TAC)) were not affected when Vit C alone was administered. However, a reduction in their levels were observed when CS was administered alone, suggesting oxidative damage in both male and female rats. This was in line with the findings of [26, 28] who recorded oxidative damage following CS administration in male and female rats. Lipid peroxidation results in the formation of malondialdehyde (MDA), which is widely used as a biomarker for assessing oxidative stress in various biological samples, including blood, urine, and exhaled breath condensate, particularly in patients with cancer and cardiovascular, pulmonary, and neurological diseases [43]. Elevated level of MDA recorded in this study following CS administration demonstrated tissue damage, which was consistent with the result of [44]. Tissue injury is often accompanied by oxidative stress, elevated LDH levels may serve as an indirect indicator of oxidative damage [45]. Increase in the level of LDH observed after CS administration suggests the occurrence of damage to the tissue in both male and female rats. In contrast, there were no changes in MDA and LDH following the administration of Vit C alone. Our results also showed that administration of Vit C and CS reversed the effects caused by CS alone on oxidative stress, suggesting the anti-oxidative potential of Vit C. This concurred with the findings of [19, 27, 46]. The effects of Vit C on cannabis-induced oxidative stress were more in female than in male rats, indicating a potential sex difference. This could be due to the widely distribution of cannabinoid receptors in male than female [47] which stimulated the activities of CS administered.

CONCLUSION

In conclusion, Vit C appeared to attenuate CS-associated oxidative stress. The observed effects were more pronounced in males compared to females, indicating a sex-dependent response. This study suggests that Vit C may be used as supplement to prevent oxidative stress which could be induced by CS. Further study is needed to show if similar effects could be observed in human subjects.

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COMPARATIVE ANALYSIS OF DEMOGRAPHIC AND COVID-19-RELATED CHARACTERISTICS AMONG STUDENTS AT NIGER DELTA UNIVERSITY CAMPUSES, NIGERIA

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ABSTRACT

This study investigated the demographic and COVID-19 related characteristics among students from the two Niger Delta University campuses - Amassoma and Yenagoa. A cross-sectional survey was administered to 514 students drawn from the College of Health Sciences (CHS), Main Campus, and the Yenagoa Law Faculty (responses recorded separately for males and females). Chi-square tests were applied to compare gender distribution, year of study, vaccination status, understanding of COVID-19 transmission, and willingness to receive the vaccine. Although gender distribution did not differ significantly ($p = 0.846$), a highly significant difference in year of study ($p < 0.001$) was observed, indicating that academic level influences COVID-19 related knowledge and behavior. In addition, significant differences in vaccination status ($p = 0.015$) and willingness to vaccinate ($p < 0.001$) were noted. These findings underscore the need for campus-specific, culturally tailored health communication and basic health education strategies to enhance COVID-19 prevention and vaccine uptake among university communities.

Keywords:
COVID-19, Vaccine, Knowledge, Attitudes, Practices, Concerns, University Students, Nigeria

INTRODUCTION

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has had unprecedented global impacts on public health, education, and economies [1]. Nigeria reported its first COVID-19 case in 2020, marking a pivotal moment for West Africa [2]. In response, measures such as lockdowns, mandatory face mask usage, and social distancing were implemented nationwide. The educational sector was profoundly affected, with many tertiary institutions forced to adopt remote learning - a challenge in Nigerian settings where digital infrastructure is often limited [3, 4].

Research on Knowledge, Attitudes, Practices, and Concerns (KAPC) regarding COVID-19 has provided valuable insights into public response to the pandemic [5, 6, 7, 8, 9, 10]. However, few

studies have focused on university students across different academic disciplines. Understanding KAPC in this population is critical for designing targeted public health interventions that improve vaccine uptake and adherence to preventive measures

Niger Delta University (NDU) offers a unique opportunity for such comparative analysis. With campuses in Amassoma and Yenagoa, NDU serves a diverse student body. This study aimed to compare the demographic characteristics and COVID-19 related perceptions and behaviors between students at the Amassoma campus and those at the Yenagoa campus. Specifically, the study examined differences in gender distribution, academic level, vaccination status, understanding of COVID-19 transmission, and willingness to be vaccinated.

Study location:

Bayelsa State, located in Southern Nigeria within the Niger Delta region, is bordered by Delta State to the North, Rivers State to the East and South, and the Atlantic Ocean to the West and South. The state was created in 1996 in part of Rivers State, and its capital is Yenagoa. As of the 2006 census, the population of Bayelsa State was 1,704,515. Recent estimates suggest that the population has grown to approximately 2,537,400 [11]. Bayelsa is the smallest state by population in Nigeria. Geographically, Bayelsa is characterized by its riverine and estuarine environment, with many communities accessible only by sea transportation. The state is rich in oil and natural gas, significantly contributing to Nigeria's petroleum industry, although it also faces challenges such as environmental pollution and poverty [11].

Niger Delta University (NDU) is a state-owned institution located on Wilberforce Island, Bayelsa State, Nigeria. Established in 2000 by the Bayelsa State government to promote higher education, NDU currently enrolls about 25,000 students and offers a wide range of undergraduate and postgraduate programs [12]. NDU operates from two primary campuses: the Amassoma campus and the Yenagoa campus. The Amassoma campus, situated on Wilberforce Island about thirty kilometers from Yenagoa, is the main hub of the institution. It comprises the Main Campus - hosting faculties such as Agriculture, Arts, Education, Engineering, Management Sciences, Pharmacy, Science, and Social Sciences - and the College of Health Sciences (CHS), which focuses on medical and health-related programs. The Yenagoa campus is dedicated to the Faculty of Law and is in the state capital, offering proximity to legal resources, government institutions, and urban amenities. Both campuses are connected by road, facilitating transportation and enhancing the overall academic experience [12].

METHODOLOGY

Study Design and Participants:

A cross-sectional survey design was employed to assess differences in COVID-19-related

behaviors and perceptions between students at the Amassoma and Yenagoa campuses of NDU. A total of 514 students were randomly selected from the following groups: CHS (College of Health Sciences), Main Campus and Yenagoa Law Faculty. Responses were recorded separately for males and females.

Data Collection:

Data was collected using a self-designed structured questionnaire administered in-person between January and March 2023. The questionnaire was pre-tested on a small sample to ensure clarity and reliability. Feedback and suggested changes were provided in writing and subsequently used to improve the final version of the questionnaire.

The questionnaire included two sections. The first section gathered socio-demographic information and assessed acceptance of COVID-19 vaccines. The second section elicited data on the respondents' Knowledge about COVID-19, understanding of COVID-19 transmission, willingness to take the COVID-19 vaccine, and explored their Attitudes, Practices, and Concerns regarding the COVID-19 vaccines.

Statistical Analysis:

Data were analyzed using the Chi-square test for independence to determine if there were significant differences between the two campuses in terms of the measured variables (KAPC). The analysis was performed using SPSS version 28, with significance level set at $p < 0.05$. Chi-square tests also compared the distributions of categorical variables between the campuses. Our statistical focus was on key areas including vaccination status, willingness to take the vaccine, and perception of vaccine usefulness.

In the analysis of respondents' Knowledge, each correct answer was coded as "1," while each incorrect answer or "don't know" was coded as "0." A 5-point Likert scale (ranging from "strongly agree" to "strongly disagree") was used to gauge respondents' Attitudes, Practices, and Concerns.

RESULTS

Demographic Characteristics:

A total of 514 students participated. Table 1 shows the demographic characteristics of campus. Overall, 347 students (67.5%) were female and 167 (32.5%) were male. Of the 514 students, 58.6% were from CHS, 25.3% from Main Campus, and 16.1% from the Yenagoa Law Faculty.

Knowledge, Attitudes, Practices, and Concerns (KAPC)

Tables 2 to 5 present detailed responses to questions on COVID-19 knowledge, attitudes, practices, and concerns. For example, regarding whether the COVID-19 vaccine is legally mandatory at NDU, 75.9% of all the students answered "No" while 14.0% said "Yes" and 10.1% "Don't know." The majority correctly

identified key aspects of COVID-19 transmission and the necessity for a two-dose regimen.

Attitudinal data (Table 3) showed that 67.8% of respondents were willing to take the vaccine, while 15.2% expressed negative attitudes. Practice responses (Table 4) indicated that a combined 77% either "strongly agreed" or "agreed" that the vaccine was useful and that they followed recommended preventive measures. Concerns about the vaccine (Table 5) varied, with 40.0% agreeing that the vaccine might not be easily available and 20.0% concerned about immediate serious side effects.

Table 1: Demographic Characteristics of Study Participants

Demographic Details	Female	Male	Total	Percentage
Residence				
CHS (College of Health Sciences)	203	98	301	58.6%
Main Campus	91	39	130	25.3%
Yenagoa Law Faculty	53	30	83	16.1%
Total	347	167	514	100%
Department (Main Campus)				
Faculty of Agriculture	14	6	20	3.9%
Dept. of Chemistry (Faculty of Science)	15	5	20	3.9%
Computer Science	12	8	20	3.9%
Faculty of Management, Social Science & Construction Mgmt.	50	20	70	13.6%
Department (CHS)				
400L Medical (A)	45	15	60	11.7%
400L Medical (B)	35	15	50	9.7%
300L Medical	35	25	60	11.7%
Biochemistry, Anatomy, Nursing & Physiology	88	43	131	25.5%
Year of Study				
First Year	90	40	130	25.3%
Second Year	50	20	70	13.6%
Third Year	60	30	90	17.5%
Fourth Year	75	45	120	23.3%
Fifth Year	72	32	104	20.2%
COVID-19 Vaccination Status				
Yes	90	50	140	27.2%
No	257	117	374	72.8%
Number of doses taken				
Once	60	30	90	64.3%
Twice	30	20	50	35.7%

Table 2: Knowledge about COVID-19 and the Vaccine

Question	Options	CHS Male	CHS Female	Main Campus Male	Main Campus Female	Law Faculty Male	Law Faculty Female	Total	Percentage (%)
1. Is it legally mandatory to take COVID-19 vaccine in NDU?	Yes	10	15	12	14	8	13	72	14.0%
	No	65	88	72	81	40	44	390	75.9%
	Don't know	8	12	10	9	6	7	52	10.1%
2. Eligibility for COVID-19 vaccine									
(i) Infants below one year of age	Eligible	4	6	5	6	4	5	30	5.8%
	Not eligible	70	90	75	85	45	55	420	81.7%
	Don't know	9	10	8	9	5	6	64	12.5%
(ii) Children and adolescents below 18 years old	Eligible	12	14	14	15	8	12	75	14.6%
	Not eligible	60	82	66	80	42	45	375	72.9%
	Don't know	11	10	8	9	4	6	64	12.5%
(iii) Adults above 18 years of age	Eligible	75	95	80	90	55	85	480	93.4%
	Not eligible	2	2	1	1	1	2	9	1.8%
	Don't know	6	9	7	8	4	6	25	4.8%
(iv) Pregnant and lactating women	Eligible	10	12	11	14	9	14	70	13.6%
	Not eligible	62	85	70	80	45	43	385	74.9%
	Don't know	11	9	7	9	6	7	59	11.5%
(v) Patients with chronic diseases (diabetes, hypertension, heart disease)	Eligible	55	70	65	75	40	55	360	70.0%
	Not eligible	18	20	15	18	10	14	95	18.5%
	Don't know	10	8	8	10	6	7	59	11.5%
(vi) Persons recovered from COVID-19 infection	Eligible	50	62	55	67	40	46	320	62.3%
	Not eligible	24	32	28	30	4	7	125	24.3%
	Don't know	9	10	7	9	6	8	69	13.4%
(vii) Persons allergic to food items or drugs	Eligible	20	24	26	28	18	19	155	30.2%
	Not eligible	60	72	65	70	22	26	315	61.3%
	Don't know	8	8	9	9	4	6	44	8.6%
(viii) Immuno-compromised persons	Eligible	22	30	28	35	30	40	185	36.0%
	Not eligible	60	72	65	75	35	48	285	55.4%
	Don't know	6	8	7	8	5	10	44	8.6%
3. What is the cause of COVID-19 infection?	Bacteria	8	9	10	8	5	5	45	8.8%
	Virus	75	85	85	90	48	42	425	82.7%
	Not sure	5	6	5	6	4	4	44	8.6%
4. What is the type of genetic material in COVID-19?	DNA	15	18	20	22	10	10	95	18.5%
	RNA	65	80	75	80	35	25	360	70.0%
	Not sure	8	10	6	8	2	5	59	11.5%
5. Are antibiotics effective in the treatment of COVID-19?	Yes	18	22	24	26	8	7	105	20.4%
	No	60	75	70	75	35	35	350	68.1%
	Don't know	10	10	7	10	4	8	59	11.5%
6. Can COVID-19 be transmitted by mosquito bite?	Yes	12	14	15	16	8	10	75	14.6%
	No	70	88	78	82	42	35	395	76.9%
	Don't know	6	7	6	7	3	5	44	8.6%
7. Can COVID-19 be spread through droplets from coughing, sneezing, and contaminated surfaces?	Yes	80	92	90	100	50	48	460	89.5%
	No	6	7	5	6	2	2	26	5.1%
	Don't know	7	8	6	6	3	4	28	5.4%
8. Protective immunity against COVID-19 will be achieved after:									
(i) First dose of vaccination	Yes	22	30	28	35	18	22	155	30.2%
	No	53	63	61	66	29	33	305	59.3%
	Don't know	8	12	10	12	6	6	54	10.5%
(ii) Second dose of vaccination	Yes	65	80	75	85	50	60	415	80.7%
	No	10	12	8	10	4	6	50	9.7%
	Don't know	10	12	9	10	5	3	49	9.5%
(iii) Fourteen days after first dose of vaccine	Yes	50	65	60	70	35	40	320	62.3%
	No	20	24	22	25	14	18	123	23.9%
	Don't know	15	15	10	10	10	11	71	13.8%
9. Influence of information sources on opinion about vaccination:									

(i) News from National TV / Radio	Insignificant effect	18	24	22	26	10	12	112	21.8%
	Somewhat significant effect	35	40	38	45	20	28	206	40.1%
	Very significant effect	32	40	32	38	20	34	196	38.1%
(ii) Government agencies	Insignificant effect	12	15	14	16	9	12	78	15.2%
	Somewhat significant effect	39	44	41	49	24	28	225	43.8%
	Very significant effect	33	44	36	42	22	34	211	41.1%
(iii) Social media (Facebook, Instagram, WhatsApp)	Insignificant effect	10	12	11	14	7	8	62	12.1%
	Somewhat significant effect	41	49	47	54	30	32	253	49.2%
	Very significant effect	33	42	33	39	18	34	199	38.7%
(iv) Discussion among friends and family	Insignificant effect	15	18	20	22	10	14	99	19.3%
	Somewhat significant effect	39	47	45	50	28	30	239	46.5%
	Very significant effect	30	38	27	36	15	30	176	34.2%

Table 3: Attitude towards the Vaccine

Question	Options	Male (CHS)	Female (CHS)	Male (Main Campus)	Female (Main Campus)	Male (Law Faculty)	Female (Law Faculty)	Total	Percentage (%)
1. When it was my turn for vaccination, I was willing to take the COVID-19 vaccine.	Yes	87	115	43	48	29	27	349	67.8%
	No	28	20	10	10	5	5	78	15.2%
	Neither agree nor disagree	29	19	14	10	8	7	87	16.9%
2. I prefer to acquire immunity against COVID-19 naturally rather than by vaccination.	Yes	65	82	23	37	14	23	244	47.4%
	No	50	24	23	14	9	12	132	25.7%
	Neither agree nor disagree	46	37	18	14	14	9	138	26.9%
3. I was willing to get the COVID-19 vaccine even if I had to pay for it.	Yes	73	109	32	46	18	27	305	59.3%
	No	29	14	9	9	9	7	77	15.0%
	Neither agree nor disagree	46	36	18	14	9	9	132	25.7%
4. I will recommend my family and friends to get vaccinated against COVID-19.	Yes	94	142	38	57	33	38	402	78.2%
	No	20	9	9	5	3	0	46	9.0%
	Neither agree nor disagree	19	14	14	9	5	5	66	12.8%

Table 4: Practices Regarding COVID-19 Vaccine

Question	Options	Male (CHS)	Female (CHS)	Male (Main Campus)	Female (Main Campus)	Male (Law Faculty)	Female (Law Faculty)	Total	Percentage (%)
(i) I think there is no harm in taking it	Strongly agree	28	32	36	38	18	18	170	33.1%
	Agree	38	45	48	50	21	23	225	43.8%
	Neither agree nor disagree	12	15	17	19	9	8	80	15.6%
	Disagree	6	8	9	10	2	4	39	7.6%
(ii) I believe COVID-19 vaccine is useful to protect me against the infection	Strongly agree	30	35	39	44	18	20	186	36.2%
	Agree	38	45	48	50	21	23	225	43.8%
	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	6	8	9	10	2	4	39	7.6%
(iii) COVID-19 vaccine is available for free	Strongly agree	28	34	37	38	18	23	178	34.6%
	Agree	35	40	44	49	22	27	217	42.2%
	Neither agree nor disagree	10	12	14	17	7	8	70	13.6%
	Disagree	7	8	10	12	5	5	49	9.5%
(iv) My health care professional (doctor/nurse/pharmacist) has recommended it to me	Strongly agree	30	35	39	44	18	20	186	36.2%
	Agree	38	45	48	50	21	23	225	43.8%
	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	6	8	9	10	2	4	39	7.6%
(v) I feel the benefit of taking the COVID-19 vaccine outweighs the risk involved	Strongly agree	31	36	40	43	20	22	192	37.3%
	Agree	40	45	48	50	21	22	226	43.9%
	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	5	6	7	8	2	4	32	6.2%
(vi) I believe that taking the COVID-19 vaccine is a societal responsibility	Strongly agree	31	36	40	43	20	22	192	37.3%
	Agree	40	45	48	50	21	22	226	43.9%
	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	5	6	7	8	2	4	32	6.2%
(vii) There is sufficient data regarding the vaccine's safety and efficacy released by the government	Strongly agree	30	35	39	41	18	21	184	35.8%
	Agree	35	40	44	49	22	24	214	41.6%
	Neither agree nor disagree	10	12	14	17	7	8	68	13.2%
	Disagree	7	8	10	12	5	6	48	9.3%
(viii) Many people are taking the COVID-19 vaccine	Strongly agree	30	35	39	44	18	20	186	36.2%
	Agree	38	45	48	50	21	23	225	43.8%
	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	6	8	9	10	2	4	39	7.6%
(ix) I think the vaccine will help in eradicating COVID-19 infection	Strongly agree	30	35	39	44	18	20	186	36.2%
	Agree	38	45	48	50	21	23	225	43.8%
	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	6	8	9	10	2	4	39	7.6%
(x) My role models/political leaders/senior	Strongly agree	31	36	40	43	20	22	192	37.3%
	Agree	40	45	48	50	21	22	226	43.9%

doctors/scientists have taken the vaccine	Neither agree nor disagree	10	12	14	16	6	7	64	12.4%
	Disagree	5	6	7	8	2	4	32	6.2%

Table 5: Concerns about COVID-19 vaccine

Question	Options	Male (CHS)	Female (CHS)	Male (Main Campus)	Female (Main Campus)	Male (Law Faculty)	Female (Law Faculty)	Total	Percentage (%)
(i) The vaccine might not be easily available to me	Strongly agree	40	50	20	30	7	7	154	30.0%
	Agree	50	60	25	35	20	16	206	40.0%
	Neither agree nor disagree	25	20	18	20	10	10	103	20.0%
	Disagree	10	8	10	12	5	6	51	10.0%
(ii) I might have immediate serious side effects after taking COVID-19 vaccine	Strongly agree	20	25	15	20	11	12	103	20.0%
	Agree	45	50	30	35	23	23	206	40.0%
	Neither agree nor disagree	20	18	18	20	13	14	103	20.0%
	Disagree	15	12	15	15	15	30	102	19.8%
(iii) COVID-19 vaccine may be faulty or fake	Strongly agree	15	18	12	15	9	8	77	15.0%
	Agree	30	35	25	30	17	17	154	30.0%
	Neither agree nor disagree	28	28	25	28	20	25	154	30.0%
	Disagree	25	22	20	23	20	19	129	25.1%
(iv) COVID-19 vaccine was rapidly developed and approved	Strongly agree	20	20	18	20	12	13	103	20.0%
	Agree	40	45	35	40	23	23	206	40.0%
	Neither agree nor disagree	15	15	12	12	11	12	77	15.0%
	Disagree	20	25	25	30	14	14	128	25.0%
(v) I might have some unforeseen future effects of the COVID-19 vaccine	Strongly agree	15	18	12	15	9	8	77	15.0%
	Agree	30	35	25	30	17	17	154	30.0%
	Neither agree nor disagree	28	28	25	28	20	25	154	30.0%
	Disagree	25	22	20	23	20	19	129	25.0%
(vi) COVID-19 vaccine is being promoted for commercial gains by pharmaceutical companies	Strongly agree	10	10	8	8	7	8	51	10.0%
	Agree	18	18	17	17	17	16	103	20.0%
	Neither agree nor disagree	30	30	25	25	22	22	154	30.0%
	Disagree	40	42	30	35	31	28	206	40.0%
(vii) Because of limited awareness on COVID-19 vaccines, I fear taking the vaccine because I am not sure whether it will protect me or not	Strongly agree	40	40	25	30	10	9	154	30.0%
	Agree	40	40	25	30	10	9	154	30.0%
	Neither agree nor disagree	20	18	18	20	13	14	103	20.0%
	Disagree	20	18	15	17	13	20	103	20.0%
(viii) After getting COVID-19 vaccine, I don't need to follow preventive measures (mask, sanitation, social distancing)	Strongly agree	10	8	8	8	8	9	51	10.0%
	Agree	15	12	13	14	11	12	77	15.0%
	Neither agree nor disagree	25	18	18	20	11	11	103	20.0%
	Disagree	50	50	40	40	40	63	283	55.1%

Group-Specific Summaries:

Responses were further stratified by campus and gender. The results are presented in the following tables. Table 6: *Summary:* Overall, 62.3% of participants demonstrated adequate knowledge about COVID-19 and its vaccine, with CHS respondents showing a slight edge.

Table 7: *Summary:* A majority (84.4%) expressed positive attitudes toward vaccination, with similar trends across all groups.

Table 8: *Summary:* Just over half (51.2%) reported good practices regarding vaccination. The trend was consistent across campuses and genders.

Table 9*: Summary:* Concerns were distributed as 37.3% low, 32.7% moderate, and 30.0% high, with CHS respondents showing a slightly higher proportion of low concern.

Table 6. Summary of Knowledge Levels Regarding COVID-19 and the Vaccine

Knowledge Level	CHS Male (n = 144)	CHS Female (n = 154)	Main Campus Male (n = 67)	Main Campus Female (n = 68)	Law Faculty Male (n = 42)	Law Faculty Female (n = 39)	Total	Overall (%)
Inadequate	20	25	8	7	4	3	67	13.0%
Moderate	45	50	10	10	7	5	127	24.7%
Adequate	79	79	49	51	31	31	320	62.3%
Total	144	154	67	68	42	39	514	100.0%

Table 7. Summary of Attitudes towards COVID-19 Vaccination

Attitude Level	CHS Male (n = 144)	CHS Female (n = 154)	Main Campus Male (n = 67)	Main Campus Female (n = 68)	Law Faculty Male (n = 42)	Law Faculty Female (n = 39)	Total	Overall (%)
Negative	24	26	7	8	7	8	80	15.6%
Positive	120	128	60	60	35	31	434	84.4%
Total	144	154	67	68	42	39	514	100.0%

Table 8. Summary of Practices Related to COVID-19 Vaccination

Practice Level	CHS Male (n = 144)	CHS Female (n = 154)	Main Campus Male (n = 67)	Main Campus Female (n = 68)	Law Faculty Male (n = 42)	Law Faculty Female (n = 39)	Total	Overall (%)
Poor	30	30	10	10	7	7	94	18.3%
Fair	45	50	20	20	10	12	157	30.5%
Good	69	74	37	38	25	20	263	51.2%
Total	144	154	67	68	42	39	514	100.0%

Table 9. Summary of Concerns about COVID-19 Vaccination

Concern Level	CHS Male (n = 144)	CHS Female (n = 154)	Main Campus Male (n = 67)	Main Campus Female (n = 68)	Law Faculty Male (n = 42)	Law Faculty Female (n = 39)	Total	Overall (%)
Low	60	65	20	20	12	15	192	37.3%
Moderate	45	50	25	24	15	9	168	32.7%
High	39	39	22	24	15	15	154	30.0%
Total	144	154	67	68	42	39	514	100.0%

DISCUSSION

The findings from this study revealed significant demographic, behavioral, and educational differences between students at the Amassoma and Yenagoa campuses regarding their responses to the COVID-19 pandemic. Analysis of the Knowledge, Attitudes, Practices, and Concerns (KAPC) among 514 respondents indicated that while overall gender distribution was similar across campuses, notable variations emerged when responses were further stratified by campus and gender.

Gender distribution:

Although there was no significant difference in overall gender distribution between the Amassoma and Yenagoa campuses, further analysis within each campus suggests that gender-specific nuances may influence COVID-19–related responses. Prior studies have demonstrated that women tend to exhibit more cautious health behaviors and more positive attitudes toward vaccination [14, 15]. In our study, both CHS and Main Campus female respondents showed slightly higher levels of adequate knowledge and positive attitudes compared to their male counterparts. These findings support the idea that tailored health campaigns that specifically address gender-related differences could enhance engagement and compliance with public health guidelines.

Year of study:

The distribution of students' years of study differed significantly between campuses. Senior students appeared to have greater access to accurate health information and resources, which likely contributed to their higher levels of knowledge regarding COVID-19, as evidenced by the knowledge summary (Table 6). This finding is consistent with previous research indicating that educational level influences health literacy and the ability to comply with health interventions [16, 17].

Senior students may be more exposed to both formal and informal sources of reliable information, which, in turn, can lead to improved perceptions and behaviors related to disease prevention.

Vaccination status:

A significant difference in vaccination status was observed between the two campuses. The

higher vaccination uptake among students at the Amassoma campus compared to those at Yenagoa may be attributable to several factors, including better access to vaccination sites, more effective campus-specific vaccination campaigns, and greater exposure to basic health education. Vaccine hesitancy is known to be influenced by misinformation, mistrust in health authorities, and perceived risks associated with vaccination [18, 19]. The observed variation in vaccination status underscores the importance of targeted outreach and educational programs aimed at addressing the unique barriers faced by different campus populations.

Understanding of COVID-19 transmission:

Differences in the understanding of COVID-19 transmission were also noted between campuses. Our data revealed that students at the Amassoma campus demonstrated higher levels of correct knowledge regarding the primary modes of transmission compared to those at the Yenagoa Law Faculty. This discrepancy suggests that basic health education - particularly regarding virus transmission - is less emphasized among non-health science students. Addressing these knowledge gaps is crucial; effective communication strategies that leverage trusted sources and provide clear, factual information can combat misinformation and promote adherence to preventive measures.

Willingness to take the COVID-19 vaccine:

Finally, the willingness to take the vaccine differed significantly between the campuses. As expected, students from Amassoma - especially those from the College of Health Sciences - showed greater willingness to receive the vaccine compared to their counterparts in the Law Faculty at Yenagoa. This finding likely reflects the influence of health-related education on vaccine confidence. Enhancing vaccine acceptance may require targeted, culturally sensitive communication that addresses specific concerns and misconceptions regarding vaccine safety and efficacy. Transparent and effective health communication, coupled with the reinforcement of basic medical education, could help bridge the gap in vaccine acceptance observed in this study.

Overall, these findings highlight the need for campus-specific public health interventions. Tailored strategies that address the unique educational and cultural contexts of each campus could improve health literacy, vaccination uptake, and overall compliance with COVID-19 prevention measures.

CONCLUSION

This study highlights the importance of understanding demographic and behavioral differences among university students to inform targeted COVID-19 intervention strategies. The significant variations in knowledge, attitudes, practices, and concerns between the Amassoma and Yenagoa campuses indicate

that culturally tailored and context-specific health communication is critical. Strengthening basic health education across all campuses could improve vaccine uptake and adherence to preventive measures, thereby reducing the risk of COVID-19 transmission within university communities.

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NURSING AND MIDWIFERY STUDENTS' PERCEPTIONS AND EXPERIENCES OF CLINICAL LEARNING IN PAPUA NEW GUINEA: A QUALITATIVE STUDY

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ABSTRACT

The clinical learning environment plays a critical role in developing the skills and competence of nursing and midwifery students. However, limitations within the clinical learning environment may impede students' ability to effectively translate theoretical knowledge into clinical practice. This study aimed to explore the perceptions and experiences of student nurses and midwives regarding their clinical learning in Papua New Guinea. A descriptive qualitative study was conducted using purposive sampling to recruit 18 nursing and midwifery students. Data was collected through individual, in-depth, semi-structured interviews and analyzed using thematic analysis. Four themes emerged from the interview data, influencing participants' perceptions and experiences regarding clinical learning: supportive learning environment, perceived bias and dismissive attitudes from clinical supervisors, inadequate supervision and unmet clinical learning outcomes, and constraints in clinical resources. Findings revealed that negative clinician attitudes, poor supervision, and limited resources hindered students' clinical learning. Strengthening collaboration between institutions and hospitals, along with improved resource support, is essential for better learning outcomes.

Keywords: Clinical learning, Nursing, Midwifery, Qualitative study, Papua New Guinea

INTRODUCTION

Clinical learning and practice constitute an indispensable component of nursing education, where students integrate cognitive, affective, and psychomotor skills to develop critical clinical competencies for practice [1]. Using the nursing competency scale, Meretoja et al. [2] described clinical competence as the ability of students to integrate knowledge, skills, attitudes, and values into nursing practices, which remain important for professional standards. Nursing and midwifery students must apply their theoretical

knowledge as they confront challenges that help build their courage and confidence in real-life clinical situations [3]. Clinical practice challenges and skill acquisition foster critical thinking, decision-making, and emotional resilience, enhancing adaptability and professional confidence for a seamless career transition [1].

The clinical learning environment comprises the dynamic factors within clinical settings where students apply theory, develop skills, and enhance problem-solving and reasoning abilities [4]. A typical clinical learning environment includes health professionals, nurse educators, and patients, which affects nursing students' careers either positively or negatively by impacting their performance [1]. Four distinct attributes of a clinical learning environment are the physical environment, interpersonal and psychosocial aspects, organizational culture, and clinical teaching components [5]. During clinical placements, the combination of practical skills, theoretical knowledge, and quality mentorship significantly bolsters nursing students' motivation, confidence, and commitment to the profession [4].

Given the significance of clinical learning, a growing body of evidence highlights a strong correlation between the quality of the learning environment and nursing students' satisfaction [6]. A supportive clinical learning environment, characterized by effective supervision, constructive feedback, and a collaborative culture, strengthens learners' coping capacities, enriches educational experiences, and fosters the development of skilled, resilient practitioners [1]. A study conducted in Spain reported that nursing students expressed high levels of satisfaction with their clinical learning environment and supervision at hospitals where they completed clinical placements, suggesting a positive relationship between these elements [7]. A strong sense of belonging during clinical placements can boost students' confidence and enhance their motivation to learn. As Mikkonen et al. [8] highlighted, clinical mentoring is crucial for the professional development of nursing students and can significantly increase their motivation to enter and remain in the profession.

Students may struggle to apply theoretical knowledge in practice when the clinical learning environment lacks sufficient support or fails to foster a positive atmosphere. Research has shown that negative experiences and poor learning environments, marked by negative attitudes from staff toward working with seniors, adversely impact students' learning [9]. Students' clinical learning can also be influenced by various interpersonal, sociocultural, instructional, environmental, emotional, and physical factors, including poor teaching materials, strained professional relationships,

inadequate supervision, limited clinical resources, and a hostile work environment [10].

Papua New Guinea (PNG) has one of the world's most geographically dispersed and culturally diverse populations. The country has a decentralized healthcare system based on primary healthcare principles [11]. Access to healthcare services is variable, as it is often affected by poor health infrastructure and limited transport access [11]. In addition, persistent health workforce shortages in PNG continue to be driven by chronic underinvestment, poor working conditions, and imbalances between workforce supply and demand [12,13]. There is considerable demand for a strengthened health workforce and equitable healthcare in the country, with existing disparities highlighting the urgent need for expanded health education and training.

Previous studies on nursing and midwifery education in PNG focused on the challenges new graduate midwives face and the broad aspects of nursing and midwifery education and regulation [14,15]. There are currently no published studies that specifically examine the perceptions and experiences of nursing or midwifery students in their clinical learning environments in PNG. Additionally, there is a lack of literature focused on nursing and midwifery clinical learning and practice in the country. Addressing this knowledge gap is crucial for enhancing the quality of nursing and midwifery education and improving clinical learning practices.

Therefore, this study aims to explore nursing and midwifery students' perceptions and experiences regarding their clinical learning practices.

METHODOLOGY:

Study design:

Informed by an interpretive philosophical paradigm, this study employed a phenomenological approach informed by Edmund Husserl [16]. Phenomenology offers a theoretical framework to scholars who seek an in-depth understanding of phenomena at the level of subjective reality. This method emphasizes interpreting personal experiences by providing phenomenological descriptions that reveal the meaning and significance of the phenomena as experienced by participants [17]. The phenomenological aspect also involves interpreting these descriptions based on the principle of understanding the experiences shared by individuals, rather than generalizing findings [17]. The phenomenological approach explored lived experiences, emphasized trust, transparency, and flexibility, which made it particularly well-suited for generating meaningful insights in nursing and midwifery education.

Sampling:

Purposive sampling was used to select 18 students from the Bachelor of Clinical Nursing programs at the School of Medicine and Health Sciences (SMHS), University of Papua New Guinea (UPNG). The students were coded for ease of reference. Three from Child Health (Participant number 1 to 3). Six from Midwifery (Participants number 4 to 9). Two from Mental Health (Participant numbers 10 to 11). Seven from Critical Care (Participant numbers 12 to 18). Purposive sampling is a type of non-probability sampling method that involves researchers selecting a specific group of individuals based on specific characteristics of interest relevant to the study's aim [18]. This approach was appropriate as it allowed for the collection of rich insights into the clinical learning experiences of nursing and midwifery students. Students from other health disciplines were excluded from this study.

Data Collection:

Following informed consent, the primary researchers used a semi-structured interview guide consisting of four main questions to guide the discussion: (1) What is your experience about the clinical setting (environment)? (2) What can you say about clinical supervision? (3). How about the resources that the setting has to enhance your clinical practice? and (4). What can you say about clinicians' or supervisors' attitudes/approaches toward your clinical placement or learning?

Participants were asked, *'Please, tell me about your clinical experiences at the hospital. Tell me more about the…'* Follow-up questions were posed based on their statements and responses. Additionally, probing questions were asked regarding the participants' responses and opinions *(e.g., "Would you elaborate more on this?" or "What did you mean by saying…?")* to obtain in-depth information.

The interviews provided participants with a platform to lead discussions and share their perceptions and experiences regarding clinical learning. In alignment with phenomenology [19], interviews fostered the development of conversational relationships with participants, allowing for a deeper exploration and reflection on the significance of their experiences. All interviews were recorded and transcribed verbatim after the interview sessions. Each interview lasted between 30 to 50 minutes. The interview process continued until data saturation was achieved. To ensure credibility, audio recordings and transcripts were consistently reviewed for alignment with data interpretation, identified codes, and emerging themes.

Data Analysis:

Using phenomenological data analysis techniques, recurring themes were identified,

leading to a 'thick description' of participants' experiences [19]. A thematic analysis was conducted using a six-step process to examine qualitative data, systematically identifying and organizing patterns of meaning into themes that provide deeper insights into participants' perspectives and experiences [20]. To develop familiarity with the data, transcripts were read systematically and with an open, reflective approach. Non-verbal cues, including pauses, laughter, vocalizations, and facial expressions, were excluded, and grammatical corrections were applied to enhance readability while preserving the original meaning of participants' narratives. Data were manually analyzed by organizing notes, codes, and categories in Microsoft Word, based on shared meanings. These were then examined for patterns, leading to the development of sub-themes and overarching themes. The themes were further refined and selected to accurately reflect participants' perceptions and experiences [20]. To enhance the credibility of the analysis, an independent reviewer, who was not involved in the study, examined the emergent themes for validation. Reporting adheres to the Standards for Reporting Qualitative Research Checklist [21].

Rigor:

This study used four criteria to establish rigor: credibility, transferability, dependability, and confirmability [22]. First, credibility was established based on several strategies throughout the study. During data collection, participants were actively engaged in interviews, followed by debriefing sessions to review the process. Four independent researchers were involved in the data analysis and coding of the themes. The researchers reviewed each theme to ensure that it accurately reflected the participants' narratives. Second, the transferability of the findings was ensured by providing a detailed description of the study context. Third, dependability was established through the dense description of the methodology used and the description of the data. All interview materials, transcriptions, findings, interpretations, and recommendations were kept accessible to the principal investigator and supervisor to allow for an audit trail; descriptions, codes, and themes were also confirmed. Finally, confirmability was enhanced by using verbatim quotations from participants' narratives and incorporating field notes, which helped minimize researcher-induced biases [22]. The data was shared among colleagues for peer review and analysis to ensure trustworthiness.

Ethical considerations:

Ethical approval for this study was obtained from the Research Ethics Committee of the SMHS, UPNG (02-07-2024). Students received information sheets and provided informed consent before participating in the study.

RESULTS

A total of 18 nursing and midwifery students (3 males and 15 females) participated in this study. Students were between the ages of 30 and 40 with more than five years of clinical experience in rural and urban healthcare facilities. Analysis of the interview data revealed four interrelated themes influencing participants' perceptions and experiences of clinical learning: (1) supportive learning environment, (2) perceived bias and dismissive attitudes from clinical supervisors, (3) inadequate supervision and unmet clinical learning outcomes, and (4) constraints in clinical resources.

Supportive learning environment:

Only a few students described the clinical environment as friendly and supportive, which facilitated the translation of theoretical knowledge into clinical competence. This supportive environment enabled students to apply theoretical concepts effectively in clinical practice, thereby enhancing their overall learning experience.

Two students (Participants No. 3 and 10) described their experiences:

> *"When I first arrived at my assigned clinical workplace, I realized it was a completely new environment. I was confused and anxious, not knowing where essential items like the emergency trolley were located…... But the staff were friendly and helpful… they showed me where medical or emergency equipment is kept... I could work confidently."*

> *"The staff has been very supportive. They guided us through their workplace orientation and called us in whenever they needed to demonstrate a procedure… they also allowed us to perform some procedures. This helped us build our confidence as students."*

Perceived bias and dismissive attitudes from clinical supervisors:

Some students reported minimal support and dismissive attitudes from the clinical staff, with perceived bias during supervision and assessment contributing to dissatisfaction and reduced learning opportunities. Students also highlighted poor clinician engagement, especially when supervision was required.

As participant No. 4 explained:

> *"Some staff members were friendly, but I noticed that*

> *others were selective in their assistance when it came to assessing students' clinical competencies. Also, some staff did not communicate well with us; they didn't greet us and responded to our questions in an unfriendly manner."*

Participant No. 5 echoed this concern, highlighting the impact on their learning:

> *"Sometimes, we were not greeted by the staff in the ward; instead, they just ignored us. I do not know why this has happened. Some would walk past us without acknowledging our presence there… it was difficult to work with them."*

Inadequate supervision and unmet learning outcomes:

Several students expressed dissatisfaction with the quality of clinical supervision, emphasizing its adverse impact on skill acquisition and competency development. In many instances, supervision during clinical placements was perceived as inadequate.

Participant No. 9 stated:

> *"We just do our clinical procedures on our own… There was no proper clinical supervision. Some nurses would tell us to do what we can with our procedures. After the procedure is completed, they sign the clinical logbooks. This is not good!"*

Some students reported being required to perform clinical tasks without direct supervision, with instructions to seek assistance only when necessary. This lack of consistent oversight made it difficult to connect theoretical knowledge with practical skills.

Participant No. 2 explained:

> *"There is no (clinical) supervision…the nurses just instructed us to perform clinical procedures independently. If you are confused or have an emergency, please call us for assistance. That was their advice to us…"*

Constraints in clinical resources:

Most of the students consistently highlighted difficulties in accessing and utilizing clinical resources during placements, coupled with concerns about inadequate infection control

practices. Limited access to essential equipment not only hindered learning but also compromised safe practice standards.

As participants No. 7 and No 8 explained:

> *"We do not have enough resources, including sterile equipment… suture trays and gloves, to do a vaginal examination, especially in the Labor ward. So, most of the time we ran out and had to use unsterile equipment to perform our clinical procedures."*

> *"We don't have enough delivery trays in the labor ward. We wash and reuse them… sometimes we only rinse them or use alcohol swabs. We don't always use sterile techniques to deliver babies."*

DISCUSSION

The study examined nursing and midwifery students' perceptions and experiences of clinical learning, highlighting both enabling and constraining factors. While this study revealed that a supportive clinical environment was associated with positive engagement and skill development, students also reported significant challenges, including limited opportunities for active participation, perceived bias and dismissive attitudes from clinicians, inadequate supervision, unmet learning objectives, and persistent resource constraints.

A supportive clinical environment promotes skill development, bridges theory and practice, and boosts student engagement and learning outcomes. In their study, Rodríguez-García et al. [7] argued that the clinical learning environment directly impacts clinical performance and learning, serving as a vital link between academic instruction and practical skill development. Evidence indicates that a supportive clinical environment, underpinned by a positive workplace culture, enables nursing and midwifery students to actively engage in clinical learning, strengthen their clinical skills and competencies, and cultivate meaningful interpersonal and professional relationships [23]. Similarly, nursing and midwifery students gain confidence and demonstrate a greater willingness to learn when the clinical environment is supportive, characterized by open communication, constructive feedback, trust, and mutual respect between clinicians and students [9]. A supportive clinical environment, effective training, and positive staff attitudes foster active participation, skill development, and deeper learning among nursing and midwifery students [1,24]. This study highlights how a supportive clinical learning environment

improves student engagement, knowledge acquisition, clinical skills, and confidence.

In contrast, the present study revealed that clinical nurses often demonstrated prejudice and negative attitudes toward nursing and midwifery students during supervision. According to Oshodi and Sookhoo [25], unethical attitudes and behaviors in healthcare can affect student learning, performance, satisfaction, and patient outcomes. Recent studies confirm that clinician biases and unsupportive attitudes toward clinical supervision can negatively affect students' clinical learning [26]. Negative attitudes from clinicians can hinder nursing and midwifery students' learning by creating an unsupportive environment that limits their ability to ask questions, practice skills, and build confidence [25]. Future research could investigate the barriers to student learning that stem from nurses' limited engagement and inadequate supervision in clinical settings.

Clinical supervisors are pivotal in cultivating students' clinical competence and confidence, thereby ensuring safe, patient-centered care. In this study, students identified inadequate supervision as the most critical determinant of their dissatisfaction with clinical learning, a finding that reflects recent studies on clinical learning [27]. One possible contributing factor to this issue is the limited competency of some clinical supervisors, compounded by a lack of authority to provide effective support to nursing students during their placements. It can be difficult for nurses to provide effective supervision while also managing their daily patient care responsibilities [28]. Another reason for inadequate clinical supervision could be the shortage of qualified supervisors and insufficient preceptorship training. This lack of oversight may stem from inadequate infrastructure and a lack of formal training, which can negatively impact students' learning and practice [27]. Clinical preceptorship plays a vital role in building students' confidence, competence, and professionalism, supporting their transition into nursing and midwifery practice [27,29].

The study demonstrated that equipment and resource limitations during clinical placements significantly impeded nursing and midwifery students' ability to translate theoretical knowledge into practice. The findings are in agreement with a similar study conducted in Tanzania [1]. Negative clinical experiences may contribute to the theory–practice gap, leading to reduced competence and confidence among nursing students upon graduation. Additionally, students in this study reported increased use of non-sterile techniques during clinical procedures, likely due to a lack of sterile equipment in clinical settings, as noted in a study from Malawi [30]. Limited clinical resources hinder effective participation in clinical practice, reducing the quality of learning experiences.

Adequate availability and accessibility of medical resources and sterile equipment in clinical settings can improve students' ability to engage effectively in clinical practice, which fosters self-motivation, confidence, and competency [31]. The study emphasized the demand for health institutions to provide adequate resources and build the capacity to improve students' learning in clinical settings.

Although the findings align with broader evidence on clinical learning, several limitations should be acknowledged. The study focused solely on students from one institution, which restricts the transferability of its insights to other programs. It also excluded clinicians' perspectives, leaving important supervision experiences and challenges unexplored. To build a more comprehensive understanding of how clinical learning environments shape outcomes, future research should involve multiple institutions and incorporate both student and clinician viewpoints.

Implications of future research and practice:

Future research should broaden its lens to include diverse institutional perspectives, integrate clinician input, and examine structural barriers, such as limited supervision capacity, heavy workloads, and resource constraints, that shape student learning in clinical settings. Collaborative efforts involving students, educators, and clinicians could help develop context-specific supervision models and resource strategies suitable to the country's nursing and midwifery context.

For practice, priority actions include reinforcing preceptorship training, formally recognizing supervision as part of clinicians' roles, cultivating respectful and inclusive workplace cultures, and ensuring adequate equipment and resources. Together, these measures can narrow the theory–practice gap and better prepare nursing and midwifery graduates to deliver safe, patient-centered care in PNG.

CONCLUSION

Clinical learning environment was generally supportive, with students' reporting positive learning experiences during their placements. However, several clinical and interpersonal challenges impeded the quality of students' learning experiences. Limited supervision and engagement from clinical staff, perceived bias in learning opportunities, and negative attitudes, including insufficient clinical resources, impacted students' ability to practice essential skills and apply theoretical knowledge. Strengthening collaboration between training institutions and hospitals is critical to advancing clinical preceptorship, improving the quality of supervision, and ensuring that educational curricula are more effectively aligned with the practical demands of clinical training. Furthermore, investing in capacity-building

initiatives and ensuring adequate resource allocation for clinical supervisors can significantly improve clinical learning outcomes.

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Competing Interest:

None.

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PARENTAL ALCOHOL USE AND ITS ASSOCIATION WITH EMOTIONAL WELL-BEING AND ACADEMIC OUTCOMES AMONG SECONDARY SCHOOL STUDENTS IN PORT MORESBY, PAPUA NEW GUINEA

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ABSTRACTS
Parental alcohol use is a growing public health concern with significant implications for the emotional well-being and academic performance of children and adolescents. Evidence from Pacific Island countries, including Papua New Guinea (PNG), remains limited. A cross-sectional study was conducted among 109 upper secondary school students in Port Moresby. Data were collected using a structured self-administered questionnaire assessing parental alcohol use, parental involvement, home environment, emotional outcomes, and academic performance. Descriptive statistics, Fisher's exact test, and binary logistic regression analyses were performed to examine associations between household factors and learning and concentration difficulties. Nearly 70% of students reported parental alcohol use, and approximately three-quarters indicated minimal or no parental involvement in their education. Emotional distress was reported by 66.1% of participants, while over two-thirds demonstrated poor to average academic performance. Logistic regression analysis showed that lack of parental guidance and support substantially increased the likelihood of learning and concentration difficulties (OR = 5.184, p = 0.002), while an unconducive home environment was associated with nearly four times higher risk (OR = 3.646, p = 0.034). Family conflict was not statistically significant (p>0.05). The findings highlight the critical role of parental engagement and supportive home environments in shaping students' emotional health and academic outcomes. Family-centred interventions that strengthen parental involvement and address alcohol-related household instability are essential for improving educational outcomes among adolescents in PNG.

Keywords**:** Parental alcohol consumption, Academic performance, Emotional wellbeing, Parental involvement, Secondary school students, Papua New Guinea.

INTRODUCTION:

Harmful alcohol consumption remains a major public health challenge globally, with significant implications for family wellbeing and children's educational pathways. The World Health Organization (WHO) [1] identifies excessive drinking as a driver of emotional distress, financial instability, and disrupted home environments factors that collectively undermine children's academic success. International evidence consistently shows that children living

with alcohol-dependent parents face higher risks of absenteeism, concentration difficulties, behavioural challenges, and reduced academic performance [2,3]. These effects are observed in both developed and developing countries, contributing to long-term socio-economic disadvantage [1].

Research from diverse settings also highlights the pathways through which parental alcohol use affects schooling. Emotional distress, neglect, and household instability have been linked to poor cognitive development and increased dropout rates among affected children [2]. Large-scale longitudinal studies from Europe and North America demonstrate that parental alcohol misuse is associated with lower educational attainment, poorer school adjustment, and diminished academic self-concept [4,5]. Other studies report increased conduct problems, impulsivity, aggression, truancy, and higher disciplinary actions among children raised in alcohol-impacted households [6,7,8]. Self-esteem, confidence, and school satisfaction may also decline, especially when children experience stigma, unpredictability, and limited emotional support at home [9,10,11].

Within the Pacific region and Papua New Guinea (PNG), alcohol misuse is a growing public health and social concern. In urban areas such as Port Moresby, parental drinking is frequently linked to domestic violence, financial strain, and child neglect are conditions that directly hinder children's educational participation and achievement [12,1]. PNG-based studies, though limited, suggest that students affected by alcohol-related household stress face greater school absenteeism, behavioural difficulties, and reduced academic performance. Economic hardship arising from parental alcohol consumption can also lead to the diversion of household resources away from education, resulting in inadequate school materials and missed learning opportunities [13]. Moreover, the psychological toll on students in the National Capital District (NCD) is substantial. Exposure to alcohol-dependent parents may lead to stress, anxiety, depression, social isolation, and reduced academic motivation [4,14,15]. These emotional burdens frequently manifest as low self-esteem and disruptive behaviours, further increasing the risk of academic underachievement [1].

In PNG, however, existing literature focuses primarily on youth alcohol consumption rather than parental drinking. Studies have shown that secondary students often engage in alcohol use due to stress, peer influence, and social behaviours, contributing to absenteeism and declining academic performance [16]. Broader national reports document alcohol's harmful impact on families and communities, which indirectly affects children's education [17]. Policy frameworks within the PNG Department of Education provide guidance on managing student behaviour, yet little emphasis is placed

on addressing the effects of parental alcohol misuse on children's learning [18]. Importantly, there are no official National Statistical Office (NSO) reports detailing the relationship between parental alcohol use and student academic outcomes in PNG, illustrating a critical knowledge gap.

Given the lack of localized evidence, there is an urgent need to examine how parental alcohol consumption affects students particularly those in upper secondary schools. This study aims to address this gap by examining the impact of parental alcohol consumption on the academic performance and overall educational outcomes of upper secondary school students in NCD, Port Moresby, PNG.

METHODOLOGY:

Study site and population: Morata Two located within the Moresby North-West Electorate in NCD, Port Moresby, PNG, was selected as the study site, due to its urban characteristics and documented exposure to social pressures, including alcohol use within households. Morata Two is estimated to have a population of approximately 18,000 – 22,000 residents (based on demographic estimates from the National Capital District Commission's urban settlement profiling reports and community assessments conducted between 2016 and 2020), making it one of the most densely populated urban settlements in the city [19,20].

The study was conducted among upper secondary school students (students enrolled in Grades 9-12) who lived in Morata Two, but attending schools. Eligibility criteria included current enrolment at the school and willingness to participate. Students who were absent during data collection or declined consent were excluded.

Study design and sampling: This study employed a descriptive cross-sectional study design**,** enabling the assessment of parental alcohol consumption and its perceived effects on students' academic performance at a single point in time, providing a snapshot of students' academic experiences. As a descriptive study, the emphasis was on documenting the prevalence of parental alcohol use and characterising its influence on students' attendance, academic achievement, behaviour, and overall school engagement.

A non-probability sampling method, specifically convenience sampling, was used to recruit participants. This approach was practical for accessing students who were available during the study period and willing to participate, particularly within communities where household alcohol use is known to be common. While convenience sampling does not ensure statistical representativeness, it is appropriate for descriptive studies seeking to gather timely, firsthand information from individuals directly affected by the issue under investigation.

Sample size: A total of 109 secondary school students living in Morata Two and attending various upper secondary schools in NCD were recruited in the study. This sample size was considered appropriate as it enabled the capture of diverse perspectives across grade levels, socio-economic backgrounds, and residential settings, while remaining feasible within the study's logistical constraints of the study, including time, school schedules, and participant availability.

Although not intended to be representative of all students in NCD, the sample provided sufficient variability to identify meaningful patterns in academic performance, attendance, and emotional or behavioural outcomes associated with parental alcohol use in a high-risk urban context.

Study variables: This study focused on two primary variables:

Independent variables: Parental alcohol use, parental involvement, home environment, family conflict,

Dependent or Outcome variables: Emotional distress, academic performance, academic satisfaction, academic confidence, learning and concentration difficulties.

These variables were chosen to better understand how alcohol use at home affects students' learning and behaviour at school. The students involved in the study lived in Morata Two but attended various schools in NCD.

Data collection tool: Data were collected using a structured, self-administered questionnaire, developed based on validated instruments from previous studies on parental alcohol use, emotional well-being, and academic outcomes [8,22,24]. The questionnaire captured: Socio-demographic characteristics, parental alcohol use, parental involvement in education, emotional well-being, academic performance, satisfaction, and confidence. The instrument was pre-tested among a small group of students from a different school to ensure clarity and relevance.

Questionnaires were distributed during school hours in classrooms designated by school administrators to ensure convenience and confidentiality. Students were briefed on the purpose of the study and assured that their responses would remain anonymous. Teachers were present only to assist with logistics and did not participate in the data collection process, helping to ensure honest and unbiased responses.

Data management and statistical analysis: All completed questionnaires were checked for completeness before data entry. Responses were coded and entered into IBM SPSS (Statistical Package for the Social Sciences) Statistics software version 22 [21]. Data

cleaning included consistency checks and exclusion of cases with missing information to ensure data quality. Data were analysed using IBM SPSS Statistics.

Univariate analysis was conducted to describe the distribution of study variables. Frequencies, and percentages were generated to summarise student demographics, parental alcohol use, academic performance, attendance, and behavioural indicators.

Bivariate analysis examined associations between parental alcohol consumption (independent variable) and students' educational outcomes (dependent variables). Cross-tabulations were performed, and Fisher's Exact Test was used to examine associations between categorical variables due to small cell sizes.

A binary logistic regression analysis was conducted to assess the influence of family-related factors on students' learning and concentration difficulties. The outcome variable was learning and concentration difficulties (Yes = 1, No = 0). Predictor (Independent) variables included lack of parental guidance and support, lack of a conducive home environment, and family conflict. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength and direction of associations, allowing for adjustment of potential confounders. Statistical significance was set at $p \leq 0.05$.

Together, these analytical approaches provided a comprehensive assessment of both descriptive patterns and predictive relationships underlying the impact of parental alcohol consumption on secondary school students in Morata Two.

Ethical clearance: Ethical approval was obtained from the University of Papua New Guinea (UPNG), School of Medicine and Health Sciences (SMHS) Ethics Committee. All procedures adhered to institutional ethical standards. Written informed consent was obtained from all participants, and participation was voluntary, with the right to withdraw at any time.

RESULTS:

A total of 140 questionnaires were distributed to students across the selected upper secondary schools. Of these, 109 questionnaires were fully completed and returned, while 31 were not returned or were incomplete, resulting in a response rate of 77.9% (109/140). The results obtained in this study are presented in the Tables below.

Participant Characteristics:

Of the 109 participants, 59.6% were female and 40.4% male (Table 1). More than half were aged 15–20 years, with most enrolled in Grades 11 and 12, reflecting a predominantly senior secondary school population.

Parental Alcohol Use and Involvement:

A substantial majority (69.7%) reported parental alcohol use. Parental involvement in education was generally low, with over 75% of students reporting minimal or no parental engagement (Table 1).

Emotional and Academic Outcomes:

Emotional distress was reported by 66.1% of students. Academic performance was predominantly poor to average, while levels of academic satisfaction and confidence were generally low (Table 1).

Associations Between Parental Alcohol Use and Educational Factors:

A statistically significant association was observed between parental alcohol use and parental involvement (Table 2; $p < 0.001$), indicating lower educational engagement among parents who consumed alcohol.

Parental alcohol use was also strongly associated with emotional distress (Table 3; $p < 0.001$). Nearly all emotionally affected students reported parental alcohol use.

Academic Performance and Satisfaction:

Academic performance was significantly associated with parental alcohol use (Table 4; $p < 0.001$). Students from alcohol-free households were more likely to report good or excellent performance, whereas those from alcohol-affected households predominantly reported average or poor performance.

Similarly, parental involvement was significantly related to academic satisfaction (Table 5; $p < 0.001$), with higher satisfaction reported among students whose parents were actively engaged in their education.

Table 1. Univariate analysis of demographic characteristics of study participants (n=109)

Variable	Frequency (n)	Percent (%)
Gender		
Male	44	40.4
Female	65	59.6
Age group (Years)		
15-20	58	53.2
21-25	28	25.7
26-30	16	14.7
>30	7	6.4
Education (Grade)		
Grade 9	18	16.5
Grade 10	22	20.2
Grade 11	34	31.2
Grade 12	35	32.1

Parental alcohol use		
Yes	76	69.7
No	33	30.3
Parental involvement in child's education		
Very involved	27	24.8
Minimally involved	38	34.9
Not involved at all	44	40.3
Emotional outcomes		
Yes	72	66.1
No	37	33.9
Academic performance		
Excellent	10	9.2
Good	27	24.7
Average	34	31.2
Poor	38	34.9
Academic satisfaction		
Completely satisfied	21	19.3
Partly satisfied	45	41.3
Not satisfied at all	43	39.4
Academically confident		
Very confident	16	14.7
Confident	17	15.6
Neutral	37	33.9
Not confident at all	39	35.8

Table 2. A bivariate analysis of association between parental involvement in education and parental alcohol use (n=109).

Parental Involvement	No Alcohol Use n (%)	Alcohol Use n (%)	Total n (%)	χ^2 value	p-value
Very involved	27 (100)	0 (0)	27 (100)	92.44	< 0.001
Minimally involved	6 (15.8)	32 (84.2)	38 (100)		
Not involved at all	0 (0)	44 (100)	44 (100)		
Total	33 (30.3)	76 (69.7)	109 (100)		

Significance level at p≤0.05

Table 3. Association Between Parental Alcohol Use and Students' Emotional Outcomes (n=109).

Emotionally Affected	No Alcohol Use n (%)	Alcohol Use n (%)	Total n (%)	χ^2 value	p-value
No	33 (89.2)	4 (10.8)	37 (100)	88.61	< 0.001
Yes	0 (0.0)	72 (100)	72 (100)		
Total	33 (30.3)	76 (69.7)	109 (100)		

Significance level at p≤0.05

Table 4. Association Between Parental Alcohol Use and Students' Academic Performance (n=109).

Academic Performance	No Alcohol Use n (%)	Alcohol Use n (%)	Total n (%)	χ^2 value	p-value
Excellent	10 (100.0)	0 (0.0)	10 (100)	90.13	< 0.001
Good	22 (81.5)	5 (18.5)	27 (100)		
Average	1 (2.9)	33 (97.1)	34 (100)		
Poor	0 (0.0)	38 (100.0)	38 (100)		
Total	33 (30.3)	76 (69.7)	109 (100)		

Significance level at p≤0.05

Table 5: Association between academic satisfaction level with parental involvement (n=109)

Academic Satisfaction	Very Involved n (%)	Minimally Involved n (%)	Not Involved at All n (%)	Total n (%)	χ^2 value	p-value
Completely Satisfied	17 (81.0)	3 (14.3)	1 (4.8)	21 (100)	50.08	<0.001
Partially Satisfied	10 (22.2)	16 (35.6)	19 (42.2)	45 (100)		
Not Satisfied at All	0 (0.0)	19 (44.2)	24 (55.8)	43 (100)		
Total	27 (24.8)	38 (34.9)	44 (40.4)	109 (100)		

Significance level at p≤0.05

Emotional Well-being and Academic Confidence:

A strong association was found between emotional distress and academic confidence (Table 6; p < 0.001).

Emotionally affected students were substantially more likely to report low confidence in their academic abilities.

Predictors of Learning and Concentration Difficulties:

Binary logistic regression analysis (Table 7) identified lack of parental guidance and support as the strongest predictor of learning and concentration difficulties (OR = 5.18, 95% CI: 1.86–14.56, p = 0.002). An unconducive home environment also significantly increased the likelihood of difficulties (OR = 3.65, 95% CI: 1.11–12.02, p = 0.034). Family conflict was not a significant predictor (p = 0.933).

Table 6: Association between Academic confident levels and emotional outcome (n=109).

Confidence in Academic Success	Not Emotionally Affected n (%)	Emotionally Affected n (%)	Total n (%)	χ^2 value	p-value
Very Confident	16 (100.0)	0 (0.0)	16 (100)	88.61	< 0.001
Confident	16 (94.1)	1 (5.9)	17 (100)		
Neutral	3 (8.1)	34 (91.9)	37 (100)		
Not Confident at All	2 (5.1)	37 (94.9)	39 (100)		
Total	37 (33.9)	72 (66.1)	109 (100)		

Significance level at p≤0.05

Table 7. Binary logistic regression of three family-related factors, lack of parental guidance and support, lack of a conducive environment at home, and family conflict on students' learning and concentration difficulties.

Predictors (Independent variables)	Odds Ratio (95% CI)	P-value
Family conflict (Yes vs No)	0.95 (0.30–3.05)	0.933
Lack of conducive environment at home (Yes vs No)	3.65 (1.11–12.02)	0.034
Lack of parental guidance and support (Yes vs No)	5.18 (1.856–14.56)	0.002
N	109	—

Footnote: OR = Odds Ratio, CI = Confidence Interval. No – Reference group for each predictor.

DISCUSSION:

This study demonstrates clear associations between parental alcohol use, parental involvement, emotional well-being, and academic outcomes among upper secondary school students in Morata Two. The higher proportion of female participants (Table 1) is noteworthy, as existing evidence suggests that girls are more likely to internalize stress in response to household adversity, whereas boys tend to externalize distress [22,23]. These gendered coping patterns may influence emotional regulation and academic engagement in alcohol-affected households.

Most participants were in late adolescence and senior secondary grades, a developmental stage marked by heightened academic demands and emotional sensitivity. Prior studies identify adolescence as a critical period during which exposure to parental alcohol use adversely affects emotional well-being, academic motivation, and behavioural adjustment [24-26]. Older students may also be

more aware of family dynamics and better able to recognize their effects on learning [8].

Parental alcohol use was highly prevalent in the study population (Table 1), consistent with global patterns [27]. This was accompanied by low levels of parental involvement in education, reinforcing evidence that alcohol misuse impairs parental functioning and reduces engagement in children's schooling [4,28,29]. The significant association ($p<0.05$) between parental alcohol use and parental involvement (Table 2) suggests that alcohol-related impairment limits emotional availability, supervision, and academic support at home [35,36].

Emotional distress was common among students from alcohol-affected households, and our findings revealed a strong association (Table 3; $p < 0.001$) between parental alcohol use and emotional distress, aligning with research showing increased risks of anxiety, emotional instability, and psychological distress among children of alcohol-dependent parents [30]. Emotional distress is known to impair concentration, academic confidence, and engagement with learning [31], and in this study, it coincided with predominantly low to moderate academic performance (Table 4; $p < 0.001$). Similar patterns have been widely reported, including higher risks of poor performance and grade repetition among children of alcohol-dependent parents [8,32].

Students' academic satisfaction further reflected these challenges. Those reporting low parental involvement were significantly more likely to be dissatisfied with their academic experience and this is consistent with evidence that children from alcohol-affected households often develop negative attitudes toward school [11].

In contrast, students with highly involved parents reported greater academic satisfaction, highlighting the protective role of parental engagement [37,38]. Similarly, our findings show that parental involvement was significantly linked to academic satisfaction (Table 5; $p < 0.001$), with higher satisfaction reported among students whose parents were actively engaged in their education.

Academic confidence was also strongly associated with emotional well-being (Table 6, $p < 0.001$). Students experiencing emotional distress were significantly more likely to report low academic confidence, supporting evidence that diminished self-esteem and unstable home environments undermine academic self-concept among children of alcohol-dependent parents [33,34].

Multivariate analysis (Table 7) identified lack of parental guidance and support as the strongest predictor of learning and concentration difficulties (OR = 5.18, 95% CI: 1.86–14.56, $p = 0.002$), while an unconducive home environment also significantly increased risk (OR = 3.65, 95% CI: 1.11–12.02, $p = 0.034$).

Family conflict was not a significant predictor (OR = 0.95, 95% CI: 0.30–3.05, p=0.933), suggesting that the absence of consistent support, structure, and emotional stability may have a more immediate impact on learning than conflict alone. This finding aligns with evidence that weakened parent-child relationships mediate the effects of parental substance use on academic achievement [39], and that environmental stressors further exacerbate learning difficulties [40].

Overall, the findings indicate that parental alcohol use undermines students' emotional well-being and academic success primarily through reduced parental involvement, emotional distress, and unstable home environments. Students with involved, non-drinking parents demonstrated greater emotional stability, academic confidence, satisfaction, and performance. These results are consistent with existing literature [24,22,8] and underscore the need for family-centred and school-based interventions that strengthen parental engagement, improve home environments, and provide targeted emotional and academic support for students living in alcohol-affected households.

Limitations of the study:

Study limitations include the cross-sectional design, which limits the ability to establish causal relationships between variables. The relatively small sample size may further restrict the generalisability of the findings. Data were based on self-reported student perceptions, which may be influenced by recall or social desirability bias, particularly when addressing sensitive issues such as parental alcohol use. The absence of parental perspectives limits a more comprehensive understanding of parental behaviours and educational engagement. Additionally, data were collected from a single geographic area in PNG, and the findings may not reflect experiences in other regions or cultural contexts.

Despite these limitations, the findings offer valuable evidence to inform policy, school-based support systems, and family-focused interventions aimed at improving student well-being and educational outcomes.

CONCLUSION:

This study demonstrates a clear association between parental alcohol use and poorer emotional wellbeing and academic performance among students in Morata two. Students from alcohol-affected households were more likely to experience anxiety, reduced academic confidence, and learning difficulties, while those with involved, non-drinking parents showed greater emotional stability and academic success. The findings align with existing literature and highlight the critical role of parental guidance and a supportive home environment in shaping students' educational outcomes.

Overall, the results emphasise the need for family-centred interventions and support systems to address alcohol-related harm and promote healthier home environments that enable students to achieve their full academic potential. Given the limited local data, more research should be conducted to explore the long-term effects of parental alcohol use on student development in PNG. This will help inform culturally relevant interventions and policies.

Recommendations:

Targeted interventions are needed to reduce the impact of parental alcohol use on students' emotional and academic well-being. Parental education programs should be strengthened to raise awareness of the effects of alcohol use on children's development and school performance. Schools should enhance counselling and mental health services to provide early emotional and academic support for affected students. Greater family engagement should be promoted through regular parent-teacher communication and home-based learning support. Students from alcohol-affected households may benefit from targeted academic and resilience-building interventions.

At the policy level, collaboration among educators, health professionals, and policymakers is essential to strengthen regulation of alcohol availability, particularly in residential areas. Further research in PNG is recommended to inform culturally appropriate, evidence-based interventions.

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A LOW-COST, CUSTOM-BUILT FEAR CONDITIONING SYSTEM FOR RODENTS: DESIGN, CONSTRUCTION AND VALIDATION

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ABSTRACT

A major goal in behavioral neuroscience is to identify the neural mechanisms underlying learning and memory. Fear conditioning models, including contextual and cued conditioning, are widely used to assess associative learning in rodents; however, commercially available systems for these experiments are often expensive (> $4000), limiting accessibility and reproducibility across laboratories. Here, we present a low-cost (about $100), modular fear conditioning platform for rodents that enables precise control of experimental parameters while maintaining compatibility with standard behavioral protocols.

The fear conditioning system consists of conditioning chambers equipped with stainless-steel grid floors, a custom-built constant-current foot-shock generator**,** and an auditory cue delivery section, all synchronized with video-based behavioral recording. Shock intensity, duration, and timing are fully programmable, and the platform supports both contextual and cued fear conditioning paradigms. To validate the setup, we designed three well-established experiments and demonstrated that this low-cost fear conditioning system produces reliable and reproducible fear conditioning behavior comparable to established commercial systems while substantially reducing system cost. Its open and flexible design provides an accessible alternative for laboratories seeking standardized fear conditioning experiments, promoting broader adoption of behavioral assays in resource-limited settings.

Keywords: Conditioning, Fear, Foot-shock, Memory, Learning, Low-cost

INTRODUCTION

Fear conditioning is a well-established behavioral model for studying associative learning and memory in rodents and has been extensively used to investigate the neural mechanisms underlying emotional learning and psychiatric disorders such as post-traumatic stress disorder and schizophrenia [1 - 3]. Standard implementations of contextual and cued fear conditioning rely on specialized

hardware, including conditioning chambers with electrified grid floors, auditory stimulus generators, and programmable shock delivery units, all of which must operate with high temporal precision and reliability.

Commercially available fear conditioning systems provide robust and standardized solutions; however, they are often cost-prohibitive, limiting accessibility for laboratories with constrained resources and reducing experimental scalability. Furthermore, proprietary hardware and software designs may restrict flexibility in protocol customization, integration with external devices, or adaptation to novel experimental needs. These limitations have motivated the development of custom-built and open hardware solutions for behavioral neuroscience, aimed at improving accessibility while maintaining experimental rigor [4,5].

A critical component of fear conditioning hardware is the foot-shock generator, which must deliver stable, reproducible electrical stimulation at low current levels through stainless-steel grid floors while ensuring animal safety and experimental consistency. Commercial shock generators, such as the SGS-003DX Shock Generator Scrambler, are commonly used as reference standards in laboratories and provide programmable constant-current output and grid scrambling to distribute current across electrodes. While effective, such systems substantially increase the overall cost of a fear conditioning setup.

In this work, we introduce a low-cost, modular fear conditioning system designed to replicate the core functional requirements of established systems while reducing cost and complexity. The platform integrates conditioning chambers with stainless-steel grid floors, a custom-designed constant-current shock generator, and an auditory cue delivery system under unified software control. We experimentally validated the system and demonstrated that the hardware successfully supports standard contextual and cued fear conditioning protocols, while providing precise control over shock intensity, duration, timing, and synchronization with behavioral recordings.

By situating our design within the context of widely used fear conditioning hardware, this system aims to facilitate standardized, reproducible behavioral experiments while lowering barriers to adoption. The system complements existing behavioral neuroscience methodologies and provides an accessible alternative for laboratories seeking flexible, open, and cost-effective experimental tools, especially in developing countries.

MATERIALS AND METHODS:

Hardware Description:

The Controller:

The controller unit was implemented as an auxiliary control circuit based on an ATmega328P microcontroller mounted on a custom printed circuit board (PCB). The system is powered by a 12 V DC external power

adapter, with on-board voltage regulation provided by a linear 7805 regulator to supply a stable 5 V rail for the microcontroller and peripheral components (Figure 1A1 – A3).

The microcontroller governs the timing and sequencing of foot-shock delivery according to user-defined experimental parameters. Specifically, the controller manages the onset and offset of shock stimuli, allowing precise control over shock duration and inter-stimulus timing. Shock parameters are selected by the user before the experiment and executed autonomously by the controller during the conditioning session.

An integrated liquid crystal display (LCD) provides real-time feedback during experiments, displaying key parameters such as cycle count, selected shock intensity, and system status while a test is ongoing. This enables experimenters to monitor protocol execution without interrupting the conditioning procedure. The controller also interfaces with an auditory cue module, triggering a buzzer used for cued fear conditioning paradigms.

Auditory stimulus timing is synchronized with shock delivery under microcontroller control to ensure consistent temporal relationships between conditioned and unconditioned stimuli.

The system supports programmable shock current amplitudes in the range of 0.1 mA to 4 mA, covering the range commonly used in rodent fear conditioning experiments.

The Shocker Rig:

The shock delivery unit, or Shocker Rig, is responsible for generating the actual foot shocks, complementing the controller, which manages input and timing. The system begins with a step-down transformer that reduces the AC mains voltage from 200 – 250 V to 110 V AC. The output is then rectified using a KBP206G bridge rectifier and filtered with a 400 V, 47 µF capacitor to provide a stable DC supply. Given the low-current requirements of the system, the voltage is further reduced to a safe output range of 40 –75 V, suitable for delivering reproducible foot shocks to rodents.

This configuration ensures that the shocks are precisely controlled, stable, and safe, while the low-current design minimizes risk to the animals and allows the unit to operate reliably over repeated cycles. By separating the control logic (managed by the ATmega328P controller) from the high-voltage output stage, the design maintains both safety and modularity.

The Conditioning Chamber:

The fear conditioning chamber was custom designed to provide a durable and transparent environment for rodents while allowing reliable shock delivery. The base consisted of a wooden platform fitted with 5 mm stainless steel rods positioned at 12 mm center-to-center intervals to create the electrifiable grid floor. The walls were made from clear acrylic, allowing unobstructed observation and video recording of behavioral responses. The overall dimensions of the

chamber are 300 mm × 320 mm × 300 mm, and the structure weighs approximately 2.5 kg, making it both lightweight and stable during experiments (Figure 1B). This design ensures consistent foot-shock delivery to support both contextual and cued fear conditioning paradigms and provides visibility for automated or manual behavior scoring, while maintaining a low-cost and easily reproducible construction.

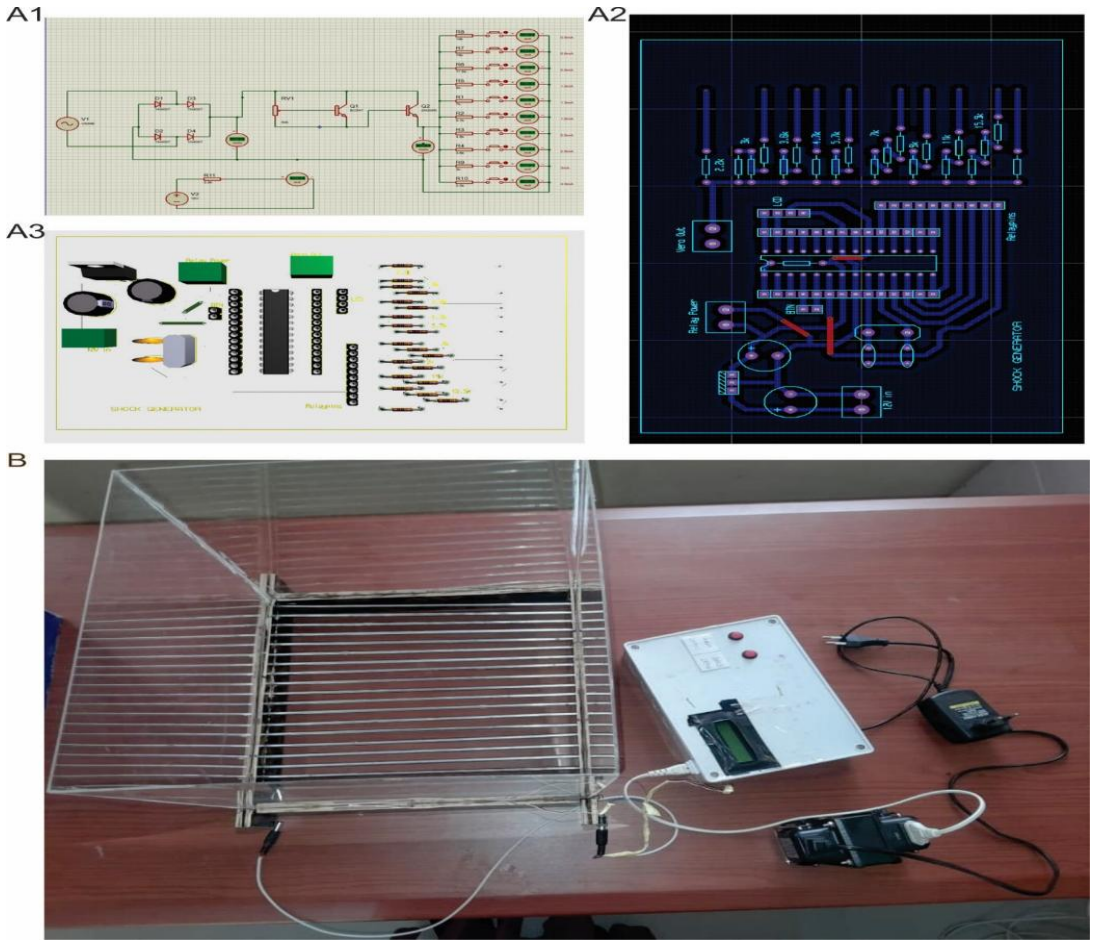

Figure 1: Hardware construction (A1) Schematic diagram of current control circuit (A2) Printed circuit board (PCB) layout showing the microcontrollers (ATMEGA328P) and their connections (A3) A 3D view of the printed circuit board (PCB) design. (B) Fear conditioning chamber

Animals:

Forty-two (42) healthy adult rats weighing 150 – 230 g were used for validation experiments. Animals were housed under standard laboratory conditions, with controlled temperature and a 12-hour light/dark cycle and were provided with food and water *ad libitum*. All procedures were conducted in accordance with the guidelines for the care and use of animals as approved by the University of Ilorin's ethical committee, Ilorin, Nigeria.

Fear conditioning protocol:

The protocol was as previously described [6] with slight modifications. We employed a 2-day conditioning protocol. On the conditioning/training\ day (DAY 1), each rat was placed in Context A and allowed to explore for 180 seconds before stimulus presentation (baseline, BL). Each rat received repeated pairings of an auditory tone (conditioned stimulus, CS) with a foot shock (unconditioned stimulus, US). Each tone presentation was co-terminated with a foot shock. Each rat was exposed to five conditioning trials (T1 – T5) with a 20-second tone and a 2-second foot-shock (55 dB; 1.5 mA) delivered at 1-minute intervals. After the final trial, rats remained in the chamber for 90 seconds before being returned to their cages.

To assess contextual fear memory, animals were re-exposed to the conditioning chamber 24 hours after the acquisition without tone and shock presentation. Freezing behavior was recorded continuously during the 8-minute session. Two hours after the context test, the cue test was conducted. Rats were placed in a neutral context (Context B). This box is similar in dimensions to those of the shock chamber (context A, Figure 1 B). This new chamber, however, was made highly different from the shock chamber. Two of its walls were transparent and had no metal grid. Each rat was allowed to explore the new context for 180 seconds before the tone presentation (baseline, BL). This phase consists of five trials (T1 – T5), with 1-minute intervals. Each rat received an auditory cue for 20 seconds every minute without a foot shock. After the final trial, rats remained in the chamber for 90 seconds before being returned to their cages.

The experimenter recorded the behaviors of the rats as freezing or not. Freezing behavior was defined as the inhibition, absence, or suppression of movement, beyond that required for autonomic nervous system functioning [7]. Head scanning and sleeping were not included as freezing. Freezing behavior was scored in the conditioned and unconditioned groups during the final 20 s of the first minute (baseline, BL), the 20 s before each foot-shock (T1–T5) and the final 20 s of the last minute (TL; [8]).

Sleep deprivation:

Sleep deprivation was induced using a modified multiple-platform technique [9,10]. The rats were placed in a plastic cage (23 × 23 cm) containing several small platforms (5 cm diameter) positioned 7 cm apart.

Food and water were provided *ad libitum* via an attached acrylic compartment and drinking bottles. When the animals entered the rapid eye movement (REM) sleep, muscle atonia caused them to lose balance and contact the water beneath the platforms, forcing them to awaken. This method selectively disrupted REM sleep while allowing non-REM sleep.

The rats underwent 18 hours of sleep deprivation daily (from 16:00 to 10:00 the next day) for seven consecutive days before behavioral testing.

After each deprivation period, the animals were gently dried with towels and returned to their home cages.

Scopolamine treatment:

To test the effect of post-training scopolamine injection on fear Conditioning, on the conditioning/training day (DAY 1), each rat was placed in Context A and allowed to explore for 180 seconds before the presentation of tone (baseline, BL). Each rat was exposed to five conditioning trials (T1 – T5) with a 20-second tone and a 2-second foot-shock (55 dB; 1.5 mA) delivered at 1-minute intervals. After the final trial, rats remained in the chamber for 90 seconds. Immediately, test rats were injected with scopolamine intraperitoneally (10 mg/kg, i.p) and returned to their home cages. The control animals received an intraperitoneal injection of normal saline. The contextual and cued tests were carried out on day 2 as described above.

On the second day, we assessed contextual fear memory by re-exposing the rats to the conditioning chamber without tone or shock presentation. Freezing behavior was recorded continuously during the 8-minute session. Two hours after the context test, the cue test was conducted. Rats were placed in a neutral context (Context B) where each rat was allowed to explore the new context 180 seconds before the tone presentation (baseline, BL). This phase consists of five trials (T1 – T5), with 1-minute intervals. Each rat received an auditory cue for 20 seconds every minute without a foot shock. After the final trial, rats remained in the chamber for 90 seconds before being returned to their cages.

Data Analysis:

These data were analyzed by a two-way ANOVA and subsequently, Bonferroni's multiple comparison tests were applied when appropriate. Data were represented as mean ± standard error of mean and the level of significance was set at $p < 0.05$.

RESULTS

Experiment 1: Low-Cost Rodent Fear Conditioning Setup: Acquisition and Expression of Tone-Shock, Contextual, and Cued Fear Memories:

The first experiment tested whether our low-cost fear conditioning apparatus could reliably demonstrate associative learning in rodents through the pairing of a tone (conditioned stimulus) with foot shock (unconditioned stimulus). Baseline freezing levels were comparable between the tone-only (control) group and the tone + shock (paired) group (n = 5/group), remaining low and consistent with typical pre-conditioning values in standard auditory fear conditioning paradigms. Following the pairing phase, however, the tone + shock group displayed significantly elevated freezing during tone presentation compared to the tone-only group (2-Way ANOVA, $F_{1,8} = 10.08$, $p = 0.0131$). This marked increase in the percentage

of freezing time spent indicates a robust conditioned fear response specific to the tone-shock association, as the tone-only group showed no such elevation. These results confirm that the rats in the paired condition successfully acquired and expressed intact Pavlovian associative learning, validating the effectiveness of the low-cost setup for detecting fear conditioning in rodents.

The context test, conducted 24 hours after the training session, involved re-exposing the rats to the original conditioning chamber for an 8-minute period without any tone or foot shock presentation. Consistent with established findings in the literature on contextual fear conditioning, the tone + shock (paired) group exhibited significantly higher levels of freezing compared to the tone-only (control) group (2-Way ANOVA, $F_{1,8} = 20.54$, $p = 0.0019$) during this exposure [11,12]. This elevated freezing response indicates the successful formation of a robust contextual fear memory, which is widely recognized as primarily dependent on the hippocampus [13,14]. These results provide further evidence that the low-cost setup reliably elicits and detects hippocampus-dependent associative learning in rodents, complementing the tone-specific (cued) fear conditioning observed in the earlier test and supporting its utility for studying fear memory mechanisms.

The cued test was performed 2 hours after the context test. Rats were placed in a novel, neutral chamber distinct from the original conditioning box (See methods). After a 180-second baseline period, the tone was presented for 20 seconds every minute, repeated five times. Results revealed significantly higher percentage freezing during tone presentations in the tone + shock (paired) group compared to the tone-only (control) group (2-Way ANOVA, $F_{1,8} = 11.07$, $p = 0.0104$). This selective increase in freezing to the tone demonstrates successful formation of a cued (auditory) associative fear memory, which is primarily dependent on the amygdala [1,2,13]. Collectively, these findings confirm that our low-cost fear conditioning setup effectively captures and quantifies key forms of associative learning in rats, offering a reliable, accessible tool for studying fear memory mechanisms.

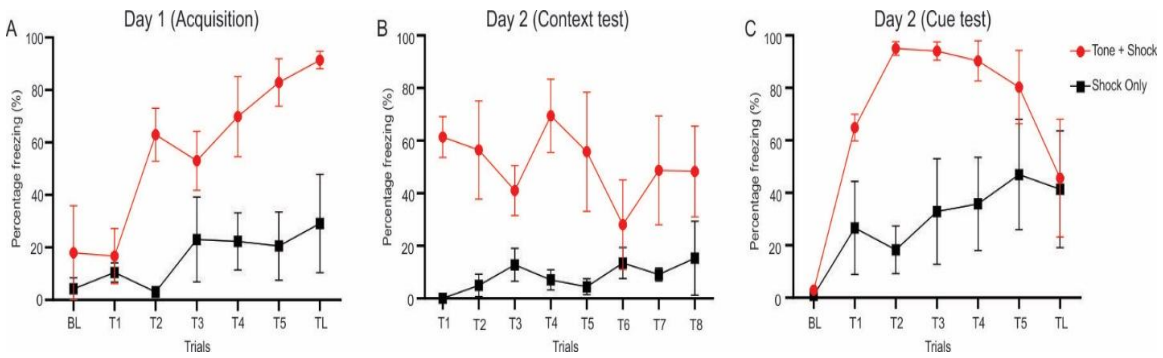

Fig. 2. Rats learned associations between the conditioned stimulus (tone) and the aversive unconditioned stimulus (foot shock). Averaged percentage of freezing displayed by rats exposed to shock and tone or tone only in the fear-conditioning paradigm (A) Training (B) context test (C) cue test. Error bars represent standard error of mean. *n* = 5; data are represented as Mean ± standard Error of mean. BL, baseline; TL, freezing level during the last trial.

Experiment 2: Selective Impairment of Hippocampus-Dependent Contextual Fear Memory Following Sleep Deprivation in Rats:

To further validate the utility of our low-cost setup for fear conditioning studies involving sleep manipulation, twenty-two rats were randomly assigned to two groups (n = 11/group): a control group and a sleep-deprived (SD) group. Testing began 2 hours after the completion of the sleep deprivation protocol (or at the equivalent time point for controls). During acquisition on day 1, both the control and SD groups exhibited progressive increases in freezing across trials, indicating gradual acquisition of the tone-shock association. Although the SD group displayed modestly lower freezing levels overall, this difference did not reach statistical significance (2-Way ANOVA, $F_{1,20}$ = 1.94, p = 0.1804). These observations suggest that pre-training sleep deprivation did not substantially impair the initial acquisition of fear learning.

On day 2, in the context test, rats were re-exposed to the original conditioning chamber for 8 minutes without tone or shock presentation. Freezing percentages were significantly higher in the control group compared to the SD group (2-Way ANOVA, $F_{1,20}$ = 21.66, p = 0.0002). This finding aligns with extensive evidence that sleep deprivation selectively impairs the consolidation and/or expression of contextual fear memory, a process heavily reliant on hippocampal function [15,16]. In contrast, during the subsequent cued test (conducted in a novel neutral chamber), both groups displayed robust and comparable freezing responses to tone presentations, with no significant differences between control and SD animals (2-Way ANOVA, $F_{1,20}$ = 0.0043, p = 0.9476). This pattern indicates that sleep deprivation did not affect the formation or expression of cued (auditory) fear memory, which is primarily amygdala-dependent and hippocampus-independent [15,16]. Taken together, these results demonstrate a selective impairment of contextual fear conditioning following sleep deprivation, while sparing cued conditioning.

This is consistent with the differential neural substrates involved (hippocampus for context vs. amygdala for cue). This further confirms the sensitivity and reliability of our low-cost fear conditioning setup for detecting hippocampus-dependent memory deficits in rodent models.

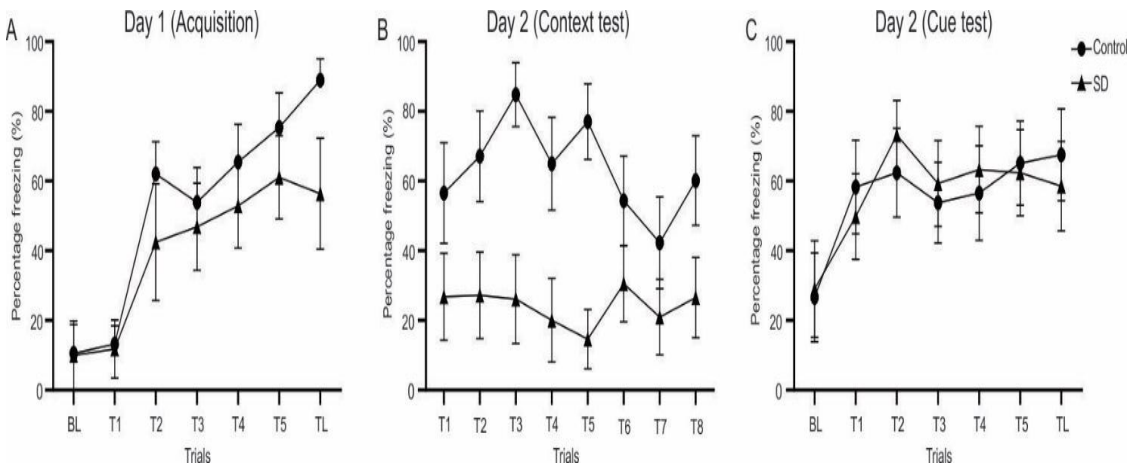

Fig. 3: Sleep deprivation selectively impairs contextual fear memory retrieval. Average percentage freezing displayed by the control or sleep-deprived rats in the fear-conditioning paradigm (A) Training (B) context test (C) cue test. Error bars represent standard error of mean. *n* = 11/group; data are represented as Mean ± standard Error of mean. BL, baseline; TL, freezing level during the last trial.

Experiment 3: Effects of Post-Training Scopolamine on Hippocampal-Dependent Contextual and Amygdala-Dependent Cued Fear Conditioning in Rats:

To further validate the pharmacological sensitivity of our low-cost fear conditioning setup, we examined the effects of post-training scopolamine administration, a muscarinic cholinergic antagonist known to disrupt memory consolidation in fear paradigms. Twelve (12) rats underwent fear conditioning via tone-shock pairings and were then randomly assigned to either a control group or a scopolamine group (n = 6/group). Immediately following training, the scopolamine group received an intraperitoneal injection of scopolamine (10 mg/kg), while the control group received an equivalent volume of normal saline intraperitoneally. On day 2, during the context test, freezing levels were significantly higher in the control group compared to the scopolamine-treated group (2-Way ANOVA, $F_{1,10} = 5.04$, $p = 0.0485$). In contrast, no significant differences in freezing were observed between the two groups during the cued test (2-Way ANOVA, $F_{1,10} = 0.22$, $p = 0.6482$). These results indicate that post-training scopolamine selectively impaired the consolidation of hippocampus-dependent contextual fear memory, while sparing amygdala-dependent cued (auditory) fear conditioning, consistent with selective hippocampal disruption in contextual but not discrete-cue fear following cholinergic blockade [6,17]. Collectively, these pharmacological

findings, alongside the prior behavioral and sleep-deprivation validation experiments, demonstrate that our low-cost apparatus reliably replicates established effects on fear memory formation and consolidation, making it a robust, accessible tool for studying cholinergic modulation of hippocampal- versus amygdala-dependent associative learning in rodents.

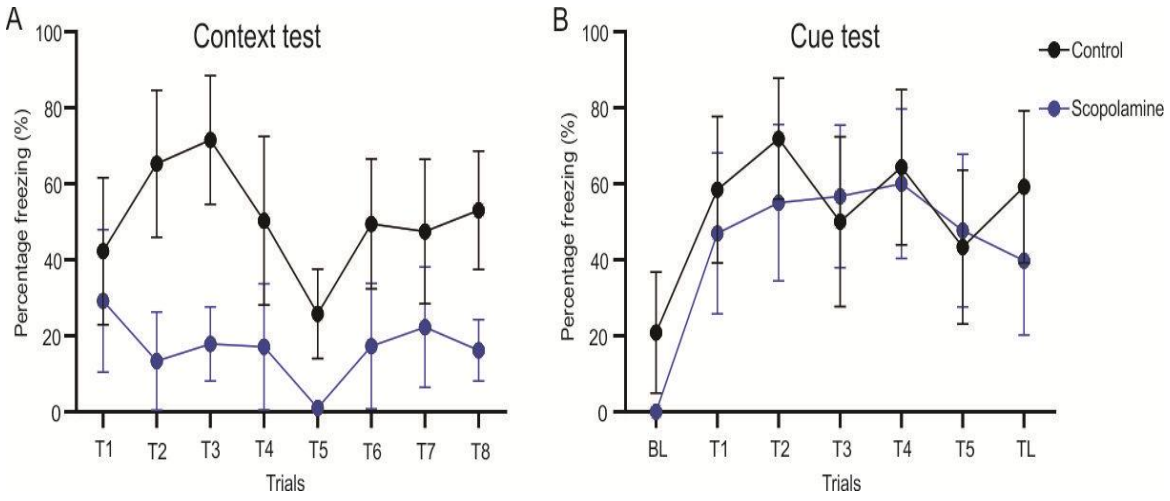

Fig. 4: Post-training injection of scopolamine affected contextual fear memory recall. Averaged percentage freezing displayed by control or scopolamine-treated rats in the fear-conditioning paradigm (A) context test (B) cue test. Error bars represent the standard error of mean. *n* = 6/group; data are represented as Mean ± standard Error of mean. BL, baseline; TL, freezing level during the last trial.

DISCUSSION:

In the present study, we successfully replicated core phenomena from classical fear conditioning paradigms, including robust tone-shock associative learning, contextual fear expression, and pharmacologically induced memory impairment by employing a novel, low-cost rodent fear conditioning system assembled from affordable, readily available components. These outcomes not only confirm the reliability and sensitivity of our economical apparatus but also highlight its viability as an accessible substitute for expensive commercial systems, thereby expanding opportunities for fear-related behavioral neuroscience research in resource-constrained environments.

In Experiment 1, our low-cost setup demonstrated effective associative fear learning in rats via a tone-shock pairing paradigm. Animals in the paired (tone + shock) group exhibited significantly elevated freezing during both acquisition and retrieval phases compared to the tone-only control group. This behavioral dissociation clearly indicates that freezing reflects a learned association between the conditioned stimulus (tone) and the unconditioned stimulus (foot shock), rather than nonspecific reactions to the auditory cue or

general arousal. Acquisition trials revealed progressive increases in freezing in the paired group, mirroring the classic pattern of Pavlovian fear conditioning where repeated CS-US pairings yield robust conditioned responses [2,7]. The tone-only group, by contrast, displayed persistently low freezing levels, consistent with prior work showing that an unpaired neutral cue fails to elicit fear without reinforcement [18,19]. Retrieval tests further substantiated memory formation: paired animals showed sustained freezing to the tone in a neutral context, while controls did not, aligning with evidence that cued fear memories are amygdala-dependent and retrievable independently of the original context [3,13]. Minimal freezing in the tone-only group across all phases ruled out baseline anxiety, habituation effects, or nonspecific stress as confounders, reinforcing freezing as a valid, specific index of associative fear [7]. Overall, these results clearly replicate canonical tone-shock conditioning outcomes, providing essential validation of the paradigm's reliability prior to more advanced manipulations.

In Experiment 2, pre-training sleep deprivation selectively impaired contextual fear memory retrieval while leaving cued fear memory intact. Both control and sleep-deprived (SD) groups acquired the tone-shock association comparably during training, with only modest, non-significant reductions in freezing among SD animals. However, during day-2 contextual testing, SD rats exhibited significantly lower freezing than controls, suggesting a targeted disruption of hippocampus-dependent processes rather than broad deficits in fear learning. This selective impairment accords with reports linking sleep deprivation to altered Hypothalamic-Pituitary-Adrenal axis activity, reduced corticosterone, and diminished phosphorylated cyclic AMP response element-binding protein (pCREB) in hippocampal CA1, which compromise synaptic plasticity and contextual memory [15,16]. In contrast, both groups displayed comparable, robust freezing to the tone in the cued test, indicating preservation of amygdala-dependent cued conditioning. This dissociation highlights the differential vulnerability of hippocampal versus amygdala circuits to sleep loss [15,16]. Additional factors, such as circadian disruptions from altered training/testing timing or varying SD durations, may modulate these effects, with longer deprivation periods more likely to induce pronounced hippocampal dysfunction [15]. Finally, in Experiment 3, post-training scopolamine (10 mg/kg, i.p.) selectively impaired contextual fear memory consolidation while sparing cued conditioning. Both control (saline) and scopolamine-treated groups tolerated training without overt motor or behavioral abnormalities, confirming the setup's tolerability. On day 2, scopolamine-treated rats showed significantly reduced freezing in the context test compared to controls, but equivalent freezing during cued tone presentations in a neutral chamber. This pattern demonstrates

cholinergic involvement in post-training memory processing, particularly for hippocampus-dependent contextual fear, consistent with evidence that systemic muscarinic blockade shortly after training disrupts later contextual memory [6,20]. The robust freezing in controls for both context and tone underscore associative learning, while the scopolamine-induced deficit validates the setup's sensitivity to established amnestic interventions. Collectively, the three experiments closely mirror findings in established literature. These congruences affirm the low-cost fear conditioning apparatus as a dependable, cost-effective platform for investigating associative fear mechanisms, neural substrates, and modulatory influences in rodents.

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Authors Contribution:

Abdulmajeed Wahab Imam conceptualized the idea, designed the experiments, supervised the experiments and prepared the manuscript; Abdulrasheed Kamil Muhammad designed and built the hardware, wrote the codes and drafted the manuscript; Adedeji Tayyib Adekunle, Osho Oluwabukola Daniel, Adesanya Fuhad Babajide, Yusuf Sekinat Funmilayo, Akinlabi Islamiyah Adenike, Nureni Mardiyat Arinola and Oseni Nuriyat Oyindamola carried out the experiments and analyzed data; Amin Abdulbasit and Ayinla Maryam Tayo designed the experiments and wrote the manuscript.

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CASE REPORT:

ATYPICAL PRESENTATION OF DENGUE FEVER IN AN OLDER PERSON: CASE REPORT

SHORT RUNNING TITLE: DENGUE FEVER IN ELDERLY

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ABSTRACT:

Dengue is an increasingly common infection among older adults, whose presentations are often atypical and easily misattributed to other causes of fever. We report the case of a 72-year-old man who presented with a short history of intermittent fever, lethargy and increased urinary frequency, initially treated as a suspected urinary tract infection. Laboratory investigations showed leucopenia, thrombocytopenia and mild anaemia. Urinalysis and cultures were negative. Dengue NS1 antigen testing confirmed the diagnosis. During the admission, the patient developed marked thrombocytopenia but did not progress to plasma leakage, bleeding, organ impairment or shock. He was managed with careful supportive care and frequent monitoring, recovering fully by Day 10 of admission. This case highlights the diagnostic challenges of dengue in older adults, whose altered physiological responses may mask classical features such as myalgia, rash and haemorrhagic manifestations. Older adults are at increased risk of severe dengue, prolonged hospitalisation and complications, thus early recognition, judicious fluid management and close observation are essential. Clinicians should consider dengue in older adults presenting with fever and cytopenias in endemic regions, even when symptoms appear non-specific.

Keywords: aged; dengue, fever, leukopenia, thrombocytopenia

INTRODUCTION:

Dengue is a mosquito-borne viral disease, with an expanding global reach and increasing incidence among older adults. Current estimates suggest that over half of the world's population is now at risk, which is expected to increase further due to climate change, urbanization and vector adaptation to new environments [1,2]. The global demographic shift towards an ageing population also means it is imperative that clinicians recognise dengue infections in older people.

Older adults usually present atypically, or have muted clinical manifestations, often lacking the classic dengue triad of fever, myalgia, and rash. Presentations tend to reflect comorbidity-related symptoms or non-specific systemic complaints [3]. Age-related immune dysregulation, multi-morbidity, decreased physiological reserve, and altered inflammatory responses contribute to diagnostic delays and worse outcomes.

Compared to younger adults, older patients have higher rates of severe dengue, dengue haemorrhagic fever (DHF), organ impairment, hospital-acquired infection, prolonged hospitalisation and dengue-related mortality [4,5]. The World Health Organization classifies dengue into febrile, critical, and recovery phases, with each phase associated with different risks. However, dengue in older adults may be less predictable, occasionally deteriorating earlier or outside these phases [6].

We report a case of dengue in a previously well 72-year-old man, whose atypical presentation was initially suggestive of a urinary tract infection. This case highlights the diagnostic challenges and key considerations in managing dengue in older adults.

Case Presentation:

A 72-year-old man presented with a two-day history of intermittent high fever with chills, poor oral intake, light-headedness and increased urinary frequency. He denied dysuria, abdominal pain, arthralgia, rash or bleeding symptoms. His past medical history was benign prostatic hypertrophy (BPH) and thalassaemia trait. He was not on regular medications, other than multivitamins. He was active and independent, working on his farm.

On examination, he was febrile, lethargic, and mildly dehydrated. Cardiovascular, respiratory, abdominal, neurological and dermatologic examinations were unremarkable. There was no lymphadenopathy or organomegaly.

Given the urinary symptoms and background of BPH, an initial diagnosis of urinary tract infection was made. Laboratory investigations showed mild anaemia (Hb 10.3g/dL), leucopenia (WBC $2.2x10^3/\mu L$) and thrombocytopenia (44 $x10^3/\mu L$). Renal and hepatic function tests were within normal limits. Urinalysis and urine culture were negative.

Dengue serology revealed a positive NS1 antigen, with both IgM and IgG initially negative. Intravenous co-amoxiclav was started but discontinued once dengue was confirmed. He received supportive therapy with careful intravenous hydration and close monitoring for bleeding manifestations and plasma leakage.

His platelet count reached a nadir of 26 $x10^3/\mu L$ on Day 3, and his haematocrit declined gradually during the initial part of the admission.

Mild transient transaminitis (ALT 66U/L) was noted, with coagulation profile showing a mildly increased INR of 1.19. The detailed results are shown in Table 1.

Despite thrombocytopenia and mild warning signs such as lethargy and reduced oral intake, he remained haemodynamically stable. He did not develop plasma leakage, mucosal bleeding, organ impairment or shock. He improved clinically and biochemically and was discharged well on Day 10.

Table 1: Blood test results during hospitalization

Parameters	Normal Values	Admit	Day 1	Day 2	Day 3	Day 6	Day 10
White blood cells	4.2-12.6 x10^3/µL	5.4	2.2	3.6	3.1	3.1	4.2
Haemoglobin	13.5-17.9 g/dL	10.3	9.9	9.9	9.5	9.0	9.3
Haematocrit	42-52%	32	31	31	29	28	32
Platelet	174-430 x10^3/µL	192	44	29	26	38	110
Lymphocyte	1.2-7.70 x10^3/µL	1.5	0.3	0.39	0.6	0.6	2.07
C-reactive protein	0.000- 0.500 mg/dL	-	1.3	4.5	2.6	1.4	1.0
Total Bilirubin	3.54-20.5 µmol/L	20	17.5		16.4	26.4	
Alkaline Phosphatase	50-116 U/L	80	49		49	59	
Alanine Transaminase	0-44.9 U/L	21	66		53	43	
Gamma Glutamyl Transferase	0-55 U/L	18	46		39	41	
INR	0.09-1.10		1.19				1.08
Sodium	136-145 mmol/ L	138	137	133	140	141	
Potassium	3.5-5.1 mmol/L	3.8	3.9	3.9	3.5	4.0	
Urea	3.0-9.2 mmol/L	4.8	3.6	3.5	3.5	3.0	
Creatinine	63.6-110.5 µmol/L	73	74	83	73	71	
Bicarbonate	22-29 mmol/L	22	21	21	22	24	
Dengue Serology			NS1 + IgG - IgM -				

DISCUSSION:

This case illustrates the diagnostic complexity of dengue in older adults, whose presentations are often non-specific and easily attributed incorrectly to other conditions, such as a urinary tract infection in this patient. Atypical presentations of dengue are well-documented among older patients, who often lack the classic triad of fever, myalgia or rash, and may instead present with confusion, respiratory or

gastrointestinal symptoms instead [3,7]. Delayed recognition contributes to worse outcomes in this population [4,5].

The NS1 antigen test is a useful test during the early febrile phase because it is detectable from the first day of illness and has a high specificity before IgM antibodies appear [6]. In this patient, while both IgM and IgG were initially negative, NS1 positivity allowed a diagnosis to be made. This case demonstrates that in early dengue infections, IgM and IgG antibodies may be falsely negative.

An important aspect of monitoring dengue infections is the interpretation of haematocrit trends, which is an indicator of plasma leakage during the critical phase. A rising haematocrit ≥20% from baseline reflects haemoconcentration and is an early indicator of potential shock [5, 6]. In our patient, haematocrit was low on admission due to known thalassaemia trait and declined rather than rising during hospitalisation. This is explained by underlying anaemia and dilutional effects of hydration, rather than an absence of severity. Older adults often have reduced bone marrow reserve and chronic anaemia; hence a lack of rising haematocrit does not exclude plasma leakage. Therefore, clinicians must integrate clinical warning signs, platelet trends and fluid balance, rather than relying solely on haematocrit in this age group [8].

Hepatic dysfunction is common in dengue, with modest elevations of AST and ALT. Our patient showed transient ALT elevation, peaking at 66 U/L, consistent with mild viral hepatitis secondary to dengue, which usually resolves as the illness recovers. Older adults are prone to hepatic dysfunction; severe transaminase elevations (>10x ULN) would suggest severe dengue, hypoxic hepatitis or alternative pathology, which were absent in this case [6,8].

Dengue patients may bleed due to thrombocytopenia, hepatic dysfunction and consumption of clotting factors, with severe dengue associated with marked coagulopathy or disseminated intravascular coagulation. Our patient exhibited only a mildly prolonged INR, which normalized on recovery. This finding is more consistent with mild, transient hepatic inflammation rather than clinically significant coagulopathy, supported by the absence of mucosal bleeding or haemodynamic instability [4,7].

Older adults are disproportionately affected by severe dengue. Studies have shown that older adults have higher rates of DHF, severe thrombocytopenia, hypo-albuminaemia, organ failure, prolonged hospitalisation and mortality [7-10]. Hypertension, chronic obstructive pulmonary disease, hypoalbuminaemia, renal impairment and hyperpyrexia have been identified as predictors of severe disease [5,10]. Severe organ impairment, especially acute

kidney injury and cardiac dysfunction, are dominant modes of severe dengue in older adults. In our patient, the favourable outcome reflects his low comorbidity burden, stable renal and hepatic function, absence of high-risk features, and early hospital presentation.

Management of dengue in older adults requires careful fluid titration. Excess intravenous hydration during the period of plasma leakage may result in pulmonary oedema, particularly in those with cardiac comorbidities, while inadequate fluids may lead to shock. Older adults also exhibit more frequent metabolic abnormalities, including hyponatremia, hypoglycaemia and hypocalcaemia, which may contribute to encephalopathy or atypical neurological symptoms [11].
Thus, close clinical observation and serial monitoring is essential.

This case illustrates several learning points: dengue should be considered in older adults presenting with non-specific febrile illness, especially when cytopenias are present. NS1 antigen testing is useful in the early febrile phase. Haematocrit interpretation must be contextualised, especially in those with baseline anaemia.

Finally, older adults require careful monitoring even in the absence of overt warning signs.

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A REVIEW:

BEYOND REPRODUCTION: A COMPREHENSIVE REVIEW OF ESTROGENIC ACTIONS IN THE MAMMALIAN BRAIN

Running title: Role of estrogens in the brain

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ABSTRACT

For decades, estrogens have been regarded as reproductive hormones. However, research in the last few years has altered this perspective. It has been shown that estrogen signaling plays a widespread and crucial role in regulating brain function throughout life in both men and women. This review summarizes recent studies from 2005 to 2025 on how estrogens work in the brain, including their mechanisms, targets, and effects. We outline where nuclear estrogen receptors (ERα, ERβ) and membrane-associated receptors (like GPER1) are found in the brain. We also highlight their roles in synaptic plasticity, adult neurogenesis, inflammation and energy use. We examine the protective and cognitive benefits of estrogens, particularly regarding neurodegenerative diseases like Alzheimer's, psychiatric issues such as depression, and brain injuries. We emphasize the importance of timing, dosage, and hormonal context within the "window of opportunity" or "critical period" hypothesis. Recent updates to hormone replacement therapy (HRT) guidelines highlight the brain health benefits of starting treatment early. Finally, we address challenges and future directions, including the development of brain-selective estrogen receptor modulators (SERMs) and tailored approaches that account for sex differences and genetic factors. The evidence clearly shows that estrogens are powerful neuroactive steroids that are vital for brain health and resilience.

Keywords: estrogen, estradiol, estrogen receptors, neuroprotection, synaptic plasticity, neurogenesis, cognition, menopause, Alzheimer's disease.

Methods: This review was conducted by systematically searching PubMed, Scopus, and Google Scholar databases using keywords such as "estrogen brain," "neuro-estrogen," "estrogen receptors," "neuroprotection," "synaptic plasticity," "neurogenesis," "cognition," "menopause," and "Alzheimer's disease." We focused primarily on peer-reviewed articles published between 2005 and 2025. Studies involving cellular, animal, and human models that discussed how estrogens work in the brain were included. We excluded non-English

publications and studies that lacked rigorous methods. More than 150 articles were screened, and 63 were selected for review. We critically evaluated the data for consistency, quality, and relevance, with special attention to recent advances to fill gaps in earlier research.

INTRODUCTION

Estrogens, especially 17β-estradiol (E2), have been traditionally defined as steroid hormones that control the development and function of the female reproductive system. The discovery of estrogen receptors (ERs) in various brain regions outside the usual hormonal control areas, like the hippocampus and prefrontal cortex, has revealed a much broader role for these hormones [1]. The last two decades have seen a surge of research in this area, driven by advancements in molecular and genetic tools, the recognition of significant differences between sexes in neurological and psychiatric disorders [2] and increased interest in women's brain health during menopause.

This wave of research has transformed our understanding of estrogens from being solely reproductive signals to recognizing them as essential neuroactive steroids that significantly influence brain structure and function throughout life for both males and females [3]. Estrogens can affect synaptic plasticity, mood, energy metabolism, provide neuroprotective effects, and support cognitive processes such as learning and memory [4]. Estrogens have been shown to modulate neuroinflammation via pathways such as PI3K/Akt and NF-κB, with important implications for aging and neurodegeneration [5-7]. This review aims to consolidate and evaluate key findings from peer-reviewed literature published between 2005 and 2025. By synthesizing evidence from cellular, animal, and human studies, the article will show that estrogen actions are crucial for brain development, balance, and resilience, affecting our understanding and treatment of various brain disorders.

Estrogen Synthesis and Receptor Systems in the Brain

Central Synthesis: The Critical Role of Neuroestrogen.

A groundbreaking discovery in the last twenty years is that the brain can produce estrogen. Neurons, astrocytes, and, to a lesser extent, microglia have the enzyme aromatase (CYP19A1), which converts testosterone into 17β-estradiol (E2) [8,9]. This local production, called "neuroestrogen," enables rapid, paracrine, sex-independent regulation of brain function. The control of aromatase in the brain differs from that in other tissues; it often increases in response to neural injury or synaptic activity, suggesting a role in neuroprotection and plasticity [10].

Neuroestrogen is important in its functional implications. E2 produced by the brain in males due to circulating testosterone is crucial in sexual differentiation and neural development of the brain, as well as adult cognitive ability [11].

Treatment with aromatase inhibitors results in impairment in spatial memory, synaptic plasticity, and male sexual behavior in rodents [12]. In females, local synthesis may help to adjust neural circuits during fluctuations in ovarian hormones across the menstrual cycle and throughout life. Research in songbirds, where neuroestrogen plays a clear role in learning and producing seasonal songs, has offered valuable insights into its mechanisms [13]. Experiments in mammals using neuron-specific aromatase knockout (ArKO) models have demonstrated that neuroestrogen is essential for synaptic plasticity, neurogenesis, and memory consolidation in the hippocampus [14,15]. Also, studies have highlighted sex differences in neuroestrogen synthesis. Males have higher aromatase levels in specific brain areas, influencing their behavior [16]

Estrogen Receptor Diversity and Signaling Mechanisms:

Nuclear Receptors (Classical Genomic Signaling): ERα and ERβ are transcription factors activated by E2 that belong to the nuclear receptor superfamily. The binding of E2 in the cytoplasm causes a conformational change and moves to the nucleus, where it attaches to specific DNA sequences called Estrogen Response Elements (EREs). This process regulates the transcription of target genes over hours to days [17]. They can also have "non-classical" effects by interacting with other transcription factors (e.g., AP-1, NF-κB, CREB) without directly binding on DNA. ERβ is particularly abundant in regions like the cortex, hippocampus, and serotonergic neurons, linking it to cognition, mood, and neuroprotection [18]. While ERα is present in these areas, its role is more prominent in hypothalamic regions that control reproductive behaviors.

Membrane-Associated Receptors and Rapid Non-Genomic Signaling:

A key advancement in the field has been identifying rapid estrogen effects (within seconds to minutes) on kinase activity, calcium movement, and synaptic strengthening. These effects come from receptors located at the plasma membrane or associated with membrane areas like caveolae.

GPER1 (GPER, GPR30):

Discovered in 2005, GPER1 is a seven-transmembrane G-protein-coupled receptor that binds E2 with high affinity [19]. It is found throughout neurons and glia in the brain. When activated, it quickly causes intracellular calcium changes and activates essential neuroprotective signaling pathways like PI3K/Akt and MAPK/ERK [20]. GPER1 is recognized as a major mediator of estrogen's rapid effects on synaptic plasticity, neuroprotection, and behavior [21]. GPER1 is known to be involved in cognitive functions, neuroinflammation and psychiatric disorders, showing promise for therapeutic uses [19].

Membrane-Initiated Steroid Signaling (MISS): Some classical ERα and ERβ can be modified to help them move to the plasma membrane [22]. These localized ERs interact with metabotropic glutamate receptors and other proteins to activate similar secondary messenger pathways (e.g., Src/ERK, PI3K) as GPER1. The MISS pathway is crucial for the rapid formation of dendritic spines in the hippocampus [23].

Estrogen-Related Receptors (ERRs):

The orphan receptors ERRα, ERRβ, and ERRγ share similarities with ERs but do not bind natural estrogens. They are highly expressed in metabolically active tissues, including the brain, where they act as transcription factors that regulate genes involved in creating energy and metabolism [24]. Their interaction with coactivators like PGC-1α positions them as significant regulators of neuronal energy use, which is increasingly tied to the neuroprotective effects of estrogen [25].

This complex array of receptors and signaling mechanisms enables estrogens to modulate brain functions, from the quick adjustments of synaptic activity to guiding long-term changes in gene expression that reshape neuronal structure and resilience.

Fundamental Neurobiological Processes that are under the control of Estrogens:

Synaptic Plasticity and Spine Dynamics

Estrogen consistently boosts strength and lowers the threshold for LTP in the CA1 region of the hippocampus [26]. This effect involves a coordinated mechanism. Rapid signaling through mERα/β or GPER1 enhances NMDA receptor activity and increases the surface expression of AMPA receptors, specifically GluA1 subunits, which improves the responsiveness of the postsynaptic neurons [27, 28]. At the same time, genomic actions via nuclear ERs lead to an increase in the transcription of NMDA receptor subunits (GluN1, GluN2B), synaptic scaffolding proteins like PSD-95, and proteins involved in spine formation [29].

Furthermore, a striking finding is that E2 can cause a rapid (within 30-120 minutes) and significant (30 - 50%) increase in dendritic spine density on CA1 pyramidal neuron apical dendrites [30,31]. This effect is robust and linked to fluctuations during the estrous cycle in rodents. The molecular cascade involves ER membrane activation of the Rho GTPase/Rho kinase/LIM-kinase pathway, which inactivates the actin-depolymerizing protein cofilin, leading to rapid actin polymerization and spine formation [32]. Additionally, E2 activates local dendritic protein synthesis through the mTOR and ERK pathways, providing the necessary components for new synapses [33].

Also, estrogens finely adjust the E/I balance within neural circuits. They can reduce inhibitory tone by lowering glutamic acid decarboxylase (GAD67) expression in specific interneurons

[34]. and by altering the composition of GABA receptor subunits. At the same time, they enhance excitatory activity by increasing NMDAR activity. This shift decreases inhibition while increasing excitation, creating a neural network state that is very conducive to plasticity and information processing [35].

Adult Neurogenesis:

The hippocampus can produce new neurons throughout life, in a process known as adult neurogenesis. Estrogens are important positive regulators of this process in the sub-granular zone of the dentate gyrus, with recent evidence showing age- and sex-specific modulation. Systemic or intrahippocampal administration of E2 increases the proliferation of neural progenitor cells and improves the survival of newly formed neurons [36,37]. This effect mainly occurs through ERα and ERβ, which are found on progenitor cells. E2 signaling promotes cell cycle progression and prevents cell death through the activation of survival pathways, such as PI3K/Akt [38]. Beyond increasing cell numbers, E2 supports the maturation and functional integration of new neurons into existing hippocampal circuits. It speeds up dendritic branching and the expression of mature neuronal markers [39]. This process is essential for certain types of memory that rely on the hippocampus, especially for distinguishing between similar experiences [40]. The drop in circulating E2 levels during menopause and aging is linked to a marked decline in markers of adult neurogenesis in animal models [41]. Exogenous administration of E2 in these models can reverse this decline. This suggests a direct relationship between hormonal loss and reduced neural flexibility in aging [6]. While measuring neurogenesis in living humans is still challenging, this connection provides insights into cognitive changes related to menopause.

Neuroprotection, Anti-Inflammation and Bioenergetics:

Estrogen is a potent inhibitor of cell death. It activates the PI3K/Akt and MAPK/ERK survival pathways, which leads to the phosphorylation and inactivation of pro-apoptotic factors like Bad and caspase-9, while increasing the expression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL [42]. This mechanism is critical in stroke models, traumatic brain injuries, and neurodegenerative diseases. E2 and its non-feminizing metabolites (e.g., 2-hydroxyestradiol, 2-methoxyestradiol) have inherent antioxidant properties. The phenolic A-ring of estradiol can directly neutralize reactive oxygen species (ROS) and lipid peroxides [43]. Furthermore, E2 increases the expression and activity of natural antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase, which strengthens the brain's defense against oxidative stress, an important factor in aging and neurodegeneration [44].

Similarly, when microglia and astrocytes, which serve as the immune cells in the brain, are activated, they release pro-inflammatory

cytokines (TNF-α, IL-1β, IL-6), chemokines, and neurotoxic factors. Estrogens primarily act through ERα and GPER1 to exert significant anti-inflammatory effects. They suppress microglial activation, stop the nuclear translocation of NF-κB (a major regulator of inflammation), and encourage a shift towards a protective, anti-inflammatory state in microglia [45,46]. These actions are particularly relevant to Alzheimer's disease, Parkinson's disease, and multiple sclerosis, which all involve significant neuroinflammation.

Neurons are highly demanding on metabolism. Estrogens help maintain neuronal energy levels by improving mitochondrial function. They boost mitochondrial respiration, ATP production, and the expression of components in the mitochondrial electron transport chain [47]. E2 also enhances glucose transport into neurons and astrocytes by increasing glucose transporters (GLUTs) and boosts aerobic glycolysis [48]. By ensuring that neurons have the energy capacity to meet demand and resist stress, estrogen signaling is a vital part of neural resilience.

Summarily, this review synthesizes emerging evidence that estrogens, besides their reproductive roles, are important neuroactive steroids that regulate synaptic plasticity, adult neurogenesis, inflammation and energy use in both sexes. In the same vein, the benefits of estrogens, which depend on timing and dosage, are critical determinants of their neuroprotective efficacy in brain injuries, psychiatric disorders and Alzheimer's diseases. While some challenges have been addressed, the development of brain-selective estrogen receptor modulators (SERMs) holds a promising future direction in addressing genetic and gender differences in treatment response.

Functional Outcomes and Translational Implications:

Cognition and Memory

There is strong clinical and preclinical evidence that supports the critical (though complicated) effect of estrogens on memory, which relies on the hippocampus and prefrontal cortex. For instance, the performance of the verbal memory and executive function tasks during the follicular phase among healthy premenopausal women is positively related to the endogenous levels of E2, and this phenomenon reflects less cognitive dysfunction [49]. Bilateral ovariectomy in the absence of estrogen treatment is equally linked with faster brain aging and the two-fold risk of impaired cognitive ability or dementia in old age. Such risks are mitigated with estrogen replacement that starts at the surgical menopause [50].

The Women's Health Initiative Memory Study (WHIMS) contributed greatly to the study of the effects of Estrogen on cognition. The study established that women between 65 and 79 years who initiated oral conjugated equine estrogens (CEE) with or without medroxyprogesterone acetate (MPA) were at increased risk of experiencing dementia and

mild impaired thinking [51]. This unexpected finding, even with a large body of preclinical neuroprotection evidence, prompted the formulation of the "window of opportunity/critical period" hypothesis [52,53]. The hypothesis is that brain functioning and maintenance can be defended by estrogen therapy at perimenopause or early postmenopause, before age 60 or within 5-10 years of menopause. Late treatment initiation when extensive age- or disease-induced changes in the brain, such as increased Ab deposition or cerebrovascular complication may exist, may be both ineffective and even detrimental. Animal experiments support this timeline, with estrogen protection following ovariectomy being more effective in middle-aged rats than in those treated later [54]. According to the Kronos Early Estrogen Prevention Study (KEEPS), early initiation of transdermal estrogen (at early menopause) could have a beneficial impact on cognitive outcomes [55]. Recent changes to the hormone replacement treatment (HRT) have suggested that hormone replacement should be initiated during the period age 10 years after menopause or prior to age 60 to achieve maximum cognitive advantages as well as have black-box warnings on timing removed.

Affective and Neuropsychiatric Disorders

Depression:

High E2 variations or declines are associated with high risks of depressive episodes in vulnerable women such as during the postpartum, perimenopausal, and premenstrual phases [56]. Mechanistically, E2 promotes serotonergic activity by increasing tryptophan hydroxylase production, the rate-limiting enzyme in 5-HT synthesis, regulation of 5-HT1A and 5-HT2A receptor expression and activity and decreasing the activity of monoamine oxidase, which breaks down monoamines [57]. Transdermal E2 has good monotherapy in PMD as well as adjunctive therapy [58], and the neuroprotective effect of estrogen has infinite opportunities to stimulate brain health, resilience, and cognitive longevity across the life span in both genders.

Schizophrenia:

Epidemiological studies indicate that sex disparity in schizophrenia is large. The diseases of women are less severe in nature but more emotionally severe, late onset and second peak incidence after 45. Gogos reported that the symptoms tend to be more severe when estrogen levels are low, after giving birth, and around the menstrual cycle [59]. It is considered that estrogen may protect by improving the efficiency of prefrontal cortex and regulating dopamine, resulting in a reduction of striatal hyper-dopaminergia. Some randomized controlled trials have found that adjuvant transdermal estradiol or selective estrogen receptor modulators, including raloxibine, can reduce positive and negative symptoms in women with schizophrenia when used in

conjunction with regular antipsychotic medication [60].

Neurodegenerative Diseases Alzheimer's Disease (AD):

Estrogens affect all the key pathogenic characteristics of AD. They stimulate the non-amyloidogenic cleavage of amyloid precursor protein (APP) via a-secretase pathway (ADAM10), thereby reducing the production of neurotoxic amyloid-β peptides [61]. They inhibit the hyperphosphorylation of tau that causes the development of neurofibrillary tangles by blocking the action of kinases like GSK-3β [62]. They also help with mitochondrial functionality and synaptic stability, as well as decreasing neuroinflammation. One of the biological risk factors of AD is E2 depletion, which is seen in the substantially higher rates of AD prevalence among postmenopausal women, despite the longer lifespan of females [63]. This is most evident in the critical period hypothesis, which is why the trials of hormone therapy in older women with probable AD have failed, though observational studies of early-initiating women have demonstrated a reduction of risk.

CONCLUSION

Within the past two decades, research has shown that the range of estrogen activity in the brain is significantly wider than reproductive neuroendocrinology. Initially, estrogens were not complex; however, various pleiotropic modulators participated in the activities of synaptic plasticity, cellular resilience, neuroimmune mechanisms, and energy homeostasis. These caused a complex program of genomic and non-genomic signaling that enhanced cognitive processes, buffered affective disorders, and highly secured the central nervous system against various insults. It is one of the enormous tasks of our reality to safely, effectively, and feasibly translate these convincing preclinical results into clinical therapy of neurodegenerative diseases, psychiatric disorders, and brain injury. This receptor-specific knowledge of the effects of estrogen and their relative significance and further development of new neural-targeted ligands offers a viable and rational future path. In fact, there is no better way of ensuring that the brain can expect health, resistance, and cognitive longevity, particularly among women and men of all age groups, than to harness this potent endogenous neuroprotective system.

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COMMENTARY:

CLINICAL INTERPRETATION OF THE UNITED STATES DIETARY GUIDELINES FOR AMERICANS 2025-2030: IMPLICATIONS FOR OLDER ADULTS

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INTRODUCTION:

The United States Dietary Guidelines for Americans (DGA) are among the most influential national nutrition guidelines globally and are frequently referenced in clinical practice, education, and research outside the United States [1]. Although designed as population-level guidance, they often inform individual dietary advice. This Clinical Note provides a brief overview of key recommendations in the updated DGA, highlights aspects particularly relevant to older adults, and discusses practical and methodological considerations relevant to geriatric practice.

Overview of key dietary guideline recommendations:

The 2025–2030 DGA maintains a life-course approach to nutrition and emphasizes overall dietary patterns rather than isolated nutrients. Core recommendations include consumption of nutrient-dense foods such as vegetables, fruits, whole grains, protein foods, dairy or fortified alternatives; limitation of sodium intake to less than 2,300 mg per day for most adults; limitation of saturated fat to less than 10% of total energy intake; and reduction of added sugars [1].

Protein intake is highlighted more explicitly, with recommended targets of approximately 1.2 – 1.6 g/kg/day. The guidelines also strongly discourage consumption of highly processed

foods, particularly those high in salt and added sugars and low in nutrient density. Full-fat dairy products are considered acceptable when unsweetened, reflecting a shift from earlier guidance that preferentially promoted low-fat or fat-free options. In practice, this requires distinguishing between occasional inclusion of saturated-fat-containing foods within a balanced diet and routine consumption that may increase cardiovascular risk.

Clinical relevance for older adults:

Although the DGA is not specifically designed for older populations, several recommendations are directly relevant to ageing and geriatric care.

Protein intake and muscle health:

Sarcopenia and frailty are prevalent among older adults and associated with increased risk of falls, disability, hospitalization, and mortality [2]. Previous editions of the DGA acknowledged the importance of adequate protein intake but did not specify higher targets for older adults. This current emphasis aligns with evidence suggesting that older adults may require greater protein intake than younger adults to preserve muscle mass and physical function, particularly in the context of chronic disease, acute illness, or reduced physical activity [3].

Achieving higher protein targets may be challenging due to reduced appetite, comorbidity, functional limitations, socioeconomic factors, and dietary preferences [4]. In clinical settings, this requires a pragmatic food-based approach, prioritizing the inclusion of protein at each meal using familiar and acceptable foods (e.g. eggs, dairy, legumes, fish, and soft-textured options). Protein advice should therefore be individualized and integrated into usual eating patterns.

Energy intake and nutritional adequacy:

Age-related changes in appetite, taste, dentition, gastrointestinal function, and functional ability may compromise dietary intake in older adults [4]. Acceptance of full-fat dairy products when unsweetened may support adequate energy and protein intake in individuals at risk of undernutrition, particularly where appetite or meal volume is limited.

There is increasing recognition that the health effects of foods, particularly dairy products, depend not only on nutrient composition but also on the overall food matrix and processing methods such as fermentation. These factors influence digestion and metabolic responses and may explain why whole or fermented foods differ in their health effects from isolated nutrients with similar fat content [5]. This supports emphasising

whole, minimally processed foods rather than focusing solely on individual nutrients.

Carbohydrate-rich foods, including whole grains and starchy vegetables, remain important sources of energy and are associated with positive health status in older adults [6]. Thus, protein-focused recommendations should not displace carbohydrate foods that contribute substantially to total energy intake and dietary fiber.

Diet quality and food processing:

Diets high in ultra-processed foods are often energy-dense but nutrient-poor. In older adults, reliance on such foods may worsen malnutrition risk and contribute to poorer functional outcomes [7]. Accordingly, the recommendation to limit highly processed foods aligns with geriatric nutrition principles that prioritize nutrient density, adequacy, and simplicity of meals.

Sodium intake:

Sodium restriction remains a consistent feature of successive DGAs and is clinically relevant given the high prevalence of hypertension and cardiovascular disease in older adults [8]. In geriatric practice, sodium reduction must be balanced against risks of reduced appetite, poor palatability, and inadequate intake. For frail or institutionalised individuals, maintaining food enjoyment and sufficient intake may take priority over strict sodium targets.

Clinical caveats and methodological considerations:

Despite their relevance, DGA recommendations should be applied cautiously to older adults. The guidelines are population-level tools intended to inform public health policy rather than individualized clinical care [9]. Higher protein targets may not be appropriate for all individuals, particularly those with advanced chronic kidney disease or specific metabolic conditions. Guidance permitting inclusion of saturated-fat–containing foods requires careful interpretation. Clinicians should clarify that such foods may be included occasionally and in small amounts within an otherwise balanced diet, rather than consumed regularly or in place of unsaturated fat sources.

Cost, access, and food insecurity may also limit the dietary approaches that emphasize increased protein intake for some older adults, reinforcing the need for flexible advice that incorporates affordable, nutrient-dense foods. Communicating these recommendations to the public may also present challenges, as concepts such as food matrix effects and context-dependent dietary risk require clinical interpretation to prevent oversimplification or misapplication.

Concerns regarding potential bias are best understood as methodological rather than ideological. The DGA relies heavily on observational evidence and intermediate cardiometabolic outcomes, reflecting the challenges of long-term nutrition trials. Outcomes of particular relevance to ageing populations, such as physical function, cognitive decline, and disability-free survival, also remain underrepresented in the evidence base [10].

CONCLUSIONS:

The 2025–2030 United States Dietary Guidelines incorporate several updates that may be clinically meaningful for older adults, particularly in relation to protein adequacy, dietary quality, and prevention of undernutrition. However, the population-wide framing requires careful, individualized application in geriatric practice. Clinicians should interpret the guidelines as a flexible framework rather than a prescriptive standard, adapting recommendations to individual functional status and nutritional risk.

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Acknowledgements:

The following should be acknowledged: Research or other financial grants; Material support, Contributions of Institutions, Colleagues, and other relevant participants.

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