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Welcome to the April 2025 edition of *The Tropical Journal of Health Sciences*. I am pleased to announce that the journal is now indexed in EBSCO, thereby increasing the visibility and global reach of our academic content. This issue presents an array of compelling research articles and reviews addressing key public health and clinical challenges.

One of the featured studies, *Prevalence of Hepatitis B Virus Infection among Attendees of Antenatal Care Clinics at Primary Health Care Centres in North-Central Nigeria*, highlights the burden and associated risk factors of HBV infection among pregnant women. Another insightful contribution, *Assessment of Prescription Quality and Incidence of Drug Therapy Problems among Patients in Selected Primary Health Facilities in Maiduguri Metropolitan Area, Borno State*, evaluates the quality of drug prescriptions and identifies prevalent drug therapy problems (DTPs), including inappropriate drug selection, untreated conditions, unnecessary drug therapy, and polypharmacy.

The study titled *A Survey of Knowledge and Perception of Surrogacy among Health Care Providers in Selected Institutions in Ilorin* presents critical findings on the awareness, knowledge, perceptions, and attitudes of health workers toward surrogacy. In the experimental research, *Newbouldia laevis Enhanced Bcl-2 Expression and Germinal Epithelial Proliferation in Adolescent Wistar Rats*, the effects of varying doses of *Newbouldia laevis* and zinc on male fertility were assessed. The study demonstrated that high doses of *Newbouldia laevis* impaired semen parameters, notably sperm motility and population, regardless of treatment duration.

The study, *Sub-Acute Toxicological Evaluation of an Artemisia-Based Moringa Supplement (AMS)*, investigates the toxicological impact of 30-day administration of AMS in male and female albino Wistar rats. The findings suggest that the supplement is relatively safe at the administered doses. The article titled *Ameliorative Effects of Nigella sativa Oil on Permethrin-Induced Neurotoxicity in the Cerebellum of Male Wistar Rats* evaluates the neuroprotective potential of *Nigella sativa* oil. The study demonstrated that it significantly enhanced glutathione peroxidase (GPx) activity and reduced tumour necrosis factor-alpha (TNF- α) levels, indicating its efficacy in mitigating oxidative stress and inflammation caused by permethrin-induced neurotoxicity.

The review article, *Review of Plasmodium vivax Malaria in Indonesia and Malaysia: Epidemiology of the Infection and the Presence of the Vector Across Time*, examines the historical and current trends in the epidemiology and entomology of *P. vivax* malaria across both countries, offering insights into disease dynamics and control efforts over time.

This edition of *The Tropical Journal of Health Sciences* offers a diverse and impactful selection of original research and review articles that not only expand existing knowledge but also underscore important clinical and public health implications. We invite you to explore these thought-provoking contributions and encourage continued research and scholarly dialogue inspired by their findings.

Prof. M.M.B. Uthman
Editor-in-Chief
Tropical Journal of Health Sciences

Prevalence of Hepatitis B Virus Infection among Attendees of Antenatal Care Clinics at Primary Health Care Centres in North-Central, Nigeria

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Abstract

Background: Hepatitis B virus (HBV) is a *Hepadnavirus* that infects liver cells, and causes an infectious disease of global significance. Several factors predisposes to HBV infection, some of these are peculiar to pregnant women, and infection in them could lead to maternal to child transmission of the disease.

The main objective of this study was to assess the prevalence and risk factors for hepatitis B Virus infection among pregnant women attending antenatal clinic (ANC).

Material and Methods: This hospital based cross-sectional study was conducted among 340 pregnant women attending antenatal care between February and March 2024. The respondents were selected using multistage sampling technique and interviewer administered semi-structured questionnaires were used to obtain information from them, after which blood samples were collected for HBsAg detection. Data was collated and analyzed using the Statistical Product and Service Solutions version 28 (SPSS-28).

Results: Respondent's age ranged from 18 to 44 years with a mean of 25 ± 5.45 years. The majority were between 20-24 years of age. The prevalence of HBV infection in the study was 12.6%. The factors found to have a statistically significant association with prevalence of HBV infection were: age, family type, previous blood transfusion, multiple sexual partner, previous hospital procedure, having a spouse with HBV infection, history of abortion, previous check of HBV status and previous result of HBV infection status.

Conclusion/Recommendations

The prevalence of HBV infection is increasing in our community and certain factors have been identified as determinants. There is a need to address risk factors. More efforts needs to be put towards preventive strategies such as increased awareness and routine screening among others. These will help to ensure adequate prevention and control of the disease.

Keywords: *Hepatitis B Virus, pregnant women, Infection, prevention.*

Background

Hepatitis is an inflammation of the liver that is caused by a variety of infectious agents (i.e., viral, bacterial, fungal, and parasitic organisms) and noninfectious agents (e.g., alcohol, drugs, autoimmune diseases, and metabolic diseases) leading to a range of health problems, some of which can be fatal.¹ There are five main strains of the hepatitis virus, referred to as types A, B, C, D and E. While they all cause liver disease, they differ in important ways including modes of

transmission, severity of the illness, population affected and prevention methods.² In particular, types B and C lead to chronic disease in hundreds of millions of people and together are the most common cause of liver cirrhosis, liver cancer and viral hepatitis-related deaths.¹ Horizontal transmission is possible in children, families and 'close personal contact'. Vertically transmission occurs perinatally from a carrier mother to her baby, through the placenta and during delivery.³

An estimated 3.6% of the global population is affected by chronic HBV infection.⁴ In Cameroon, the prevalence of HBV among pregnant women were 5.7% and 7.5% in two health care facilities.⁵ Nigeria accounts for 8.3% of the global burden of chronic HBV infection.⁶ A meta-analysis in Nigeria reported a prevalence of 14.0% among blood donors and 14.1% among pregnant women attending antenatal clinics.⁷

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Studies in Abuja and Ilorin Nigeria, among the general population revealed prevalence of 12.9% and 10.9% respectively.^{8,8} About 10% of pregnant women who are positive for HBsAg will become chronic carriers, and 10% of these chronic carriers will develop cirrhosis and hepatocellular carcinoma.¹⁰

In highly endemic areas, HBV is most commonly spread from mother to child at birth from exposure to maternal blood and secretions at delivery, or from person to person in early childhood.¹¹ In areas of low endemicity, perinatal or early childhood transmission of HBV may be responsible for over a third of chronic infections. Transmission early in life is also associated with a higher risk of (lifelong) chronic infection.¹¹ As a result of the poor screening and low vaccination rate, the vertical transmission of HBV has become a major route of transmission of the virus. This leaves the children born at risk of developing chronic liver disease later in life.¹² It is therefore important that the most effective interventions to prevent mother-to-child transmission (MTCT) of HBV are identified and utilized. In-utero transmission is relatively rare and is not the major means of transmission of HBV from mothers to infants, although it may occur if intrauterine placental leakage arises as a result of threatened preterm labour.

A number of risk factors have been shown to be predictors of HBV carrier state in the general population and these include ethnicity, history of sexually transmitted infections (STI), blood transfusion, hepatitis exposure and occupational exposure.¹³ Risk factors identified in pregnant women include: multiple sexual partners, early age at sexual debut, past history of STI, and poor knowledge of HBV infection.^{14,15} A study in South-Western, Nigeria among pregnant women revealed that multiple sexual partners (MSP) and early age at sexual debut were independent risk factors for HBV infection.¹⁶

This study set out to determine the prevalence of hepatitis B Virus infection among pregnant women attending antenatal care (ANC) clinics at primary health care (PHC) centers in Kwara central zone and to identify risk factors associated with HBV infection among them.

Methodology

Description of the Study Area

The study was done in Kwara central zone, Kwara State, Nigeria. The projected population of the state based on 2006 census is 3,438,079. There are PHC facilities located within the local governments in Kwara Central where ANC take place along with provision of other health care services. Routine ANC takes place at PHCs in Kwara central daily except

weekends. The prevalence of HBV infection of 11.2% was found in a cross sectional study among pregnant women in Kwara State.³

The study was a descriptive cross-sectional study carried out using quantitative method of data collection. This involved questionnaire administration and sample collection. The study population comprised of pregnant women 18 years and above, attending ANC clinics at PHC centers in Kwara Central senatorial district. Very sick pregnant women who were not able to answer the questionnaire were excluded.

Sample Size Determination

The sample size was calculated using Fisher's formula:

$$n = z^2 pq/d^2$$

Final sample size calculated was **340**.

Sampling technique

Multistage sampling technique was used.¹

Stage 1: Selection of local government areas (LGAs): Two LGAs (Ilorin East and Asa) were randomly selected from the four LGAs in Kwara Central zone using simple random technique (balloting).

Stage 2: Selection of PHC centre: From each of the two LGAs selected, two PHC centres (one rural and one urban) were selected from the twenty and twelve PHC in Ilorin East and Asa respectively using simple random technique (balloting). Proportional allocation was then used to distribute the sample size across the health facilities.

Stage 3: Selection of Respondents: at the PHC facilities, systematic random sampling technique was used to select patient after a list daily clinical attendance of the patients in the clinic was obtained and used as the sampling frame. Double sampling was prevented by putting an identifier on the folder. Also regular data cleaning and validation was done to identify and remove any duplicate entries or potential cases of double sampling.

Proportional allocation to calculate sample size for each health facility.

Using proportional allocation: 120 participants were recruited from Okelele health centre, 55 from Oke Oyi Health, 85 from Afon District Health Centre and 80 from Ogele PHC Afon.

$$\text{Sampling interval} = \frac{\text{Population size}}{\text{Sample size}}$$

$$\text{Sampling interval} = 1840$$

340=5.4. i.e. 6

Hence, sampling interval of six was used at the four facilities.

Samples were collected between February and March 2024.

Data Collection Instrument

The data was collected using the following instruments:

- 1) Semi-structured interviewer administered questionnaires
- 2) Hepatitis B Surface Antigen Test Strip (by Atlas Link Biotech Co., LTD USA)

The questionnaire was structured to have four sections: Section A was used for respondent's socio-demographic data. Section B was used to collect data on obstetrics and gynaecology history while section C was used to collect data on the potential risk factors associated with HBV infection. Section D was used to document the respondent's hepatitis B status.

Hepatitis B Surface Antigen (HBsAg) Detection

Blood sample (4 ml) was collected aseptically by the researcher from all participants from the ante-cubital vein, using sterile plastic disposable syringes. The sera collected was tested for HBsAg using NOVA test® HBsAg serum strip Kit manufactured by Atlas Link Biotech Co., LTD USA, in the laboratory at the facilities within 15-30 minutes of sample collection. The relative sensitivity and specificity of the rapid test kit have been found to be greater than 99.9% and 99.6% for serum or plasma specimens; and greater than 99.9% and 99.5% for whole blood specimens.^{17,18}

Preparation for Data Collection, Pretesting and Data Collection Procedure

Advocacy visits were paid to the hospitals of the selected local governments, intimating them with the research and soliciting for their support. The tool of data collection was pretested using 10% of the sample size, at Offa LGA.

Four trained research assistants participated in the data collection process. A detailed explanation as to the purpose of the study was given to all the eligible respondents. Data collection was done within 8 weeks.

Data Analysis

Data collected was checked daily for correctness and completeness. Then it was coded and analyzed using statistical package for social science (SPSS version 28.0). Inferential statistics to test for associations between categorical variables was done using Chi-square test. Summary of the statistics has been presented using frequency tables, charts, means and rates. Logistic regression analysis was done to identify independent factors for HBV infection. Level of

statistical significance was set at $p < 0.05$ at 95% confidence level.

Ethical Approval and consent to participate

Ethical approval was obtained from the Ethical Review Committee of Faculty of Clinical Sciences, University of Ilorin.

Results

A total of 340 pregnant woman participated in the study. Respondent's age ranged from 18 to 44 years with a mean of 25 ± 5.45 . In figure 1, forty-one of the respondents tested positive for HBV infection, thus giving a prevalence rate of 12.6%. As shown in table 2a, 112 (32.9%) of respondents were aware of HBV infection. About 77 (22.6%), 147 (43.2%) and 8 (2.4%) had tonsillectomy, scarification arks and tattoo in the past respectively.

Table 1: Socio-demographic characteristics of respondents n=340

Variables	Frequency (%)
Age groups (years)	
< 20	25 (7.4)
20 – 24	126 (37.1)
25 – 29	106 (31.2)
30 – 34	48 (14.1)
35 – 39	28 (8.2)
≥ 40	7 (2.0)
Mean ± SD	25 ± 5.45
Range	18 – 44
Ethnicity	
Yoruba	326 (95.9)
Igbo	3 (0.9)
Hausa	4 (1.2)
Others	7 (2.1)
Marital Status	
Married	303 (89.1)
Separated/Divorced/Widowed/Never Married	37 (10.9)
Type of marriage	N=303
Monogamy	269 (88.8)
Polygamy	34 (11.2)
Level of education	
Tertiary	70 (20.6)
Secondary	214 (62.9)
Primary	36 (10.6)
No formal education	20 (5.9)
Occupation	
Unemployed	21 (6.2)
Self employed	145 (42.6)
Civil servant	120 (35.3)
Others ^a	54 (15.9)
Income in one month^b	n=312
≤ 30,000	210 (67.3)
> 30,000	102 (32.7)
Family type	
Nuclear	178 (52.4)
Extended	162 (47.6)

^a Including private company workers, labourers

^b The cut-off of thirty thousand naira per month was based on the national minimum wage

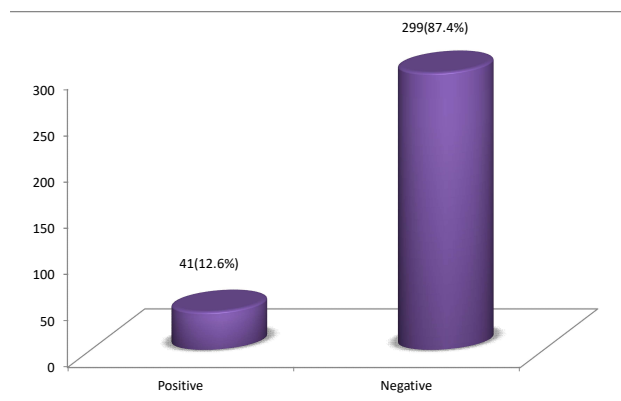


Figure 1: Prevalence of HBV Infection.

Table 2a: Potential risk factors of HBV infection among pregnant women n=340

Variables	Frequency (%)
Aware of the infection viral HBV	
Yes	112 (32.9)
No	228 (67.1)
Past history of Local Tonsillectomy	
Yes	77 (22.6)
No	263 (77.4)
History of Local Scarification	
Yes	147 (43.2)
No	193 (56.8)
Tattoo	
Yes	8 (2.4)
No	332 (97.6)
Previous blood transfusion	
Yes	10 (2.9)
No	330 (97.1)
Number of blood transfusion	n=10
Once	7 (70.0)
More than once	3 (30.0)
Purpose of transfusion	
Cesarean section	2 (20.0)
Sickness	6 (60.0)
Others	2 (20.0)
More than one current sexual partner	
Yes	23 (6.8)
No	317 (93.2)
Number of current sexual partners	n=23
Two	2 (8.7)
Three	19 (82.6)
Four or more	2 (8.7)

Table 2b revealed that 261 (76.8) of participants had a previous check of their HBV status mostly due to previous pregnancies. Among these, positive results were seen in 12 (4.6%) of the women. Table 2c shows that 224 (65.9) had more than one pregnancies with 147 (64.8) having more than one child. Participants with gestational age greater than 24 weeks constituted 211 (62%) of respondents. Furthermore, participants with

Table 2b: Potential risk factors of HBV infection among pregnant women n=340

Variable	Frequency (%)
Previous check of HBV status	
Yes	261 (76.8)
No	79 (23.2)
Outcome of result of previous check	
Positive	12 (4.6)
Negative	249 (95.4)
Spouses HBV status known	
Yes	10 (2.9)
No	126 (37.1)
Don't know	204 (60.0)
Spouse is positive to HBV	
Yes	2 (20.0)
No	8 (80.0)
Sharing of sharp Objects	
Yes	132 (38.3)
No	208 (61.2)
Intravenous drug use	
Yes	0 (0)
No	340 (100)
Hospital Procedure in the past	
Yes	26 (7.6)
No	314 (92.4)

Table 2c: Obstetric and gynecological factors of Hepatitis B virus infection among pregnant women

Variables	Frequency (%)
Gravidity	N=340
One	116 (34.1)
> 1	224 (65.9)
Parity	n=224
One	80 (35.7)
> 1	144 (50.9)
Gestational age	N=340
≤ 24 weeks	129 (38)
> 24 weeks	211 (62)
History of abortion	
Yes	47 (13.8)
No	293 (86.2)

positive history of abortion constituted 47 (13.8%) of respondents. In table 3 prevalence of HBV infection was highest among participants above and equal to 40 years of age (57.1%) and least (7.5%) among those with ages 25-29. Prevalence of HBV was highest in the participants with extended family set up. This was seen in 33 (20.4%). These associations were statistically significant.

Table 3: Association between hepatitis B status and socio-demographic variables n=340

Variables	HBV Status		χ^2	p-value
	Positive (%)	Negative (%)		
Age groups (years)			16.157	0.024^f
< 20	4 (16.0)	21 (84.0)		
20 – 24	15 (11.9)	111 (88.1)		
25 – 29	8 (7.5)	98 (92.5)		
30 – 34	7 (14.6)	41 (85.4)		
35 – 39	3 (10.7)	25 (89.3)		
≥ 40	4 (57.1)	3 (42.9)		
Mean ± SD	27 ± 6.83	25 ± 5.25	1.382	0.168
Marital Status			0.061	0.850
Married	37 (12.2)	266 (87.8)		
Separated/Divorced/Widowed/Never married	4 (10.8)	33 (89.2)		
Ethnicity			1.995	0.574
Yoruba	38 (11.7)	288 (88.3)		
Igbo	1 (33.3)	2 (66.7)		
Hausa	1 (25.0)	3 (75.0)		
Others	1 (14.3)	6 (85.7)		
Type of marriage		N=303	0.66	0.417
Monogamy	34 (12.6)	235 (87.4)		
Polygamy	6 (17.6)	28 (82.4)		
Level of education			1.142	0.767
Tertiary	8 (11.4)	62 (88.6)		
Secondary	27 (12.6)	187 (87.4)		
Primary	5 (13.9)	31 (86.1)		
No formal education /Quranic	1 (5.0)	19 (95.0)		
Occupation			1.649	0.648
House wife/ Unemployed	2 (9.5)	19 (90.5)		
Self employed	16 (11.0)	129 (89.0)		
Civil servant	18 (15.0)	102 (85.0)		
Others	5 (9.3)	49 (90.7)		
Family type			20.158	0.001
Nuclear	8 (4.5)	170 (95.5)		
Extended	33 (20.4)	129 (79.6)		
Income			0.860	0.354
≤ 30	25 (11.9)	185 (88.1)		
> 30	16 (15.7)	86 (84.3)		
> 30	16 (15.7)	86 (84.3)		

% = percentage of respondents in category; χ^2 = Chi square test; p = significance; f=Fisher's exact

As shown in table 4a, previous blood transfusion had a statistically significant association with hepatitis B status as 40% of those with previous blood transfusion were positive for HBV infection. Similarly, 34.8% of participants who had MSP were positive for HBV infection. In table 4b, a higher prevalence of HBV was seen among participants who had not taken HBV screening in the past 15 (19%). Those who had initial positive HBV result 12 (100.0) as well as those whose spouse were positive for HBV infection 2 (100.0), all

had a positive HBV result. Among participants who had hospital procedure in the past, 13 (50%) had HBV infection. In table 4c, 23.4% of participants with previous history of abortion were positive for HBV infection. These associations were statistically significant. In table 5, family type was a strong predictor of HBV infection with P of less than 0.05. History of previous blood transfusion, multiple sexual partner, hospital procedure in the past, having a positive spouse and history of abortion all had a p value below 0.05 and odd ratio above 1.

Table 4a: Association between potential factors of HBV infection and HBV infection Status

Variables	HBV Status		χ^2	p-value
	Positive (%)	Negative (%)		
Aware of the infection viral HBV			0.280	0.597
Yes	15 (13.4)	97 (86.6)		
No	26 (11.4)	202 (88.6)		
Local Tonsillectomy as previous procedure			0.013	1.000
Yes	9 (11.7)	68 (88.3)		
No	32 (12.2)	231 (87.8)		
Scarification			1.211	0.271
Yes	21 (14.3)	126 (85.7)		
No	20 (10.4)	173 (89.6)		
Tattoo			1.294	0.255
Yes	2 (25.0)	6 (75.0)		
No	39 (11.7)	293 (88.3)		
Previous blood transfusion			7.585	0.006
Yes	4 (40.0)	6 (60.0)		
No	37 (11.2)	293 (88.8)		
Number of blood transfusion			3.750	0.053
Once	2 (25.0)	6 (75.0)		
More than once	2 (100.0)	0 (0.0)		
More than one sexual partner			12.012	0.001
Yes	8 (34.8)	15 (65.2)		
No	33 (10.4)	284 (89.6)		

% = percentage of respondents in category; X^2 = Chi square test; p = significance; f=Fisher's exact

Table 4b: Association between potential factors of HBV infection and HBV infection

Variables	HBV Status		χ^2	p-value
	Positive (%)	Negative (%)		
Previous check of HBV status			4.659	0.031
Yes	26 (10.0)	235 (90.0)		
No	15 (19.0)	64 (81.0)		
Result of previous check of HBV status			113.69	0.001
Positive	12 (100.0)	0 (0.0)		
Negative	14 (5.6)	235 (94.4)		
Spouse is positive to HB			4.727	0.030^f
Yes	2 (100.0)	0 (0.0)		
No	0 (0.0)	8 (100.0)		
Sharing of sharp objects			1.792	0.181
Yes	12 (9.1)	120 (90.9)		
No	29 (13.9)	179 (86.1)		
Intravenous drug use			0.007	0.933
Yes	0 (0)	0 (0)		
No	41 (13.9)	299 (86.1)		
Hospital Procedure in the past			38.216	0.001
Yes	13 (50.0)	13 (50.0)		
No	28 (8.9)	286 (91.1)		

% = percentage of respondents in category X^2 = Chi square test p = significance f=Fisher's exact

Table 4c: Association between HBV status and Obstetrics and Gynaecological factors

Variables	HBV Status		χ^2	p-value
	Positive (%)	Negative (%)		
Gravidity			0.121	0.861
One	13 (11.2)	103 (88.8)		
> 1	28 (12.5)	196 (87.5)		
Parity			0.807	0.369
One	12 (15.2)	67 (84.8)		
> 1	16 (11.0)	129 (89.0)		
Gestational age			1.489	0.222
≤ 24 weeks	12 (9.3)	117 (90.7)		
> 24 weeks	29 (13.7)	182 (86.3)		
History of abortion			6.620	0.010
Yes	11 (23.4)	36 (76.6)		
No	30 (10.2)	263 (89.8)		

% = percentage of respondents in category

X² = Chi square test

p = significance

Table 5: Predictors of HBV status

Variables	B	p-value	Odd ratio	95 % C I
Age groups				
< 20	-1.571	0.107	0.208	0.031 – 1.404
20 – 24	-1.834	0.029	0.158	0.030 – 0.825
25 – 29	-2.387	0.006	0.092	0.016 – 0.513
30 – 34	-1.652	0.071	0.197	0.034 – 1.149
35 – 39	-1.964	0.052	0.140	0.019 – 1.0202
≥ 40	RC			
Family type				
Nuclear	-1.581	0.001	0.206	0.091 – 0.466
Extended	RC			
Previous blood transfusion				
Yes	1.664	0.013	5.279	1.424 – 19.577
No	RC			
More than one sexual partner				
Yes	1.524	0.001	4.590	1.810 – 11.641
No	RC			
Previous check of HBV status				
Yes	-0.751	0.034	0.472	0.236 – 0.944
No	RC			
Outcome of result of previous check				
Positive	-24.006	0.998	0.010	0.001 – 0.100
Negative	RC			
Hospital Procedure in the past				
Yes	2.324	0.001	10.214	4.317 – 24.166
No	RC			
Spouse is positive to HB				
Yes	3.584	0.001	36.00	7.336 – 176.655
No	RC			
History of abortion				
Yes	0.985	0.013	2.679	1.236 – 5.807
No	RC			

β = Regression coefficient; p = significance; CI= Confidence interval; RC: Reference Category

Discussion

There were 340 participants recruited into the study with a mean age of 25 ± 5.45 .

In this study, the prevalence of HBV infection was found to be 12.6% as 41 of the respondents had positive

hepatitis B test result. This means approximately, one out of eight pregnant women have hepatitis B virus infection. This is comparable to a prevalence of 11.2% found in a cross sectional study among pregnant women in Kwara State.³ Although this study was carried out about two years prior to the index study, prevalence of HBV infection has increased despite efforts by governmental and non-governmental organizations. The prevalence is not too far from prevalence of 10.5%, 10.2%, 9.2%, 8.0%, 7.89%, and 7.2% in South-West Nigeria, Benue, Gambia, Eastern Ethiopia, China, and Jos Nigeria^{-10,19,22}. It is however different from prevalence of 4.4% and 4.98% found in cross sectional studies among pregnant women attending ANC in Ghana and Cameroun.^{15,23} The similarities in the study population, and study design may be reasons for the close range of prevalence.

Prevalence of HBV infection was highest among women equal and above 40 years of age (57.1%) and least among pregnant women between 25-29 years (7.5%). This association was statistically significant. This finding is similar to findings in a cross-sectional study in China where prevalence of hepatitis was more at advanced age (greater than 35 years).²⁰ The finding is however different from findings in a cross sectional study in Jos, Nigeria where a higher prevalence of hepatitis was found in pregnant women between 15 and 30 years.²⁴ The possibility that older women had more exposure overtime than young women could be possible reasons for the course observed in the index study and similar studies.

The family type had a statistically significant association with prevalence of hepatitis. The highest prevalence of hepatitis were found in the participants who lived in an extended type of family 33 (20.4%). This means a family type with more members living together. This association was statistically significant. However Talla et al, in Benue Nigeria did not find a statistically significant association between family type and prevalence of HBV infection.²⁵ Findings in the index study may be due to increase in the incidence of transfer of communicable diseases like hepatitis through various means within the family. Previous studies in Oman and Indonesia showed that HBV carriers in the household significantly increase the risk of interfamilial transmission.^{26,27} In a retrospective chart review of all adult Omani patients diagnosed with chronic hepatitis at two tertiary hospitals in Oman, Busafi et al concluded that direct contact of infected individuals within a family is a main reported risk factor for HBV infection.²⁶ Hence, this facts may be possible reasons for the high prevalence observed in the extended family type with more family members compared to the nuclear type.

History of previous blood transfusion had a statistically significant association with prevalence of HBV infection as 4 (40%) of those with previous blood transfusion were positive for HBV infection compared with 11.2% of those without initial blood transfusion. This is similar findings in South-Western Nigeria, Eastern Ethiopia and Jos, Nigeria where a higher prevalence of hepatitis was found in pregnant women with initial blood transfusion.^{19,22,24} However, it is different from findings in North-West Ethiopia, where prevalence of hepatitis was more among participants who had not receive blood transfusion in the past.²⁸ Findings from the index study can be explained by the fact that HBV can be transmitted through any fluid/mucosal/blood contact from infected patients to others.

In this study, 34.8% of participants who had MSP were positive for HBV infection compared to 10.4% of those who had a single partner. This is comparable to findings in two tertiary hospitals in Uganda and Bauchi, Nigeria where participants with MSP had a higher prevalence of hepatitis compared to those with single partners.^{29,30} Bittaye et al in Bauchi Nigeria, however, did not find a statistically significant association between number of sexual partners and prevalence of HBV infection.¹⁰ According to WHO, sexual transmission of HBV is more prevalent in persons with MSP.³¹ This may be the reason for the consistent statistically significant finding in the index study and similar ones done in various regions of the world.

In the index study, a higher proportion of participants who had no previous check of their HBV infection status had a positive HBsAg result. This is similar to findings in South-West Nigeria, where a higher proportion of participants with no previous screening had a positive HBsAg result.¹⁹ It is however different from findings in Ghana where a higher proportion of participants who had HBV screening prior, had a positive HBs Ag result.²³ The findings in the index study might indicate that prior screening is a positive initiative as such persons may have taken further preventive measures to prevent infection hence a lower proportion compared to the initially unscreened participant.

Furthermore, all participants with previous positive hepatitis B status had a positive HBV test in the index study. This is similar to a study in South-West Nigeria where 100% of initially positive participant also had a positive HBsAg result.¹⁹ This may indicate a high level of sensitivity in the kit used.

This study revealed that all participants whose spouse had a positive hepatitis B result were positive for HBsAg test. A study by Talla et al in Benue Nigeria,

revealed a similar finding of increased prevalence of hepatitis B among women with spouse who had a positive hepatitis result.²¹ Possible reason for this finding could be increased risk of transmission among the couples from sexual as well as other forms of contact.

Participants in this study who had previous hospital procedure had a higher prevalence of HBV infection (50%) compared to those that did not (8.9%). This is consistent with findings in Eastern Ethiopia and Jos, Nigeria, where participants with previous hospital procedure had higher prevalence of hepatitis compared to those without previous hospital procedure.^{22,24} However, finding in North-West Ethiopia and Bauchi, Nigeria, did not reveal a statistically significant association between HBV prevalence and presence of hospital procedure in the past.^{28,30} Improper sterilization of hospital equipment and improper screening of transfused blood may be risk factors for transmission of HBV infection following hospital procedures.

A larger proportion of participants with previous history of abortion had a higher prevalence of hepatitis 11 (23.4%) compared to those without history of abortion (10.2%). This is comparable to findings in Eastern Ethiopia where a higher proportion of participants with previous history of abortion had a positive hepatitis B test result (22.2%).²² Possible source of infection in such cases might include: procedures like dilatation and curettage or blood transfusion in cases of associated moderate to severe blood loss.

The introduction of significant variables into logistic regression further showed that the family type was a stronger predictor of HBV infection. Likewise history of previous blood transfusion, multiple sexual partner, hospital procedure in the past, having a positive spouse and history of abortion. This further showed the strength of the associations.

The study had a descriptive cross-sectional study design, hence the outcome may not necessarily carry a causal relationship with the tested variables. A longitudinal study design would be required to establish causal relationships. This study is however intended to add to the body of knowledge that will form a template for future studies.

Conclusion

The prevalence of HBV infection is 12.6% among the study population. The main risk factors to HBV infection were: age, family type, previous blood transfusion, multiple sexual partner, previous positive HBV status, previous hospital procedure and history of abortion among others. These findings calls for

increasing need to improve the prevention and control of HBV infection. Measures need to be put in place to reduce the risk factors in order to allow for effective control. These strategies may help to reduce the prevalence of HBV infection and eventually lead to adequate prevention and control of the disease, and hopefully meet the goal of eliminating viral hepatitis as a public health problem by 2030.

There is a need to address risk factors. More efforts need to be put towards preventive strategies such as increased awareness and routine screening among others. These will help to ensure adequate prevention and control of the disease.

Competing interests: The authors declare that they have no competing interests

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Assessment of prescription quality and incidence of drug therapy problems among patients in selected primary health facilities in Maiduguri metropolitan area, Borno State

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Abstract

Background: The quality of medicine utilization at PHCs has been a matter of concern because of lack of skilled healthcare professionals and challenges with government funding. There is little information on quality of drug utilization following the use of PHCs as the major provider of basic medical services for internally displaced persons in Borno State. This study therefore aims to assess quality of drug prescriptions and also identify drug therapy problems (DTPs).

Material and Methods: This was a cross sectional retrospective review of prescriptions obtained from the medical records of 1000 patients in five PHC. Demographic data, drugs prescribed, diagnosis and other relevant information were extracted from medical records. WHO/INRUD core prescribing indicator was used to assess prescription quality, while DTPs were identified using Beers criteria, STOPP/START, NORGEF criteria. The data was entered into Microsoft excel and cleaned before being loaded into SPSS version 21 for analysis using descriptive statistics.

Results: The most prescribed drugs included analgesics (100%), antibiotics (79.6%), antimalarials (48.6%) and antihypertensives (25.9%). DTPs were identified in more than half of all prescriptions (52.5%) and mostly involved antibiotics (38.8%), analgesics (28.5%), proton pump inhibitors (23.3%) and corticosteroids (10.4%). DTPs found included wrong drug, untreated conditions, unnecessary drug therapy and polypharmacy.

Conclusion: Drugs prescribing quality was poor and characterized by high level DTPs, irrational antibiotic use and polypharmacy. This may increase the risk of microbial resistance to commonly used antibiotics and adverse drug reactions.

Keywords: Antibiotics, Drug therapy problems, Drug prescriptions, Primary healthcare, WHO/INRUD core indicators

Introduction

The primary health care system in the country is plagued by inadequate funding, lack of basic equipment and technologies as well as absence of skilled manpower. These challenges are now compounded by massive influx of internally displaced persons (IDPs) created by the worst humanitarian crisis in the country affecting millions of people.¹ The primary healthcare (PHC) system is responsible for basic medical care of both IDPs and local residents.^{2,3} Humanitarian organizations are providing significant support for the maintenance of services at PHCs,

however coping with increasing patient inflow remains a major challenge.⁴

There have been frequent reports infectious diseases such as cholera, measles, gastroenteritis, sexually transmitted infections and non-communicable diseases.^{5,6} The increased demand for healthcare services is occurring against the background of damaged facilities, lack of qualified staff and violence related disruptions to service delivery.⁷

The incidence of DTPs at PHC level has been an issue of concern in many developing countries, although figures vary widely in literature.^{8,9} The most frequently reported DTPs include polypharmacy, unnecessary drug therapy, adverse drug reactions, untreated conditions, ineffective drug(s) and wrong drug dosages.¹⁰⁻¹² These DTPs compromise the quality of medicine utilization and have been reported to cause

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poor clinical outcomes, adverse drug reactions, increased risk of emergency hospitalization, deaths and unnecessary medical costs.^{13,14}

Drug therapy comes with some safety concerns from known pharmacological effects and unpredictable adverse effects, so the presence of DTPs is believed to significantly increase the risk of more side effects and drug interactions both of which can cause reduction in patient's quality of life. The misuse of medicines at PHCs can be linked to the use of low cadre healthcare workers many of whom have limited training, skills and expertise to prescribe drugs, manage complex diseases and effectively respond to complications. While Nigeria's national health policy allows nurses, community health workers to prescribe medicines for simple ailments, there is little oversight on prescription quality and tracking the incidence of DTPs. This study is therefore aimed at assessing prescription quality and incidence of DTPs

Materials and Methods

Study setting: The study was carried out in five major PHCs located in Maiduguri the capital city of Borno State. The selection of PHCs was based on location in densely populated areas, patient traffic at least 500 per month and availability of good medical records. The selected PHCs included Abbagaram PHC, 1000 housing estate PHC, Gwange PHC, Jidari Polo PHC and Gamboru PHC located in major residential districts of Maiduguri metropolitan area.

Study design: This was a cross sectional retrospective review of drug prescriptions obtained from medical records of patients over a six month period (May and November 2023). The study duration was chosen to align with higher patient traffic and completeness of medical records as older records appeared to have missing information.

Sample size/Sampling: The sample size was determined using the formula $n = Z^2 PQ/e^2$ where n = sample size, $Z = 1.96$ at 95% confidence interval, P = proportion of prescriptions with DTPs, $Q = 1 - P$, e = margin of error (5%). The sample size was determined to be 384; however 1000 medical records were selected by simple random sampling method at the rate of 200 per facility.

Assessment tools: Quality of prescriptions was determined using the World Health Organization and International network for rational use of drug (WHO/INRUD) core indicators¹⁵ which provided standards for number of drugs, percentage of antibiotics and percentage of injectable drugs per encounter as well as generic prescriptions and percentage of drugs prescribed from the essential drugs

list. While DTPs was assessed with the use of Beers criteria¹⁶, the screening tool of older persons prescriptions (STOPP)/ Screening tool to alert doctors to right treatment (START)¹⁷ and the Norwegian general practice criteria (NORGEPI).¹⁸

Outcome variables: The prescriptions were reviewed for incompatibilities between diagnosis and drugs identified when there is a mismatch based on national standard treatment guideline. Dosage errors were determined when dosage is outside recommended range for patient's age, body weight, frequency of dosage and duration of therapy. The potential for drug interactions was identified when two or more co-prescribed drugs may chemically react to amplify or reduce the pharmacological effect with potential for toxicity or ineffective therapy. A medical condition is regarded as untreated if no specific medication was prescribed for it and when more than one drug is prescribed to manage one clinical condition – it was considered duplicate therapy. A drug is considered to be contraindicated if there is documented evidence that it will possibly result in adverse reactions, allergy, worsen symptoms of disease(s), teratogenic in pregnancy and toxic in children. Polypharmacy was considered to have occurred when there are ≥ 4 drugs per patient encounter.

Data collection: The materials used for data collection included Medical folders, attendance records and dispensing ledgers. Patients medical records were selected using simple random sampling method and relevant demographic, drug prescription, and clinical data was extracted. The presence of drug(s) considered inappropriate by Beers criteria, STOPP/STOPP and NORGEPI criteria in light of patient variables was determined to be a DTP.

Analysis: The data was entered in to SPSS version 22 and analyzed using descriptive statistics for quality of drug prescription, polypharmacy and DTPs. The prescriptions were reviewed for DTP using the three tools, while WHO/INRUD core prescribing indicator tool was used to determine prescription quality.

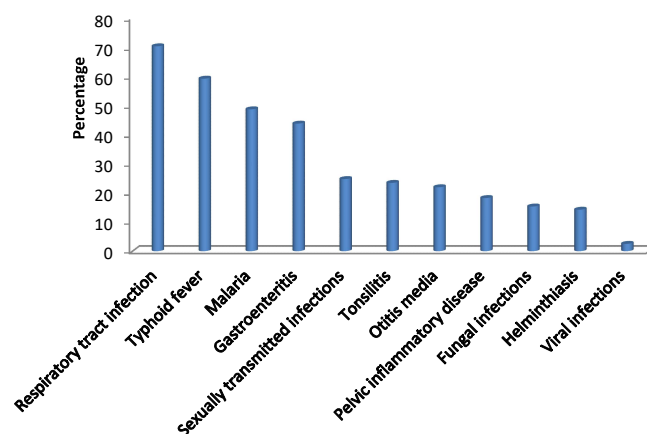
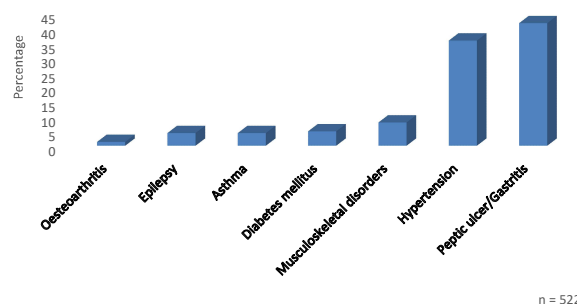
Ethical issue: The approval for this study was obtained from Borno State Ministry of Health (MOH/GEN/6679/1) and Borno State primary healthcare development agency (SPHCDA/GEN/181)

Results

Majority of patients were females (65.2%) and most of them were also (63.3%). The average duration of patient visits to these facilities was 4.7 years (**Table 1**). The results showed bacterial infections (67.6%), malaria (48.5%) and fungal infections (15.3%) were the most frequently encountered among patients.

Table 1: Demographic data

Variable	Frequency (%)
Gender	
Male	348 (34.8)
Female	652 (65.2)
Marital status	
Single	319 (31.9)
Married	633 (63.3)
Divorced	3 (0.3)
Widowed	45 (4.5)
Education	
Illiterate	690 (69)
Primary	104 (10.4)
Secondary	160 (16)
Tertiary	46 (4.6)
Age (years)	
≤ 20	253 (25.3)
21 – 30	191 (19.1)
31 – 40	181 (18.1)
41 – 50	133 (13.3)
51 – 60	96 (9.6)
61 – 70	89 (8.9)
≥ 71	57 (5.7)
Mean age	36.6 ± 18.6
Attendance at PHCs (years)	
1 – 2	59 (5.9)
3 – 4	369 (36.9)
5 – 6	498 (49.8)
≥ 7	74 (7.4)
Mean	4.7 ± 1.6 yrs.

**Figure 1:** Prevalence of infectious diseases (n=642)**Figure 2:** Prevalence of non-communicable diseases

Overall, more than half of patients (64.2%) were treated for communicable diseases. The most common bacterial infections were respiratory tract infections (70.7%), typhoid fever (59.1%), gastrointestinal infections (43.7%) and sexually transmitted infections (24.7%). Others included tonsillitis (23.4%), otitis media (21.9%) and pelvic inflammatory disease among females (18.2%) (**Figure 1**).

The most frequently encountered non-communicable diseases were peptic ulcer/Gastritis (41.6%), hypertension (35.7%) and musculoskeletal disorders (7.9%). While diabetes mellitus (4.9%), asthma (4.3%) and epilepsy (4.3%) were less frequently found among patients (**Figure 2**).

A total of 2350 prescriptions consisting 11045 individual drugs were reviewed and analgesics (100%), antibiotics (79.6%), antimalarials (48.6%), gastrointestinal drugs (41.8%), antihypertensives (35.7%), antifungals (15.3%) and anthelmintics (14.2%) were the most prescribed class of drugs. Anxiolytics were prescribed for 6.8% patients, while 4.9% were for diabetes mellitus (**Table 2**).

Drug therapy problems were found in more than half of all prescriptions (52.5%) of which wrong drug (86.8%), untreated conditions (21.6%), unnecessary drug

therapy (21.6%) and duplicate therapy (11.1%). The need for additional drug(s) was found among 9.4% of patients while contraindication was identified in 1.8% of prescriptions (**Figure 3**).

The drugs most implicated in DTPs were antibiotics (38.8%), analgesics (28.5%), proton pump inhibitors (23.3%) and corticosteroids (10.4%) (**Figure 4**).

The quality of prescriptions was generally poor as results were well above the standard reference values set by WHO/INRUD core indicator. The percentage of antibiotics per encounter (79.6%), injectable drugs (48.5%), number of drugs per encounter (4.6) and generic prescription (78.4%) were above recommended standard values (**Table 3**).

Discussion

The quality of drug prescriptions was generally poor and comparably similar to reports from many developing countries,¹⁹ although contrasting figures have also been published.^{20,21} Most prescription audits in Nigeria largely focused on secondary and tertiary health facilities,²² with little emphasis on PHC facilities which provide basic medical care to majority of the population. While diseases were generally comparable to other studies, greater proportion of patients were females,^{23,24} as reported in previous studies.^{25,26}

Table 2: Drugs prescribed according to pharmacological classification

Class of drug	Frequency (%)	Drugs	n (%)
Analgesics	11045 (100)	Paracetamol	4615 (41.8)
		Ibuprofen	2847 (25.8)
		Diclofenac	3189 (28.9)
		Paracetamol + Codeine	394 (3.5)
Antibiotics	8791 (79.6)	Amoxicillin	538 (6.1)
		Ampicillin/Cloxacillin	423 (4.8)
		Ciprofloxacin	1328 (15.1)
		Levofloxacin	696 (7.9)
		Amoxicillin/Clavulanic acid	2749 (31.3)
		Cefuroxime	1765 (20.1)
		Doxycycline	362 (6.4)
		Cotrimoxazole	247 (2.8)
		Metronidazole	483 (5.5)
Antimalarials	5081 (48.6)	Artemether/Lumefantrine	2724 (53.6)
		Artesunate injection	646 (12.7)
		Artesunate/Piperaquine	492 (9.7)
		Dihydroartemisinin	150 (2.9)
		Arteether injection	1069 (21.1)
Antidiabetics	541 (4.9)	Metformin	435 (80.4)
		Glibenclamide	85 (15.7)
		Glimepiride	21 (3.9)
Antihistamines	232 (2.1)	Chlorpheniramine	179 (77.2)
		Promethazine	53 (22.8)
Anthelminthics	1568 (14.2)	Praziquantel	289 (18.5)
		Albendazole	857 (54.6)
		Mebendazole	422 (26.9)
Antifungals	1689 (15.3)	Griseofulvin	394 (23.3)
		Fluconazole	1200 (71.1)
		Nystatin	95 (5.6)
Anxiolytics	751 (6.8)	Diazepam	230 (30.6)
Antiepileptics	475 (4.3)	Bromazepam	521 (69.4)
		Carbamazepine	384 (80.8)
		Phenobarbitone	91 (19.2)
Antiasthmatics	475 (4.3)	Salbutamol	132 (27.8)
		Salbutamol inhaler	343 (72.2)
Antivirals	276 (2.5)	Acyclovir	276 (100)
Cardiovascular drugs	3943 (35.7)	Amlodipine	2027 (51.5)
		Nifedipine	546 (13.8)
		Methyldopa	344 (8.7)
		Atenolol	396 (10.1)
		Amiloride/Triamterene	630 (15.9)

Corticosteroids	243 (2.2)	Prednisolone	184 (75.7)
		Hydrocortisone	59 (24.3)
Gastrointestinal drugs	4595 [41.6]	Omeprazole	1973 [42.9]
		Rabeprazole	2126 [46.3]
		Loperamide	342 [7.4]
		Hyoscine N methyl bromide	154 [3.4]

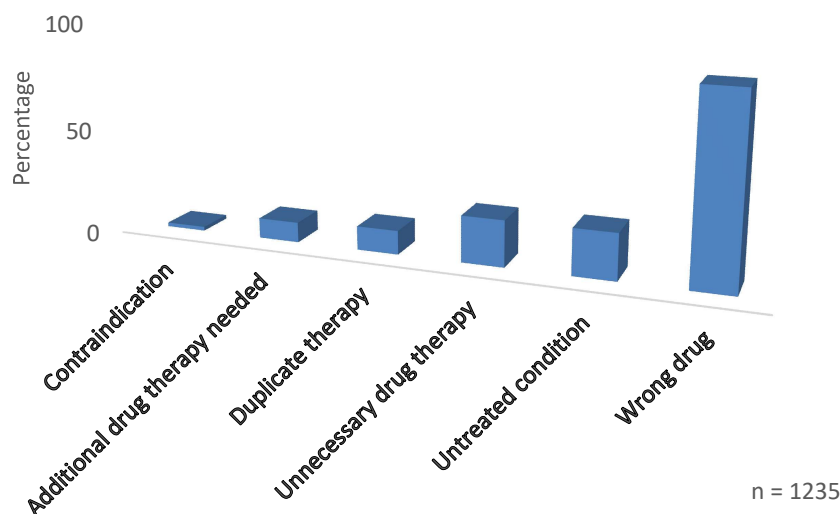


Figure 3: Frequency of drug therapy problems

Similarly bacterial infections and malaria appear to be the most encountered infectious diseases,²⁷ while peptic ulcer and hypertension were the most non-communicable diseases among patients.²³

The most prescribed drugs were analgesics and antibiotics similar to previous studies,^{28,29} contrasting rates have also been reported.^{30,31} The poor quality of prescriptions using WHO/INRUD indicators was similarly reported^{32,33} and several factors have been given for this consistent finding some of which include prescriber qualification, medicine availability, preferences and level of adherence to standard treatment guidelines.³² The deployment of low cadre health workers with limited training and inadequate oversight by physicians and pharmacists also create favourable environment for poor prescription practices.³⁴

While empirical prescription is widely practiced in most PHCs in the country, the problems associated with patient exposure to medication errors, irrational drug use and drug misadventure remains an endemic public health challenge.^{35,36} For instance, antibiotics were inappropriately prescribed for fevers associated with malaria and common cold.³⁷⁻³⁹ This observation may be partly responsible for the high antibiotic prescriptions in this study with its associated risk of resistance to commonly used antibiotics.⁴⁰ Furthermore, the clinical

competence and prescribing skills of low cadre health workers does not equip them with knowledge needed to optimize prescription quality.

The high prevalence of DTPs in this study have been previously reported,^{14,40} however mixed results have been previously reported due to differences in assessment tools, patient variables, study settings and DTP classification system.⁴² While the most common DTPs were wrong drug(s), unnecessary drug therapy and untreated conditions, individual drugs implicated vary widely between studies.^{42,43}

The number of drugs per encounter was considerably higher than WHO/INRUD standard (1.6–1.8),⁴⁴ so also it is for including injectable drugs,^{45,46} generic drug,⁴⁷ polypharmacy^{40,48} and prescription from essential drugs list.⁴⁹ The poor prescription quality coupled with the high prevalence of DTPs clearly indicates that significant challenges remain with drug utilization at PHC facilities. There is need for better training and supervision of prescribers at this level of the healthcare system if improvements are to be made to the quality of drug utilization.

Conclusion

Drug prescription quality was poor and characterized by high level of DTPs, polypharmacy and irrational antibiotic use. These significantly increase the risk of

adverse reactions, unnecessary cost of drugs and poor clinical outcomes.

Conflict Of Interest: None declared

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A Survey of knowledge and perception of surrogacy among health care providers in selected institutions in Ilorin

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Abstract

Background: Surrogacy is a type of Assisted Reproductive Technology (ART) whereby a third party carry a pregnancy on behalf of another couple, delivers a baby and hands the child to the commissioning parents at birth. Surrogacy is becoming increasingly popular in developed countries with a well-structured legislation and laws. However, surrogacy is a relatively new practice in Nigeria and no legislation are guiding any form of ART in Nigeria. This study aimed to assess the awareness, knowledge, perceptions and attitudes of health workers toward surrogacy.

Materials and Methods: This was a cross-sectional survey of Health workers in University of Ilorin Teaching Hospital (UTH) and University of Ilorin Health Centre. A self administered questionnaire was used to obtain information on the knowledge of surrogacy, perception and attitudes toward surrogacy. Data was analysed using SPSS version 20. A total of 402 health workers of various categories participated in this study.

Results: Majority (85.5%) of respondents had previous knowledge of surrogacy with the commonest source of information being the health facility (33.3%). Less than 20% of the respondents had previously seen a baby born through surrogacy. About two-thirds of respondents (66.7%) opined that the intended mother should be the legal mother of the child in full surrogacy, while 54.2% supported the intended mother being the legal mother in partial surrogacy. Majority of respondents (79.4%) opined that the intended father should be the legal father of the child. Knowledge and awareness of surrogacy among the respondents was high.

Conclusion/Recommendations: There is high level of awareness and knowledge of surrogacy among the respondents. The high level of knowledge and awareness did not reflect in the attitudes and perception toward surrogacy. There is also low acceptability of surrogacy as a means of assisted reproductive therapy among our respondents.

Keywords: Surrogacy, Knowledge, Perception, commissioning parents

Introduction

In recent times there has been improvement in medical knowledge via technological advancements to improve the healthcare delivery system. This advancement has also led to a revolution in human reproduction which allows a woman to carry and give birth to a baby that is the product of another couple that wants to have a child. Surrogacy, a type of assisted reproductive technique (ART) can be described as a form of arrangement that usually involves a third party called the surrogate

mother. The surrogate mother is the woman who becomes pregnant, carries the pregnancy and gives birth to the child on behalf of another couple, the commissioning parents.¹³

There are various types of surrogacy arrangements: Full or gestational surrogacy which involves transfer of embryos created from gametes of either the commissioning parents or gamete(s) from a parent and another donor to the surrogate via In vitro fertilization (IVF) and the surrogate mother has no genetic link to the child. Partial, genetic or traditional surrogacy involves artificial insemination of the surrogate by the commissioning father, thus making her the genetic mother.^{4,7} Therefore, a surrogate mother is a woman who carries a pregnancy for the intended parents. Surrogacy arrangements are increasing and becoming popular worldwide because they allow couples who,

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for whatever reason, cannot procreate naturally to build a family.² It also enables couples to have a child that is genetically related to them by using their gametes (gestational surrogacy); which is usually carried out through IVF technology.^{8,9} Prior to the advent of IVF, only traditional surrogacy was practiced where the child was usually genetically related to the surrogate because her egg was used.⁸⁻¹⁰ The practice of commercial surrogacy has also contributed to the increasing popularity of surrogacy.¹¹ This involves compensating the surrogate for her services and not merely her medical expenses alone as is obtained in altruistic surrogacy arrangements where the surrogate mother does not receive financial compensation. In commercial surrogacy, the commissioning parents may opt for cross border surrogacy in which they make surrogacy arrangements with a surrogate from another country, and thereafter travel to the surrogate's country, or may opt to use a surrogate within the same country.¹² Surrogacy arrangements are sometimes made through some organizations.¹³ Surrogacy arrangements are usually bound by contract but this can only be legally enforceable in countries where surrogacy is legally practiced. Furthermore, surrogacy has gained popularity in situation whereby it is medically impossible for a woman to give birth or the woman would be risking her life by becoming pregnant due to some medical reasons.^{2,8,14,15} Surrogacy gives opportunities to heterosexual couples, homosexual couples, single mothers, or fathers to become parents. It can be achieved by using donor sperm, using donor oocytes, oocytes from the commissioning mother or using the oocytes of the surrogate mother.^{2,12}

Despite surrogate arrangement becoming popular, there are still challenges of this type of arrangement being widely accepted because surrogate arrangements are illegal or unethical in some communities, while some accept it to a certain extent. Its acceptability in some areas is also frowned at due to cultural and religious reasons.⁴ Hence, there are controversies surrounding this arrangement, and the complicated legislation around the world is not helping matters.^{10,16,17} Apart from the publicity, the laws surrounding surrogacy are complicated and strict and vary from one jurisdiction to another. In the UK, although single parents can commission a surrogate child, parental responsibility cannot be granted, since the law states that it can only be granted to couples or two people living in an enduring relationship.¹⁴ The United Kingdom (UK) has the most articulated legislation on surrogacy.¹⁸ Unfortunately, there is no law guiding ART in our country (Nigeria).¹⁹ Consequently, most people in Nigeria will see it as a taboo for using a Surrogate. Therefore, this study aims to assess the health worker's knowledge and awareness of surrogacy as well as their perception and attitudes toward surrogacy.

Materials and Methods

The study was a cross-sectional survey of 402 Health workers at the University of Ilorin Teaching Hospital (UITH) and the University of Ilorin Health Centre, Nigeria. This study was conducted in 2018 using self-administered questionnaires as the data collection method. This comprises doctors, nurses, pharmacists and laboratory scientists. A stratified sampling method was used, the health workers were grouped into four strata namely, doctors, nurses, pharmacists and laboratory scientists. A table of random numbers was then used to randomly select the participants to obtain the total sample size of 402.

Quantitative methods of data collection were used to obtain information from the study participants. An adapted and validated questionnaire on knowledge, attitude and perception was used to obtain all related information for this study.²⁰ Prior to the data collection period, ethical approval was obtained from university of Ilorin ethical review committee. Participation was voluntary and data obtained were treated with absolute confidentiality. Data entry and management were done using Statistical Package for Social Science (SPSS) version²⁰ software (IBM Corporation, Armonk, USA). Descriptive statistics was used to summarize the results which were presented with the aid of pie charts, bar charts, and frequency tables.

Written informed consent was obtained from each study participants before they completed the questionnaire. Ethical approval for the study was granted by the Ethical Review Committee of the University of Ilorin (Approval Number: UERC/ASN/2017/1087; Protocol Identification Code: UERC/BMS/088). Participation was entirely voluntary, and respondents retained the right to withdraw their consent at any time without any consequences.

Results

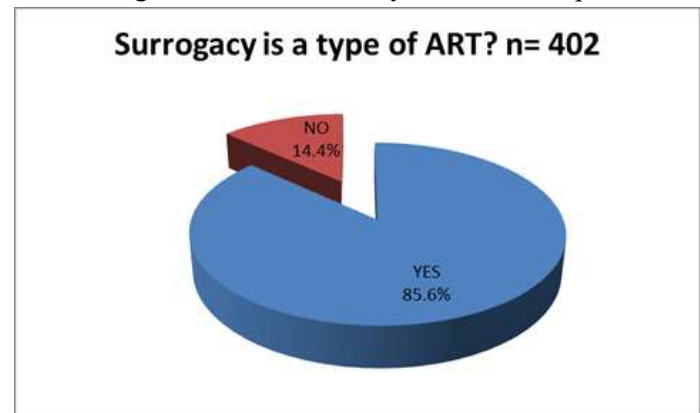
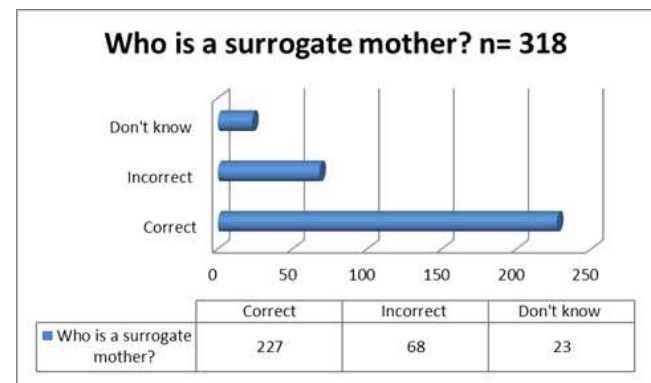
A total of 402 health workers were interviewed. Two hundred and fifty-one (62.4%) of respondents were between the ages of 21-40 years age group. Close to two-thirds (64.7%) were male, and more than half (56.0%) of the respondents were married. Doctors constituted over a third (35.3%) of respondents, and majority (84.3%) practiced monogamy. (Table 1).

Table 2 shows the respondents' awareness of surrogacy. Majority of respondents (85.5%) were aware of surrogacy, and with 33.3% hearing about it at health facilities. However, less than 20% of the respondents had seen a baby born through a surrogacy arrangement. Figures 1 and 2 show respondents' knowledge on surrogacy. Three hundred and forty-four (85.6 %) of

Table 1. Socio-demographic characteristics of participants

Socio-demographic characteristics	n (%)
Age group (years):	n = 402
<20	44 (11.00)
21 – 30	126 (31.30)
31 – 40	125 (31.10)
41 – 50	74 (18.40)
51 – 60	26 (6.50)
60 – above	7 (1.70)
Sex:	n = 402
Female	141 (35.10)
Male	260 (64.70)
No response	1 (0.20)
Profession:	n = 402
Doctors	142 (35.30)
Nurses	116 (28.90)
Pharmacists	39 (9.70)
Lab scientists	17 (4.20)
Others	74 (18.40)
No- response	14 (3.5)
Marital status:	n = 402
Single	171 (42.50)
Married	225 (56.00)
Divorced	2 (0.50)
Separated	2 (0.50)
Others	2 (0.50)
Religion:	n = 402
Christianity	218 (54.23)
Islam	179 (44.53)
Traditional	0 (0.00)
No Response	5 (1.24)
Family type:	n = 402
Monogamy	339 (84.33)
Polygamy	46 (11.44)
No Response	17 (4.23)
Have children?	n = 402
Yes	224 (55.72)
No	166 (41.29)
No Response	12 (2.99)

the respondents knew that surrogacy is a type of ART (figure 1). Figure 2 shows the participant's response to the question “Who is a surrogate mother?”. Tables 3 shows respondents' perception on surrogacy, majority of the respondents 42.8% in full surrogacy and 43. 0 in partial surrogacy feels decision for antenatal care of the surrogate should be made by both intended parents

**Figure 1. Respondent's knowledge on surrogacy: Is surrogacy a type of ART?****Figure 2. Respondent's knowledge of a surrogate mother****Table 2. Respondents' awareness of surrogacy**

Variable	n (%)
Ever heard of surrogacy:	n = 402
Yes	344 (85.57)
No	50 (12.44)
No Response	8 (1.99)
Where did you first hear about surrogacy?	n = 402
Friends/family	32 (7.96)
Colleague	56 (13.93)
Mass media	71 (17.66)
Social media	51 (12.69)
Health facility	134 (33.33)
No Response	58 (14.43)
Ever seen a baby born through surrogacy?	n = 402
Yes	75 (18.66)
No	298 (74.13)
No Response	29 (7.21)

Table 3. Respondents' Perception on surrogacy n=402

Variable	Full Surrogacy n (%)	Partial Surrogacy n (%)
Decision on antenatal care		
Intended parent	120 (29.9)	83 (20.5)
surrogate mother	63 (15.7)	68 (17.0)
both	172 (42.8)	173 (43.0)
not sure	31 (7.7)	56 (14.0)
undecided	16 (4.0)	22 (5.5)
Legal mother of the child?		
Intended mother	268 (66.7)	218 (54.2)
surrogate mother	65 (16.2)	75 (18.7)
not sure	48 (12.9)	87 (21.6)
undecided	21 (5.2)	22 (5.5)
Legal father of the child?		
Intended father	319 (79.4)	
Husband of Surrogate	28 (7.0)	
not sure	29 (7.2)	
undecided	26 (6.4)	

Table 4. Respondents' Attitudes toward surrogacy n=402

Variables	Agree n (%)	Neutral n (%)	Disagree n (%)
Surrogacy should be a means of assisted reproduction	278 (69.2)	80 (19.90)	44 (11.0)
If you/your partner found yourself to be infertile, would you consider having a child via surrogate mother?	142 (33.3)	129(32.08)	131 (32.6)
Would you prefer surrogacy to adoption?	158 (39.3)	95 (23.46)	149 (37.1)
After a surrogacy agreement has been reached between two parties and the surrogate mother is pregnant do you think the intended parents should be able to opt out of the agreement if they change their mind about wanting a child?	51 (12.7)	96 (23.88)	255 (63.4)
Should a sister of the intended mother be allowed to be the surrogate mother?	142 (35.3)	118(29.35)	142 (35.3)
Surrogacy should not be allowed because it is against my religion	100 (24.9)	137(34.07)	165 (41.0)

and surrogate mother while 29.9% in full surrogacy and 20.5 % in partial surrogacy feels decision for antenatal care should be done by Intended parents only. Only 15.7% in full surrogacy and 17.0% in partial surrogacy feels decision should be made by the surrogate mother only. Majority of respondents 66.7% in full surrogacy and 54.2% in partial surrogacy feels the intended mother should be the legal mother of the child at birth and 79.4% feels the intended father should be the legal father at birth.

Table 4 shows the respondents' attitude toward

surrogacy, almost two third of respondent 69.2% agree that surrogacy should be a means of assisted reproduction but only 33.3% agreed to consider having a child via surrogacy if they have infertility, 39.3% prefers surrogacy to adoption. However, 63.4% of the respondents disagree that intended parents can opt out of the surrogacy agreement if they change their mind of wanting to have a child after agreement has been reached between the two parties. 35.3% agrees that sister to the intended mother should be allowed to be the surrogate mother while 35.3% disagrees. 41.0% disagrees that surrogacy should not be allowed if it is

against their religion while 24.9% agrees that surrogacy should not be allowed if it is against their religion.

Discussion

The results of this study show a high level of awareness among health workers. This may not be surprising because most assisted reproductive therapies are probably hospital-based. However, the awareness level in this study is lower than that reported in Greece among medical students which revealed more than 90% were aware of surrogacy,²¹ and higher than that reported (35.2%) by Bello and co-workers among women attending infertility clinics in Ibadan.²² The Greek study reported internet, books, journals/and TV (37%) as the major sources of ART-related information²¹ as opposed to our study in which health facility was the leading source of surrogacy-related information. The awareness of surrogacy may be overwhelming among health workers as indicated by the findings from this study, but it is not common in our society when compared to other types of ART such as In vitro fertilization (IVF), Intracytoplasmic sperm injection (ICSI), Artificial insemination (AI). Even though surrogacy is hospital-based, less than 20% of health workers in our study have ever seen a baby born through a surrogacy arrangement. This low surrogacy practice and acceptance in our communities may be in part influenced by cultural norms, religious beliefs, psychological, ethical, and legal issues.^{9,19,23,24}

Surrogates could be genetic (involving artificial insemination of the surrogate by the commissioning father, thus making her the genetic mother) or gestational (via IVF by the commissioning parents and embryo transfer to the surrogate; or transfer of embryos created from gametes donated by others to the surrogate).^{5,7} Therefore, a surrogate mother is a woman who carries a pregnancy for the intended parents. Surprisingly, more than 20% of the health workers in our study did not know who a surrogate mother was. However, majority of the respondents know that surrogacy is a type of ART. The significance of this high level of awareness is the increase in understanding and acceptance among families. This also helps the intending parents to be well informed and prepare emotionally therefore reducing stress and anxiety.

Medically, as awareness about surrogacy grows, more people may seek out surrogacy services, this can lead to better medical care and support for surrogates and intended parents. It was expected that the high level of knowledge and awareness about surrogacy of respondents documented in our study should reflect in respondents' perception and attitudes toward surrogacy. However, a mixed attitude toward surrogacy was observed in our environment as most of attitude

related questions posed, revealed respondents' differing attitudes. We observed that 70% of our respondents agreed that surrogacy should be a means of ART, and 35.3% would consider having a child via a surrogate mother if their partner is infertile. This was similar to the findings by Bello et al in Ibadan, Southwest Nigeria²² Our findings on respondents' perception of surrogacy revealed that there was a significant opposition to the regulations on surrogacy as is obtainable in advanced countries such as the UK (where an intended parent is the legal parent of the child at birth)²⁵ as majority of our respondents think both the intended parent and surrogate mother should be the legal parents in Full surrogacy and the intended mother should be the legal mother in partial surrogacy. Both surrogate mother and intended parents should decide the antenatal care. This opinion may contribute to legal issues following surrogacy arrangements in our locality as there is currently no known law or parliamentary act guiding and regulating ART in Nigeria.^{9,19,24} However, our respondents' perception of who should determine and decide the antenatal care of the foetus was in agreement with international best practice in which both the intended parent and surrogate jointly decide the antenatal care of the foetus. Overall, participant's knowledge of and awareness of surrogacy was high. This may stem from the fact that the participant's cohorts were health workers where surrogacy-related issues may be commonly discussed before ART is offered. However, the knowledge and awareness of these participants do not translate to their attitude toward surrogacy. This, however, may be linked to religion and or cultural beliefs.

One limitation of this study is the use of a closed-ended questionnaire as the sole instrument for data collection. This may have constrained participants' ability to fully express their personal views and nuanced experiences, which might have been better captured through qualitative approaches such as in-depth interviews (IDIs) or focus group discussions (FGDs). Nonetheless, a key strength of the study lies in the systematic sampling method employed in the selection of participants, which enhances the representativeness and reliability of the findings.

Conclusion

The findings of this study showed a high level of awareness and knowledge of surrogacy among the respondents. However, the high level of knowledge and awareness did not reflect in their attitudes and perception toward surrogacy as we documented somewhat mixed results on attitudes and perceptions. Our results also show a low acceptability of surrogacy as a means of assisted reproductive therapy among our respondents. More awareness on surrogacy is needed in our society. Health workers are well positioned in the

society to provide ART related information as communities would most likely believe and trust surrogacy related information coming from them than anyone else. Therefore, more awareness should be raised through the health workers in partnership with civil societies and non-governmental organizations (NGOs) to increase the acceptability in the society and hence helps in its legislation.

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***Newbouldia Laevis* Enhanced Bcl-2 Expression and Germinal Epithelial Proliferation in adolescent Wistar rats**

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Abstract

Background: In traditional medicine, numerous plant extracts have been used as infertility treatments to enhance chances of procreation over the years. The use of *Newbouldia laevis* as an adjuvant has been shown in experimental animal models to have the ability to either stimulate or suppress male reproductive processes at specific dosages. This study aimed to compare the effects of graded dosages of *Newbouldia laevis* and zinc on male fertility.

Materials and Methods: Thirty-six male adolescent Wistar rats, weighing between 55 - 125 g were randomly divided into six groups of five rats each. They were treated with normal saline, Zinc, and *Newbouldia laevis* at different doses and duration, viz: low-dose short-term (LS) and high-dose short-term (HS) for a period of 28 days, and low-dose long-term (LL), and high-dose long-term (HL) for of 56 days. The semen parameters, histomorphometric analysis, and Bcl-2 expression, a protein that controls apoptosis, were studied to assess the testicular health in experimental animals.

Results: The results demonstrated that high doses of *Newbouldia laevis* impaired semen parameters, particularly the motility and population of sperm cells, irrespective of treatment duration. The population of germinal epithelial cells was unchanged. However, the expression of Bcl-2 was reduced in the high dose (HS and HL) groups when compared to other groups.

Conclusion/Recommendations: The study suggests that moderate use of *Newbouldia laevis* extract for a limited period may have beneficial effects on male fertility potential with effects comparable to those of Zinc.

Keywords: *Newbouldia laevis*; semen parameters, germinal epithelium, Zinc, Bcl-2

Introduction

Nowadays, infertility has become a real public health problem because of its increasing prevalence, widespread distribution, and the difficulties inherent in its management. Infertility affects millions of people of reproductive age worldwide and has an impact on their families and communities. It is estimated that 48 million couples and 186 million individuals live with infertility globally.¹ Epidemiological data conclude that about 15% of couples go through troubles in

having children and refer to physicians in search of solutions.² Infertility imposes stress on infertile couples and in different ways threatens their mental health in the form of emotional and psychological problems such as frustrations, fear and anxiety, depression, anger and aggression.³ Infertility can be caused by female reproductive issues, as well as male reproductive problems.

Male factor infertility (MFI) is thought to be the cause of up to 50% of all cases of infertility globally and at least 30 million men worldwide are infertile with the highest rates in Africa and Eastern Europe.⁴ There are potentially many different causes of male infertility, including hormonal, anatomical, and secondary exposure to exogenous substances. MFI is typically diagnosed through an abnormal semen analysis. While the semen analysis has multiple measured parameters, the most important are abnormalities in sperm count, ranging from fewer than normal sperm (oligospermia) to undetectable sperm cells (azoospermia), sperm

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motility, and sperm morphology.⁵ Many medications have been invented to tackle infertility, including clomiphene citrate and Tamoxifen. Some other trace elements have also been discovered to positively affect male fertility and are even contained in human semen. Examples include calcium (Ca), magnesium (Mg), selenium (Se) and particularly Zinc (Zn).⁶

Zinc is an essential mineral required for prenatal and postnatal development. It is a trace mineral, necessary for many enzyme activities and chemical reactions in the body. It is a major player in the creation of DNA, the growth of cells, the building of proteins, the healing of damaged tissue, and supporting a healthy immune system. As a hormone balancer, it helps hormones such as testosterone, prostrate and sexual health and functions as an antibacterial agent in men's urea system. It plays a role in epithelial integrity, showing that Zn is essential for maintaining the lining of the reproductive organs and may have a regulative role in the progress of capacitation and acrosome reaction.⁷ In contrast, Zn deficiency impedes spermatogenesis is a reason for sperm abnormalities and has a negative effect on serum testosterone concentration. Based on these findings, the Zn micro-element is very essential for male fertility. It could be considered as a nutrient marker with many potentials in the prevention, diagnosis, and treatment of male infertility).⁸

In some parts of Africa and indeed Nigeria, a wide variety of plants are of great medicinal and nutritional importance. It has been documented that from time immemorial, plants have been used medicinally. Many plants/plant extracts have been used as fertility agents in folklore and traditional medicines without producing apparent toxic effects. Several plants have been used to enhance fertility, producing results similar to that of Zinc, an example of which is the *Newbouldia laevis* (*N. laevis*) plant. *N. laevis* is a rapidly growing perennial shrub that belongs to the Bignoniaceae family and extensively found in West and Central Africa. It is popularly referred to as the 'tree of life' or 'fertility tree' in Nigeria. It is locally referred to as ewe Akoko, Aduruku, or Ogirisi by the Yoruba, Hausa and Igbo tribes respectively.

Most importantly, *N. laevis* has been shown to have a great impact on improving fertility in both males and females. According to the research carried out by Sansone and colleagues, the result reveals that *N. laevis* extract may produce inhibitory effects or induce hormonal imbalances in males at certain dosages, as exemplified in the experimental animal models.⁹ According to empirical evidence, in women, the *N. laevis* plant is used to treat a range of gynaecological issues, including miscarriage, irregular menstruation, suppression of menstrual flow, and others. These

effects have been attributed to the rich phytonutrient mix of the herb.¹⁰ Although *N. laevis* leaves modify several reproductive hormones in males, its overdose can modestly lower testosterone levels.¹¹ This research aims to determine the comparative effect of Zinc and graded doses of *N. laevis* on male fertility.

Materials and Methods

Collection and aqueous extraction of Newbouldia laevis

The leaves of *Newbouldia laevis* were harvested in Oke-ose Ilorin, Kwara State, Nigeria. The plant was authenticated at the Botany Department of the University of Ilorin, Nigeria. Extraction was done by methods described by Akande et al., 2020.¹⁰ The fresh leaves of the harvested *N. laevis* were washed with distilled water to remove dust and other foreign particles. The leaves were then air-dried on a clean surface at room temperature and ground into a fine powder using a blender. Exactly 400 g of the powdered leaf sample of *N. laevis* was soaked in a clean container with distilled water. The mixture was allowed to stand for 72 hours with constant stirring. The filtrate was then separated by repeated filtration with sterile filter paper. The filtrates were concentrated at room temperature; then, the aqueous part was allowed to dry.¹² The crude extracts obtained were stored in a tight container and kept in a refrigerator at 4°C for further use.

Animal care

Thirty-six (36) male Wistar rats were purchased from Ilorin. The animals were housed at the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. The rats were immediately weighed, and their weights recorded. They were kept in well-ventilated plastic cages with wood shavings. The Ethical Committee (Institutional Animal Care and Use Committee - IACUC) of the University of Ilorin approved the protocol with reference number UERC/ASN/2022/2362.

Animal grouping and treatments

The thirty-six (36) male Wistar rats weighing between 55 - 125g were obtained and bred in the animal house of the College of Health Sciences, University of Ilorin and were used for the research. The rats were randomly assigned into 6 groups of 5 rats each, viz: Normal Saline (NS), Zinc (ZN), Low dose short-term (LS), High dose short-term (HS), Low dose long-term (LL), High dose long-term (HL). The rats were fed daily with rat pellets grower mash. All animals were given water ad libitum. The animals were allowed to acclimatize for a period of 2 weeks. Animals were weighed once every week during this acclimatization period. Female Wistar rats were later purchased for mating as the research proceeded. The 50 mg-strength Zinc tablets were used

in this study.

Experimental design

Animals were randomly placed into six groups of five animals each, according to the treatment given. Group NS had normal saline, ZN had 100mg/kg of Zinc,¹³ and LS and HS were given 200mg/kg and 400mg/kg⁹ of *Newbouldia laevis* extract, respectively, daily for 28 days. While LL and HL had 200mg/kg and 400mg/kg daily for 56 days, respectively. The animals were weighed weekly for dose adjustments.

Animal sacrifice and tissue collection

The animals were sacrificed at 28 days and 56 days following daily administration via cervical dislocation. The rats' limbs were pinned in supination for easy access to the organs. A distal abdominal incision was made close to the urogenital area, and a cut was made along the abdomen, cutting the dome of the diaphragm and exposing the thoracic cavity, after which blood was carefully withdrawn from the apex (left ventricle) of the heart using a 5 ml syringe into an EDTA bottle. The testes were carefully excised from the abdomen and weighed using a sensitive scale to the nearest 0.1g. The relative testicular weights (RTW) were calculated (using the formula; $RTW = \text{Testicular weight} / \text{Final body weight}$)

The cauda epididymis was separated from the testis and minced in 2 ml of normal saline. The resulting epididymal sperm suspension was used in determining the semen parameters. The excised testes were fixed in 10% formaldehyde solution.

Semen Analysis

The percentage of progressively motile sperm was evaluated using a light microscope at 400× magnification. For this procedure, one drop of sperm suspension was placed in the Neubauer's counting chamber. Sperm motility was divided into four levels according to certain criteria such as slowly progressive forward movement, rapid progressive forward movement, residual motion and those motionless were counted in several microscopic fields and percentages of motile and immotile sperm cells were recorded.

In counting sperm cells, a small amount of prepared epididymal sperm suspension was diluted with 10% formalin in phosphate-buffered saline in the ratio 1:20. An aliquot of the diluted suspension, about 10ul was transferred into a Neubauer's counting chamber using a Pasteur pipette, and the solution was allowed to remain for seven minutes. Then the sperms at the four corners and the centre of the central square were counted.

Tissue processing for histological Analysis

The fixed testes underwent a series of histological

processes to enable viewing of the cytoarchitecture. Histological slides were examined under a light microscope at low (X10) and high (X40) magnifications and photographed using a USB camera and then comparisons were made between the control and treated groups.

Bcl-2 Expression

Sections of 5 µm thickness obtained from routine paraffin were deparaffinized and subjected to antigen retrieval by heating in a citrate-based antigen unmasking solution, pH 6.0 (Vector Labs, CA, USA) for 30 minutes in a steamer and allowed to cool on the bench at room temperature for another 30 minutes. Endogenous peroxidase blocking was performed in 0.3 % hydrogen peroxide in phosphate-buffered saline (PBS, pH 7.4) for 10 minutes. Sections were then incubated at room temperature for 2 hours in primary rabbit antibodies diluted in a universal antibody diluent and blocking reagent, UltraCruz® Blocking Reagent (Santa Cruz, USA). The primary antibody used was Bcl-2 (Novus Biologicals, USA; NB100-56098) at 1:2500, sections were washed in PBS and incubated in ImmPRESS™ HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, made in horse (Vector Labs, USA). Colour was developed with DAB Peroxidase (HRP) Substrate Kit (Vector Labs, USA), and sections were counter-stained in hematoxylin^{14,15}

Statistical Analysis

Data was expressed as mean ± standard error of the mean (SEM). The difference between means was determined using Analysis of Variance (ANOVA). A p-value < 0.05 was considered significant.

Results

Relative Testicular Weight

Animals in groups LS, HS and HL showed rapid weight gain, but this was not reflected in the testicular weights. There were no significant differences in the relative testicular weights across the groups. (See table 1)

Semen Analysis

Sperm motility

Groups ZN, LS and LL had significantly higher numbers of progressively motile sperms compared with the NS group. Group HS has the lowest percentage. HS and HL displayed significantly lower motility than the Zn group ($p < 0.05$). See Figure 1

Sperm Count

Findings in the sperm concentration are similar to those with the motility. The Zinc group had the highest concentration, with comparable counts in the LS and LL groups. The HS and HL groups have lesser numbers of sperm cells per milliliter of semen. (See Figure 2)

Table 1: Relative Testicular Weight across all animal groups

Groups	Weight gain	Final Body Weight	Testicular Weight	Relative Testicular Weight
NS	66.00 ± 13.79	173.8 ± 15.37	1.79 ± 0.434	1.338 ± 0.06
ZN	73.200 ± 36.18	210.6 ± 26.87	2.43 ± 0.200	1.190 ± 0.075
LS	101.80 ± 9.29*	241.8 ± 11.70	2.99 ± 0.22	1.246 ± 0.107
HS	94.80 ± 6.08*	236.4 ± 9.08	2.64 ± 0.06	1.124 ± 0.048
LL	76.40 ± 14.47	233.0 ± 14.15	2.53 ± 0.21	1.094 ± 0.088
HL	98.60 ± 11.84*	247.2 ± 16.59	2.90 ± 0.14	1.180 ± 0.054

* significant difference compared to NS

Table 2: Population of BCL-2-stained cells in the germinal epithelium

Group	Mean ±SEM
NS	790.00 ± 15.15
ZN	1028.00 ± 6.87*
LS	1035.00 ± 7.40*
HS	1043.40 ± 4.91*
LL	1131.00 ± 7.74*
HL	769.80 ± 14.32*

*Significantly different compared to NS

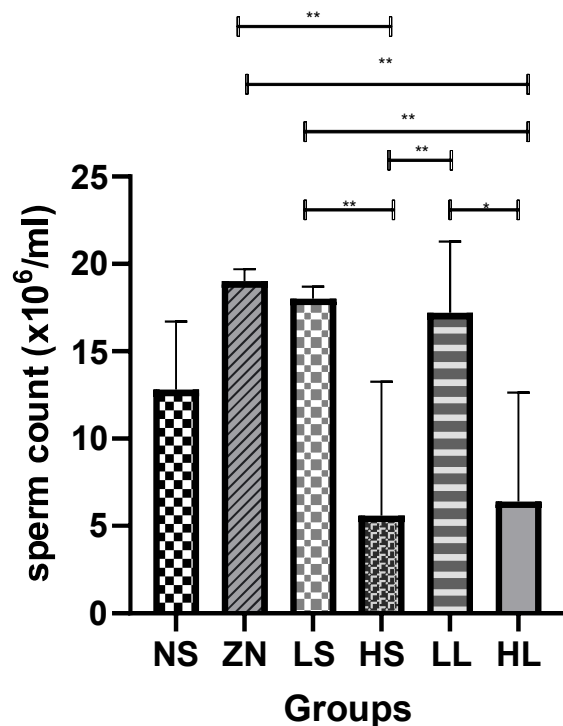


Figure 2: Sperm count for animals in groups NS, ZN, LS, HS, LL and HL. * indicates significant difference between two groups at $p < 0.05$, ** indicates significant difference between two groups at $p < 0.01$.

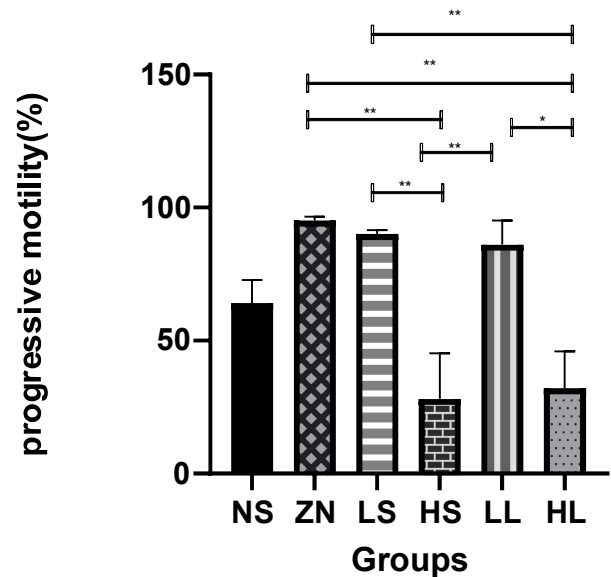


Figure 1: Progressive sperm motility for animals in groups NS, ZN, LS, HS, LL and HL. * indicates significant difference between two groups at $p < 0.05$, ** indicates significant difference between two groups at $p < 0.01$.

Histological Observation

Histological examination of tissue sections from the testes of animals in the experimental groups displayed varying observations. The NS group displayed normal progression of the cells of the spermatogenic series from the spermatogonic cells line lines on the basement membrane to the mature spermatozoa in the lumen. Testicular tissues of the rats receiving low and high doses of *Newbouldia laevis* displayed a depleted and distorted progression of the cells of the germinal epithelium. While the distortion in the high dose groups appeared to be accompanied by hyperchromasia, the low dose groups were associated with widening of the lumen and vacuolation in the germinal epithelium (see Figure 3).

Bcl-2 expression

BCL-2 is found on the outer membrane of cells' mitochondria, where it functions to support cellular viability and impede the activities of proteins that encourage apoptosis. Immunohistochemical

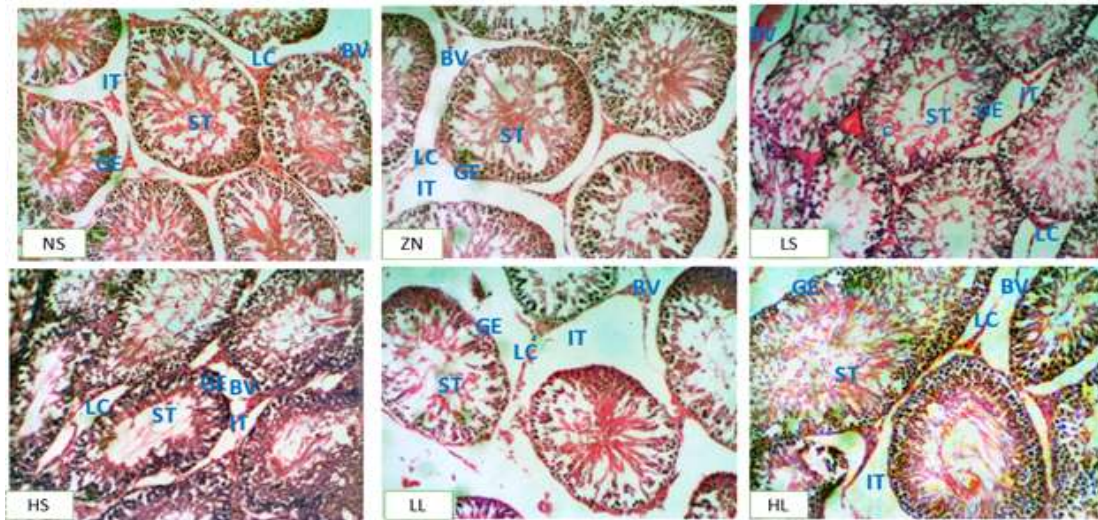


Figure 3: Histology of the testes of the Control and Experimental groups (H and E 100x magnification). NS – Control group, ZN – Zinc group, LS – Low dose of *N. laevis* in the short term, HS – High dose of *N. laevis* in the short term, LL – low dose of *N. laevis* in the long term, and HL- high dose of *N. laevis* in long term. GE- germinal epithelium, ST- spermatid, LC- Leydig cells, IT- Interstitium, BV- Blood vessels

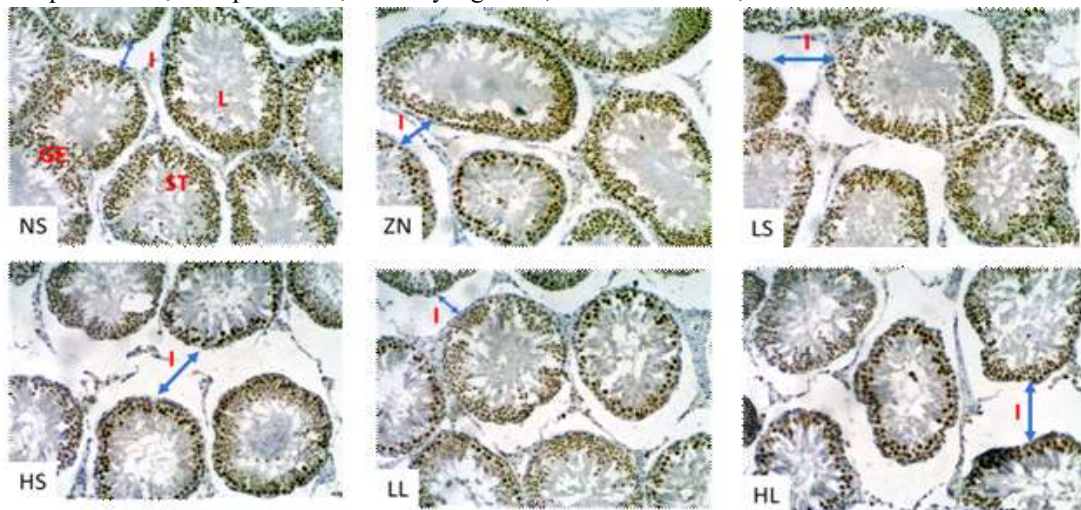


Figure 4: Immunohistochemical view of the testes of the Control and Experimental groups (BCL2 100x magnification). NS – Control group, ZN – Zinc group, LS – Low dose of *N. laevis* in the short term, HS – High dose of *N. laevis* in the short term, LL – low dose of *N. laevis* in the long term, and HL- high dose of *N. laevis* in the long term. GE- germinal epithelium, ST- seminiferous tubule, LC- Leydig cells, I- Interstitium.

expression of Bcl-2 in tissues, particularly the germinal epithelium of the testes, is an indication of the proliferative properties of its cells. In this examination, a 100x magnification was used to assess the expression of Bcl-2 in the seminiferous tubule of the rats' testes. The study revealed that rats in groups HS and HL displayed fewer Bcl-2 stained cells when compared to NS and ZN groups. The expression in LS and LL were comparable to that of the ZN group (see Figure 4).

BCL2 staining cell count

The number of Bcl-2-stained cells in the HL group was low compared to those of the other groups (NS, ZN, LS, and LL). The LS expressed the highest Bcl-2 in the germinal epithelium (see Table 2).

Discussion

The present study was carried out to determine the effect of *Newbouldia laevis* on the testicular histoarchitecture and compare its effect with that of Zinc, a trace element known for the vital role it plays in the development of the three major accessory sex glands in male reproduction (the testis, epididymis, and prostate). It plays a key role in the initial stages of germ cell development and spermatogenesis.⁶

Several pathological conditions such as varicocele and orchitis, as well as exposure to therapeutic and toxic environmental agents, can lead to testicular dysfunction through multiple mechanisms including oxidative stress, inflammation and apoptosis, which ultimately will lead to male infertility.¹¹ A search for

agents that can prevent and protect against reproductive challenges becomes imperative. Natural agents have become a thing in many health conditions today.^{16,17} In recent years, *Newbouldia laevis* has gained attention due to its massive and profound health benefits.¹⁸ Previous reports show the potency of *Newbouldia laevis* for the management of male infertility.¹⁶ However, its definitive role in male reproductive biology has not yet been evaluated.

The organ weight serves as a reliable as well as reasonably sensitive marker of testicular health.¹⁹ Testis size, weight, and tubular diameter often decrease as a result of the loss of germ cells from the seminiferous tubules and decreasing seminiferous tubule fluid secretion. Certain toxicants cause a brief rise in testis size due to an increase in interstitial fluid, but a drop in weight will always accompany the degeneration of the seminiferous epithelium.²⁰ In the study, the administration of *Newbouldia laevis* did not affect the testicular weight. The testicular weights were maintained even in the groups with altered testicular histoarchitecture.

Zinc is also involved in various biochemical processes of the body and is said to positively affect male fertility. Hence, in this study, it was found that Zn induced increases in sperm motility and count. A comparable effect was observed in low-dose *Newbouldia laevis*, irrespective of the duration, while high doses have shown deleterious effects on sperm motility. This was also observed in the effects on sperm count. Likewise, a recent study on rabbits revealed similar findings.²¹ In recent years, *Newbouldia laevis* has gained attention due to its massive and profound health benefits. Previous reports show the potency of *Newbouldia laevis* for the management of male infertility.¹⁶ Different animal species have been used to demonstrate the fertility-enhancing properties of *Newbouldia laevis* in males. Studies done in rabbits,²² Wistar rats,^{23,24} and albino rats¹⁷ suggest the beneficial properties of the herb. However, its definitive role in human reproductive biology has not yet been evaluated. The findings of these research also conclude that the low doses appear to be safer and more beneficial to the reproductive system.^{17,24}

The testicular histoarchitectural findings were also in tandem with the reports from semen analysis. The testes of the rats treated with a high dose of *Newbouldia laevis* displayed distorted germinal epithelium with very large interstitium and smaller seminiferous tubules. While this study has revealed both the beneficial and deleterious effects of *Newbouldia laevis* on male reproductive potential, many authors have reported the positive effects of this plant on fertility.^{18,21} *Newbouldia laevis* maintained the normal

histoarchitecture of the testes at modest doses, while degenerative changes were observed at higher doses.

The immunohistochemical study of Bcl-2 expression reveals that the protein was mostly seen in cell populations with a long lifespan and cells with the capacity to proliferate.²⁵ It has been shown that a reduction in Bcl-2 levels causes cell death through apoptosis.²⁶ Additionally, Bcl-2 overexpression has been shown to prevent epithelial cells from dying.^{27,28} In this study, Bcl-2 expression was higher with the use of *Newbouldia laevis*. This was observed in the spermatogonia cells and the primary spermatocytes. High doses of the herb exhibited depletion of the spermatogonia population in the germinal epithelium, which also indicates a reduction in cellular proliferation. The length of exposure to *Newbouldia laevis*, however, reveals no effect on the expression of Bcl-2 in the cells. Moderate uses of *Newbouldia laevis* may promote tissue regeneration and maintenance in the testes. This agrees with the findings of Adebisi et al., 2024.²³ Similarly, in a study of the effects of glyphosate-induced testicular toxicity in rabbits, *Newbouldia laevis* restored the testicular histoarchitecture.²²

This study revealed that low doses of *Newbouldia laevis* have a beneficial effect comparable to the use of Zinc on male fertility potential. However, the herb has been shown to have dose—and duration-dependent adverse effects on reproductive functions in male rats. A more detailed study of the biochemical effects of this herb will help analyze the exact mechanism for the observed results. Similarly, in a study of the effects of glyphosate-induced testicular toxicity in rabbits, *Newbouldia laevis* restored the testicular histoarchitecture.

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Sub-Acute Toxicological Evaluation of An Artemesia-Based Moringa Supplement (AMS)

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Abstract

Background: In developing nations, several natural medicine practitioners explore the use of plant-based supplements and other forms of phytotherapies to combat viral infection via immune-boosting mechanisms. One such supplement is a locally compounded and consumed Moringa supplement containing *artemisia annua* and named, artemisia-based Moringa supplement (AMS). The aim of this study was to evaluate the effect of a sub-acute (30 days) administration of AMS on the toxicological indices in albino Wistar rats of both sexes.

Materials and Methods: Thirty-two albino Wistar rats (170-220 g) were randomly assigned into four experimental groups: Group 1, served as control, while Groups 2-4 were administered 125, 250 and 500 mg/kg/day of AMS consecutively for 30 days. The animals were anesthetized, and cardiac puncture was used to obtain whole blood for hematology and serum biochemistry. The *in vivo* liver antioxidant assays were also carried out on the liver homogenates.

Results: There were no adverse hematological effects because of AMS treatment. Serum electrolytes levels were not affected across the doses of AMS administered for 30 days. Creatinine levels were not significantly altered, however a significant ($p = 0.037$) reduction in urea level was observed in the highest dose of 500 mg/kg. Liver function assays showed no significant alterations in the liver function enzymes, although, a significant decrease in the liver malonaldehyde (MDA, product of lipid peroxidation) level was observed. There was no adverse effect on kidney function parameters and serum lipid profile.

Conclusion/ Recommendations: The artemisia-based polyherbal supplement seems to be relatively safe at the doses considered for this experiment. However, further safety studies will be required to ascertain more detailed organ safety.

Keywords: *Artemisia annua*, Nutraceuticals, Hepatotoxicity, Serum biochemistry, Hematology

Introduction

The use of herbal medicines and other forms of phytotherapies, provides ample potentials for the development of new agents either as supplements, drugs, extract, pure compounds and their respective derivatives.^{1,2} Over the years, there has been a folkloric misconception that natural originating supplements are safe for medicinal purposes. This however lacks scientific validation.^{3,4} Several herbal therapies used in

folk medicine have compelling proof regarding their bio-medicinal activities.^{5,6} However, the major limitation to the use of these remedies is the paucity of scientific, mechanistic and clinical data in support of the efficacy and safety of these drugs.⁷ Toxicological evaluations of plant-derived medicinal in the form of supplements and therapeutic agent must be carried out to ascertain their preclinical safety profile, as this will increase the assurance of their safety in humans, particularly for use in the development of pharmaceuticals.

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Herbal formulations, medicinal plants or dietary supplements constitute a major part of our existence.^{8,9} Investigation of plants or their constituents as medicinal foods dates to the very history of man. Research findings suggest that a great percentage of Africans and Asians depend on natural product medicines as a key element of therapy and not just a

complementary aspect.¹⁰ The increased subscription for herbal medicines is linked to their accessibility, availability and affordability. Many constructs for the use of nutraceuticals/phytomedicines as sources of therapeutic agents are being explored, profiled and scientifically validated to herbal-based pharmacopoeia, like the West African Herbal Pharmacopoeia.¹¹ This affords a compendium of several plants, their derivatives, and vital information about their safety and possible efficacy. Plants produce several secondary metabolites, forming complex compounds that may be harmful or beneficial to humans.

Artemisia annua usually referred to as “annual absinthe” is an annual herbaceous plant, where it derived its name, “annua”. The plant is grown in Asia, Africa, Central Europe and Australia tropical regions.^{12,13} It is commonly used as a dietary condiment, herbal tea and medicinal plant in the mild climates of Asia, such as China and Korea; and some African countries, such as Nigeria.^{14,15} For several decades, *Artemisia annua* plant extract has been a main therapy in Asia and Africa for the treatment of malaria and fever, in the form of tea or herbal concoction.^{16,17}

We previously reported a 30-day toxicological assessment of a Nigerian polyherbal Moringa supplement,¹⁸ this present study evaluates a 30-day preliminary safety profile of a new *Artemisia*-based Moringa supplement (AMS). This will help understand the safety role of *artemisia* or otherwise in the previously reported herbal formula.

Materials and Methods

Experimental animals and Sub-acute Toxicological Evaluation: Rats (32) of both sexes were randomly assigned to four groups, one as control and the others for doses of 125, 250 and 500 mg/kg of AMS respectively. AMS extract and vehicle (distilled water, 600 µL for the control), were administered daily for 30 days using an orogastric tube. Wistar albino rats of both sexes weighing between 180 to 220g were purchased from *McTenny Farms*, a commercial private colony near Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria, and housed under natural light and temperature conditions within the Laboratory Animal House facility of the College of Health Sciences, University of Ilorin, Nigeria. Rat pellets (Growers' Mash, Ogo Oluwa, Ilorin, Kwara State, Nigeria) and water were made available *ad libitum*. The animals were acclimatized for one week prior to the experiment. Procedures involving animal handling followed the guidelines published by the National Institute of Health (NIH, 1996) and the University of Ilorin Ethical Review Committee (UIERC) with approval number:

UERC/ASN/2020/2034.

Reagents and Solvents: All reagents used were of analytical grade. Anesthetic agent (pentobarbital) and absolute ethanol, were obtained from Sigma Aldrich, USA.

Preparation of *Artemisia* based Moringa supplement (AMS): AMS, a natural product supplement containing, medicinal plants: leaves of *Moringa oleifera* (75%w/w), *Artemisia annua* (5%w/w) *Ocimum gratissimum* (5%w/w), *Garcinia kola* (bitter kola) (5%w/w), and *Zingiber officinale* (ginger) (5%w/w) were compounded by Biofuel® and Natural Product Herbal Supplement; NAPERBS). AMS 500g was constituted in absolute ethanol (98.7 %) for 72hr, filtered using Whatman filter paper 1. The filtrate was concentrated using a rotary evaporator at 40°C (BUCHI Rotavapor® Model R-215, Switzerland) with the vacuum Model V-801 EasyVac® Switzerland. The concentrate was weighed and tagged AMS extract.

$$\text{Percentage yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of PHS powder (g)}} \times 100$$

Hematological Assays: After 30 days of AMS administration, the rats were sacrificed by an intraperitoneal administration of 30 mg/kg pentobarbital and blood was drawn by cardiac puncture. A portion of the blood sample was put in a heparinized bottle for hematological assays, while the other portion was centrifuged for 20 min at 3000 g, and the serum was harvested and frozen for serum biochemical assay.

A wide-range of investigative hematological parameters was explored to access the percentage lymphocyte (% LYM), white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), hemoglobin concentration (HBG), etc., using an automated hematology analyzer from *HORIBA Pentra™ XL 80*, USA.

Serum Biochemical Assays: As previously described by Afolabi et al., serum electrolyte levels were assessed using instruments from TECO Diagnostics (CA, USA). Renal function determinants such as serum creatinine and urea levels were evaluated using kits supplied by *Spinreact™ autoanalyzer* (Girona, Spain). Liver function enzymes such as alanine transaminase (ALT), aspartate transferase (AST), and bilirubin levels were evaluated using the *Beckman Coulter LH 780 analyzer*. Lipid profile [total cholesterol (TC), triglycerides (Trig), high-density lipoprotein (HDL-c), and low-density lipoprotein (LDL-c)] were analyzed using commercial kits obtained from Randox Laboratories Ltd (Crumlin, UK), with strict adherence to the

manufacturer's protocol.¹⁸

Liver Antioxidant assays and MDA levels: Catalase, Superoxide dismutase (SOD) and reduced glutathione (GSH) activities were carried out on the liver homogenate post 30 days administration with AMS. Analytical kits obtained from *MyBiosource Inc.* (San Diego, USA) were used for the assay. Glutathione peroxidase and malonaldehyde (a marker of lipid peroxidation) levels were estimated using analytical kits obtained from *MyBiosource Inc.* (San Diego, USA) and *Elab Sciences* (Texas, USA) respectively, and following the manufacturer's protocol as previously reported.¹⁸ To assay for the product of lipid peroxidation (MDA levels), three major reagents containing clarificant, acid reagent (thiobarbituric acid) and a chromogenic agent were added successively to each experimental group in triplicate (including standard and experimental controls) according to the manufacturer's protocol, and absorbance was read at 532 nm with a microplate reader.

Statistical Analysis: The statistical analysis used was the one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test (*GraphPad prism IX software*, Boston, USA). Significance was considered at $P < 0.05$. All results are expressed as the mean $\pm$ standard error of the mean

Results

General Observation: A percentage extract yield of 15.25 % w/w was extrapolated for AMS extract. On an observatory note, the control and treatment groups did not show any physical sign of pain by writhing, stretching, or paw licking during the 30-day administration. There was no daily observance of fatigue or anxiety during the course of the experiment. Food and water intake was not different from the control group (data not presented).

Hematological Assays: After administering doses of AMS daily for 30 days, there were no statistical differences in the hematological indices such as WBC, LYM (%), PCV, RBC, MCV, HGB, etc. (Table 1).

Serum Electrolyte: At the dose of 250 mg/kg/day, the sodium level was significantly ($P = 0.032$) decreased (Table 2). Potassium ($P = 0.12$), calcium ($P = 0.09$) and bicarbonate levels ($P = 0.11$) were not significantly affected by the 30-day administration of AMS.

Liver and Kidney Function Parameters: Across the groups, ALT, AST, and bilirubin levels were not altered by the 30-day AMS treatment. In the same trend, for the kidney function indices, creatinine and urea concentrations were not significantly ($P < 0.05$) altered

Table 1. Effect of AMS on hematological indices

Parameters	Control (n = 4)	AMS (125 mg/kg) (n = 4)	AMS (250 mg/kg) (n = 4)	AMS (500 mg/kg) (n = 4)	p values
PCV	51.50 $\pm$ 1.60	50.67 $\pm$ 1.74	46.17 $\pm$ 2.34	52.33 $\pm$ 1.94	0.9318
HBG (g/dL)	16.65 $\pm$ 0.47	16.57 $\pm$ 0.48	15.41 $\pm$ 0.77	17.30 $\pm$ 0.49	0.7990
RBC ($10^6/\mu\text{L}$)	8.46 $\pm$ 0.21	8.34 $\pm$ 0.19	7.70 $\pm$ 0.40	8.59 $\pm$ 0.30	0.6021
WBC ($10^3/\mu\text{L}$)	5.72 $\pm$ 0.20	5.38 $\pm$ 0.16	5.27 $\pm$ 0.61	4.60 $\pm$ 1.01	0.1171
Platelet ($10^3/\mu\text{L}$)	67.83 $\pm$ 3.56	67.33 $\pm$ 12.42	66.00 $\pm$ 10.54	71.00 $\pm$ 10.72	0.3249
Lymphocytes (%)	77.50 $\pm$ 1.36	76.33 $\pm$ 2.02	74.00 $\pm$ 0.57	76.83 $\pm$ 1.19	0.0732
Neutrophils (%)	18.67 $\pm$ 1.36	19.67 $\pm$ 2.70	23.67 $\pm$ 0.61	20.17 $\pm$ 1.37	0.2320
Monocytes (%)	1.83 $\pm$ 0.30	2.17 $\pm$ 0.48	1.83 $\pm$ 0.40	1.67 $\pm$ 0.33	0.0928
Eosinophils (%)	2.00 $\pm$ 0.58	1.83 $\pm$ 0.79	1.17 $\pm$ 0.17	1.33 $\pm$ 0.42	0.0768
MCV (fL)	60.87 $\pm$ 0.57	60.71 $\pm$ 1.17	59.93 $\pm$ 0.38	60.90 $\pm$ 0.44	0.2196
MCH (pg)	19.69 $\pm$ 0.16	19.86 $\pm$ 0.31	20.01 $\pm$ 0.14	20.15 $\pm$ 0.18	0.5386
MCHC (g/dL)	3.23 $\pm$ 0.01	3.27 $\pm$ 0.03	3.34 $\pm$ 0.03	3.31 $\pm$ 0.05	0.1570

n = 4, * $P < 0.05$; statistical analysis: One-way Analysis of Variance (ANOVA)

Table 2. Effect of AMS on serum electrolyte indices.

n = 4, * $P < 0.05$; statistical analysis: One-way Analysis of Variance (ANOVA)

Parameters	Control (n = 4)	AMS (125 mg/kg) (n = 4)	AMS (250 mg/kg) (n = 4)	AMS (500 mg/kg) (n = 4)	p values
Na (mg/L)	149.50 $\pm$ 1.70	147.83 $\pm$ 1.85	145.16 $\pm$ 1.10	148.83 $\pm$ 1.77	0.5147
K (mg/L)	7.95 $\pm$ 0.14	7.70 $\pm$ 0.12	7.60 $\pm$ 0.04	7.80 $\pm$ 0.11	0.3277
Ca (mg/L)	11.28 $\pm$ 0.36	11.00 $\pm$ 0.29	11.00 $\pm$ 0.24	11.41 $\pm$ 0.37	0.8935
HCO ₃ (mg/L)	24.50 $\pm$ 0.62	23.50 $\pm$ 0.76	22.17 $\pm$ 0.54	22.82 $\pm$ 0.31	0.5863

Table 3. Effect of AMS administration on liver and kidney function indices
 $n = 4$, * $P < 0.05$; statistical analysis: One-way Analysis of Variance (ANOVA)

Parameters	Control ($n = 4$)	AMS (125 mg/kg) ($n = 4$)	AMS (250 mg/kg) ($n = 4$)	AMS (500 mg/kg) ($n = 4$)	p values
AST (U/L)	109.87 $\pm$ 3.62	101.82 $\pm$ 9.82	96.93 $\pm$ 7.17	93.10 $\pm$ 2.80	0.1857
ALT (U/L)	67.98 $\pm$ 2.95	61.89 $\pm$ 8.31	65.07 $\pm$ 5.71	55.22 $\pm$ 2.69	0.2235
Creatinine (mg/dL)	46.83 $\pm$ 3.27	56.83 $\pm$ 4.72	43.50 $\pm$ 9.38	51.00 $\pm$ 4.28	0.3161
Total Bilirubin (mg/dL)	3.16 $\pm$ 0.26	3.09 $\pm$ 0.33	3.24 $\pm$ 0.38	3.10 $\pm$ 0.24	0.8708
Urea (mg/dL)	52.68 $\pm$ 1.90	53.66 $\pm$ 1.10	55.77 $\pm$ 1.39	44.95 $\pm$ 5.35*	0.0365

Table 4. Effect of AMS on lipid profile indices

Parameters	Control ($n = 4$)	AMS (125 mg/kg) ($n = 4$)	AMS (250 mg/kg) ($n = 4$)	AMS (500 mg/kg) ($n = 4$)	p values
Triglyceride (mg/dL)	73.79 $\pm$ 4.89	79.51 $\pm$ 9.21	79.52 $\pm$ 6.48	70.23 $\pm$ 4.32	0.6028
Total cholesterol (mg/dL)	99.07 $\pm$ 9.48	92.18 $\pm$ 9.07	91.87 $\pm$ 2.61	86.62 $\pm$ 4.12	0.1701
HDL (mg/dL)	24.50 $\pm$ 1.53	26.01 $\pm$ 2.98	28.58 $\pm$ 2.06	24.31 $\pm$ 1.71	0.9992
LDL (mg/dL)	59.36 $\pm$ 8.94	50.27 $\pm$ 8.35	47.40 $\pm$ 2.72	48.27 $\pm$ 4.52	0.2593
VLDL (mg/dL)	14.76 $\pm$ 0.98	15.90 $\pm$ 1.84	15.90 $\pm$ 1.30	14.05 $\pm$ 0.87	0.6094
AI	2.00 $\pm$ 0.23	2.20 $\pm$ 0.45	1.86 $\pm$ 0.33	1.93 $\pm$ 0.19	0.5147
HDL/LDL	0.51 $\pm$ 0.14	0.60 $\pm$ 0.13	0.61 $\pm$ 0.06	0.55 $\pm$ 0.09	0.5608
CRI	4.01 $\pm$ 0.35	3.62 $\pm$ 0.30	3.27 $\pm$ 0.18	3.68 $\pm$ 0.37	0.7070

 $n = 4$, * $P < 0.05$; statistical analysis: One-way Analysis of Variance (ANOVA)Table 5. Effect of AMS treatment on liver antioxidant enzymes and MDA levels.
 $n = 4$, * $P < 0.05$; statistical analysis: One-way Analysis of Variance (ANOVA)

Parameters	Control ($n = 4$)	AMS (125 mg/kg) ($n = 4$)	AMS (250 mg/kg) ($n = 4$)	AMS (500 mg/kg) ($n = 4$)	p values
GSH (μ mol/ mg protein)	4.91 $\pm$ 0.17	6.64 $\pm$ 0.29	7.20 $\pm$ 0.19	7.08 $\pm$ 0.19	0.8060
MDA (η mol/ mg protein)	1.56 $\pm$ 0.24	1.09 $\pm$ 0.13	0.57 $\pm$ 0.10*	0.40 $\pm$ 0.10*	0.0027
Nitrite (μ mol/ mg protein)	1.78 $\pm$ 0.23	1.74 $\pm$ 0.17	1.78 $\pm$ 0.41	1.79 $\pm$ 0.18	0.4112
Catalase (U/ mg protein)	2.84 $\pm$ 0.19	5.44 $\pm$ 0.62	5.97 $\pm$ 0.56	5.52 $\pm$ 0.32	0.2796
SOD (U/ mg protein)	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.06 $\pm$ 0.01	0.07 $\pm$ 0.02	0.5232

when compared with the experimental control.

Serum Lipid Profile: Across the treatment groups, there was no statistically significant ($P < 0.05$) difference in the concentration of triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), Atherogenic index (AI) and coronary risk index (CRI) when compared with the control.

Liver Antioxidant Parameters and MDA Levels:

There was a significant ($P < 0.05$) increase in the reduced glutathione (GSH) levels across the treatment groups, when compared with the control (Table 5). The product of lipid peroxidation, malonaldehyde levels were also significantly ($P < 0.05$) decreased in both 250 and 500 mg/kg/day treatment groups. The concentration of catalase enzyme was also significantly ($P < 0.05$) increased across the treatment

groups when compared to the control. Sodium dismutase (SOD) and nitrite concentrations were not significantly altered as a result of AMS treatment, when compared with the control.

Discussion

This study evaluated the sub-acute toxicity profile of an artemisia based moringa supplement (AMS) consumed for its immune-boosting folkloric claims. In the sub-acute toxicological assessment, AMS did not affect the PCV, RBC, WBC counts and its differentials such as the lymphocytes, monocytes and granulocytes across the groups. Studies have shown that a reduction in neutrophils could denote an immunocompromised status in the animals, leading to susceptibility to several opportunistic infections.¹⁹⁻²⁰ An increase in WBC and lymphocytes suggests an active immunologic response to infections or stress, while a decrease in these differentials may connote a chronic state of infection.²¹ AMS does not portend an adverse effect on the animal's hematological indices which is crucial for optimal wellness.

Serum lipid profile is a vital investigatory tool to ascertain optimal cardiac functions, cardiovascular well-being and more pertinently, the risk of developing atherosclerosis and other cardiac-related diseases.²² Coronary risk index (CRI) (TC/HDL-C) and Atherogenic index (AI) (LDL-C/HDL-C) are vital indices for checking the risk of coronary heart diseases and atherosclerosis.²³ Our findings show that AMS did not significantly alter all the lipid parameters assayed for and their corresponding index, after the 30 days administration. Marked increase in serum LDL-c and total cholesterol levels have been linked to increased cardiovascular morbidity and mortality, whereas a high HDL-c level is cardioprotective.²⁴ This suggests that AMS does not increase the risk of atherosclerosis and other cardiovascular diseases.

At the doses considered in this study, AMS did not affect the serum electrolytes: Na^+ , K^+ , Ca^{2+} and HCO_3^- levels. assayed for. For example, Sodium ion is an osmotically active cation and a main electrolyte in the extracellular fluid. It is responsible for the maintenance of the extracellular fluid volume and regulating the membrane potential of cells.²⁵ A significant decrease in the serum concentration of Na^+ could be the result of increased Na^+ excretion alongside water, similar to a natriuretic or diuretic effect, or an underlying kidney or heart failure.²⁶ However, AMS didn't cause any electrolyte imbalance.

Creatinine levels as indicators of kidney functions was not significantly altered by AMS administration. Creatinine is a known indicator of chronic kidney disease (CKD).²⁷ Creatinine is a metabolic byproduct

in the serum, produced by the catabolism of muscle cells. Physiologically, optimal-functioning kidneys clear creatinine out of the blood and excrete it from the system via the urine.²⁸ In the case of an impaired kidney function, creatinine level increases because of its build-up in serum. AMS at the highest dose of 500 mg/kg caused a significant reduction in the level of urea.²⁹ A reduction in serum urea levels alone cannot be used as standalone diagnostic parameter. The assessment of liver and kidney functions is crucial, as these metabolic organs have several detoxifying mechanisms.³⁰ While the liver and kidney are pivotal to the process of detoxifying, the organs are also susceptible to damage during the process of detoxification. Therefore, several biochemical parameters are used in the assessment of hepatic dysfunction and liver cell damage.³¹ Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are accessors of liver functions. An increase in ALT, AST, ALP, and bilirubin denotes hepatocellular disease. An elevation in ALP and bilirubin suggests cholestatic disease.³² AMS did not significantly alter these liver enzymes.

Free radicals are produced in the body as a result of several metabolic processes. The body mops up these radicals via the machinery of antioxidants. The impaired ability of these antioxidants to clean up these radicals lead to oxidative stress, which underlies several cardiovascular and metabolic disorders.³³ Our findings showed no significant alterations in the liver antioxidant enzymes evaluated. Catalase is a vital antioxidant enzyme that catalyzes the conversion of hydrogen peroxide to oxygen and water; thus, neutralizing the cytotoxicity of peroxy radicals. Catalase deficiency in mice has been shown to increase the possibility of developing type 2 diabetes, obesity and fatty liver.³⁴ Malonaldehyde (MDA) concentration was dose-dependently reduced in the 250 and 500 mg/kg groups. MDA, a product of lipid peroxidation gives a scientific idea of a functional antioxidant system. A reduction in the MDA levels suggest a robust antioxidant defense system.³⁶ AMS could prevent lipid peroxidation in the liver, which is a major hepatoprotective mechanism.

Conclusion

Our findings suggest that AMS could be hepatoprotective via antioxidant mechanisms, although there is the possibility of hyponatremia at doses considered for this study. Further research into more specific toxicities such as neurotoxicity, genotoxicity reproductive toxicity and histological examination of vital organs will establish a more robust safety profile.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ameliorative Effects of *Nigella Sativa* Oil on Permethrin-Induced Neurotoxicity in the Cerebellum of Male Wistar Rats

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Abstract

Background: Permethrin, a pyrethroid insecticide commonly used for indoor residential treatment and outdoor applications can cause neuroinflammation and oxidative stress in rodent brains, resulting in a variety of neurodegenerative alterations. *Nigella sativa* oil (NSO) and its phytochemical components have been reported to possess anti-inflammatory, anti-hyperlipidemic, anti-oxidative and other beneficial properties. The goal of this study is to examine how NSO affects permethrin-induced neurotoxicity in the cerebellum of adult male Wistar rats.

Materials and Methods: Sixteen (16) young male rats were divided into four groups, with each group containing four rats. Group A (control) was fed on a regular rat diet, Group B was fed with rat diet mixed with 1000 mg/kg of 0.6% permethrin, Group C was fed a regular diet with 14 ml/kg of NSO, Group D was fed with rat diet mixed with 1000 mg/kg of 0.6% permethrin and 14 ml/kg of NSO. All administration lasted for 14 days. The experimental animals were sacrificed and their cerebelli were excised for post-mortem studies and biochemical analysis.

Results: Permethrin-treated animals presented with a reduction in the cerebellar level of glutathione peroxidase (GPx) with a corresponding increase in tumour necrosis factor alpha (TNF- α). Histological observations showed impaired cellular characterization in the cerebellum of permethrin-treated rats. *Nigella sativa* oil administration mitigated permethrin effects by increasing GPx level, decreasing TNF- α , and limiting cerebellar histoarchitectural alterations.

Conclusion/Recommendations: In conclusion, our study demonstrated that *Nigella sativa* oil significantly enhanced the activity of GPx (glutathione peroxidase) and reduced levels of TNF- α , suggesting its potential neuroprotective effects. These results underscore the therapeutic potential of *Nigella sativa* oil in mitigating oxidative stress and inflammation caused by neurotoxins like permethrin.

Keywords: Permethrin, *Nigella sativa* oil, neuroinflammation, oxidative stress, toxicity

Introduction

The nervous system's normal histoarchitecture and functions can be disrupted by exposure to harmful substances (neurotoxins), which can cause neurons and other brain cells to malfunction. Exposure to heavy metals like lead and mercury, substances used in chemotherapy, radiation treatment, drug therapies, organ transplants, certain foods and food additives, pesticides, industrial or cleaning solvents, cosmetics, and some naturally occurring chemicals can cause neurotoxicity.¹

Exposure to environmental toxins, such as pesticides, has been linked to an increased risk of developing neurological dysfunction.² Although the cause of neurodegenerative conditions is unknown, evidence regarding energy metabolism, excitotoxicity, and oxidative damage is becoming more persuasive.³ These processes interact in a sophisticated way. Neuronal depolarization, activation of N-Methyl-D-aspartate excitatory amino acid receptors, and increases in intracellular calcium buffered by mitochondria may result from a failure in energy metabolism. Mitochondria are the main intracellular source of free radicals; higher mitochondrial calcium concentrations enhance free radical production. However, mitochondrial DNA is particularly vulnerable to oxidative stress.⁴ Oxidative stress and mitochondrial damage have been implicated in the pathogenesis of many neurodegenerative conditions, such as Alzheimer's disease, amyotrophic lateral

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sclerosis, and Parkinson's disease.⁵

Due to its strong insecticidal action and minimal mammalian toxicity, permethrin, the most commonly used synthetic Type I pyrethroid insecticide, is widely utilized worldwide. Initially, permethrin was thought to have low toxicity in non-target animals. However, as its usage became more widespread globally, evidence emerged that it might cause neurotoxicity, immunotoxicity, hepatotoxicity, cytotoxicity, digestive system toxicity, reproductive effects, effects in the body.⁶ It affects the neurological systems of insects, disrupting neuronal functions by interfering with sodium channels, resulting in muscular spasms, paralysis, and death.⁷ Permethrin, a synthetic pyrethroid insecticide can be absorbed through various routes such as dermal, inhalation and oral routes with the oral route constituting the most effective route through which it enters systemic circulation (60-70 %).⁸

Once absorbed, it is distributed throughout the body as a result of its lipophilic nature. It also has the ability to accumulate in tissues including fats and brain tissues, crossing biological membrane as well as blood-brain barrier.⁸ It is metabolized in the liver and plasma through metabolic pathways such as hydrolysis and oxidation process leading to metabolites such as 3-phenoxybenzyl alcohol glucuronide in urine and others such as *cis*- and *trans*-dichlorovinyl chrysanthemic acids (DCVA) and phenoxybenzoic acid derivatives.⁸ The primary route of elimination is through excretion via urinary excretion which peaks within 12 to 24 hours post exposure.⁸

Permethrin has been shown to cause neuroinflammation in brain areas involved in memory creation, processing, and consolidation. Permethrin acts on the axons in the peripheral and central nervous systems, causing prolonged opening of sodium channels.⁸ Since neuroinflammation and cognitive impairment are inextricably linked, this clarifies one of the possible mechanisms by which permethrin influences behavior.⁹ The harmful effects of permethrin are increasingly being linked to oxidative stress, according to mounting data. In the newborn rat brain, permethrin causes neurotoxicity and oxidative stress. Prenatal permethrin exposure may impair brain development due to vascular alterations.¹⁰ More importantly, permethrin treatment throughout childhood may have long-term consequences in adulthood, affecting homeostatic processes, physiological indices, and oxidative status.¹¹

Phytochemical elements of certain medicinal plants, such as NSO, could be used in the treatment of

neurodegenerative conditions.¹² For millennia, NSO (*Ranunculaceae*) has been used to treat various animal and human maladies in diverse cultures worldwide. NS is consumed in different forms which includes seeds, oils and extracts. Previous studies have shown that *Nigella sativa* seeds contain both fixed and essential oils, proteins, alkaloids, and saponin. However, most of the biological activities of *Nigella sativa* are attributed to its main active constituent, thymoquinone.¹³ Thymoquinone is absorbed through the gastrointestinal tract when ingested with the oils through its lipophilic nature permits better solubility and uptake in the intestinal mucosa.¹⁴ Once absorbed, they are distributed throughout the body where they bind to plasma protein and cross biological and blood brain barrier to accumulate in various tissues.¹⁵ The active component of NSO is metabolized in the liver. Thymoquinone undergoes biotransformation through oxidation and conjugation through which therapeutic properties and inactive forms are prepared for excretion.¹⁶ Elimination of thymoquinone and other metabolites occurs through elimination in the urine and feces with a half-life which spans from a few hours to several days.¹⁷

NSO is medically effective against a wide range of clinical conditions, such as inflammatory conditions, infectious diseases, infertility, and neurological and mental illnesses, among others.¹² The goal of this study was to ascertain the role of NSO in alleviating neurochemical and histochemical alterations associated with permethrin-induced cerebellar injury in male Wistar rats.

Materials and Methods

Sixteen (16) young male Wistar rats (*Rattus norvegicus*), averaging 89.5 g were used. They were housed in plastic rectangular cages with gauze nets and fed standard rat feed on a daily basis containing 15% crude protein, 7% fat, 10% crude fiber, 1% calcium, 0.35% phosphorus, and a caloric measurement of 2.55 kcal/kg, purchased from Ogo-Oluwa Feeds and Flour Mill Limited, Ilorin, Kwara State. They had unlimited access to clean water and clean surroundings, with bedding changed everyday. Before the trial began, the rats were allowed to acclimatize for seven days.

Treatment of animals

A Rambo bug powder (RamboR®; Gongoni Company Limited, Kano, Nigeria), containing permethrin 0.6% and inert carriers 99.40%, was used. The animals were divided into four groups, each with four animals, labeled A-D respectively, with n=4/group. Group A (Control) had a normal diet. Group B had 0.6% permethrin (1000 mg/kg body weight) mixed with their diet.⁹ Group C received *Nigella sativa* oil (NSO)

(Hemani International KEPZ, Karachi, Pakistan) at 14 ml/kg via oral cannula. Group D was given a combination of a 0.6% permethrin diet and NSO at similar doses. The treatment lasted for 14 days to investigate the prolonged effects of NSO on permethrin toxicity in the cerebellum of male Wistar rats.

Tissue processing for histological, histochemical and immunohistochemical demonstration

The animals for qualitative analysis (4 rats per group) were sacrificed via cervical dislocation once the study was completed. On ice, the cerebellum was removed, and the cerebellar hemispheres were separated. For biochemical tests, the left cerebellar hemispheres were immediately placed in a 30% cold sucrose solution, while the right cerebellar hemispheres were fixed in 10% formal saline for histological and immunohistochemical examinations. The tissues were prepared for histology, embedded in paraffin, and sectioned using a rotary microtome at a thickness of 5 µm. Hematoxylin and eosin (H&E) staining was used for general histology, and cresyl fast violet (CFV) staining was used for the histochemical demonstration of Nissl substances.

Colorimetric assay for biochemical studies

Enzymatic experiments were conducted on right cerebellar hemispheres maintained in a 30% cold sucrose solution. The cerebellum of rats was assessed using a spectrophotometric approach employing a glutathione peroxidase assay kit (ELISA reader Diatech DR200BS, UK)¹³. Before beginning the procedures, all reagents and samples were brought to room temperature. Cerebellar tissues were weighed and homogenized with an automated homogenizer (Teflon homogenizer GR3200, China) at 4°C in an ice-cold 30% sucrose solution. The homogenate was poured into a 5 mL specimen container, which was then placed in an ice-filled centrifuge tube (IEC central GPSR, USA). The homogenate was centrifuged at 3,000 rpm for 15 minutes. The supernatants were aspirated into labeled glass cuvettes that were kept in

the freezer. The assay was conducted according to the instructions on the assay pack provided by the manufacturer. A pro-inflammatory cytokine, termed tumor necrosis factor alpha, was also used to assess the degree of neuroinflammation

Light microscopy

Histological sections were captured using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, USA).

Data analysis

This research was conducted at the Animal facility situated at the College of Health Science, University of Ilorin, Nigeria, and was approved by the University of Ilorin Ethical Review Committee (UERC) following the recommendation of the College of Health Sciences Ethical Review Committee, in compliance with the Institutional Animal Care and Use Committee (IACUC).

The numerical data obtained were analyzed statistically using one-way ANOVA, followed by Tukey's post hoc test with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA; version 8 for Windows). Results were expressed as mean ± SEM (standard error of the mean). Statistical differences between control and treated groups were determined at $p < 0.05$ and $p < 0.01$, indicating statistical significance.

Results

The body weights were measured after 14 days of treatment. The control group gained the most weight (Table 1), followed by permethrin, NSO, and the group co-treated with NSO and permethrin (0.002). The permethrin group had a reduced weight difference compared with the Control, but higher than the NSO group. The group co-treated with permethrin and NSO had the lowest weight difference.

Table 1: Mean of the initial and final weights for each group, mean weight difference, Mean cerebellar weight, and cerebellar/body weight ratio

Groups	Mean initial weight (g)	Mean final weight (g)	Mean weight difference (g)	Mean cerebellar weight (g)	Mean cerebellar/body weight ratio	p value
A: Control	85.25± 11.20	120.0± 2.79	34.75± 9.52	0.185 ± 0.015	0.001	$p < 0.05$
B: Permethrin (1000 mg/kg)	87.25± 9.49	113.0± 11.60	25.75± 2.98	0.240 ± 0.051	0.002	$p < 0.05$
C: <i>Nigella sativa</i> oil (14ml/kg)	89.20± 8.92	99.5± 9.36	10.25± 3.30	0.202 ± 0.026	0.002	$p < 0.05$
D: <i>Nigella sativa</i> (14ml/kg) oil + Permethrin (1000 mg/kg)	90.50± 7.41	101.0± 10.25	10.50± 3.50	0.157 ± 0.023	0.002	$p < 0.05$

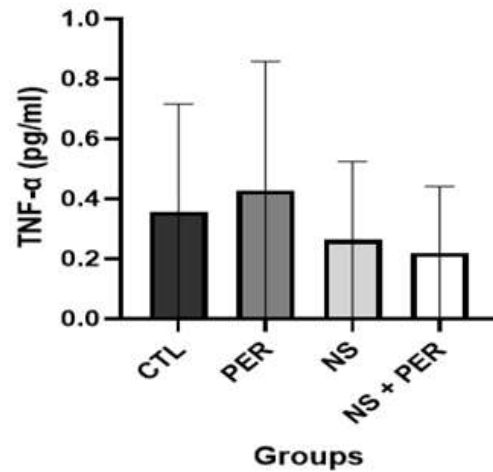
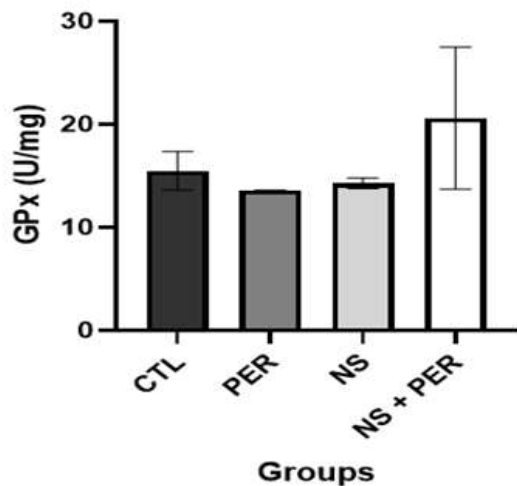


Figure 2. Graphical representation of cerebellar glutathione peroxidase (GPx) activity assayed across the four groups. **Figure 3.** Graphical representation of cerebellar tumor necrosis factor (TNF- α) activity assayed across the four groups.

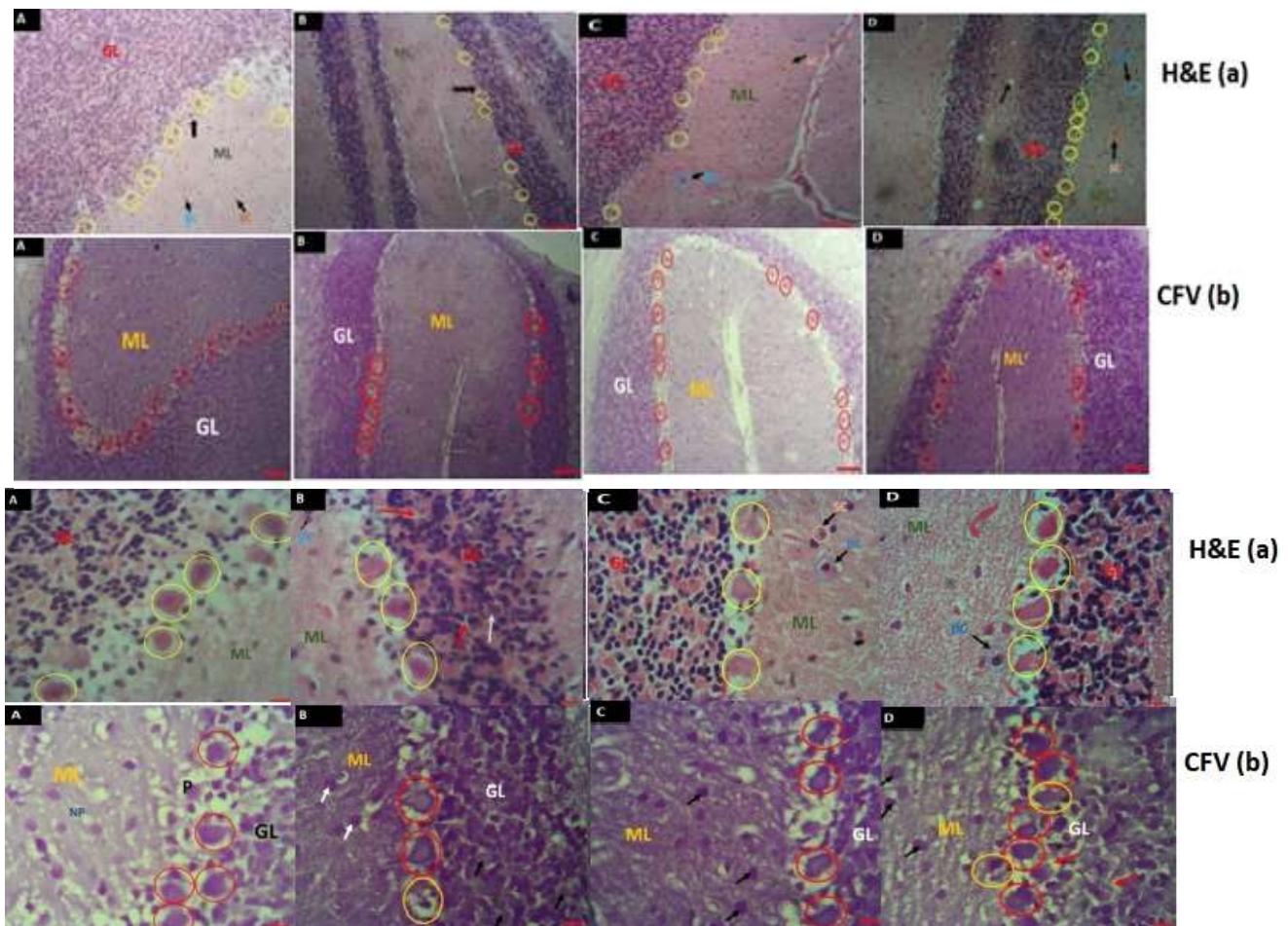


Figure 5. Representative photomicrographs of the cerebellum of the experimental animals stained with hematoxyline and eosin (a) and cresyl fast violet (b). (25 μ m scale bar).

***Nigella sativa* oil counterweights permethrin-induced oxidative stress in the cerebellum**

Cerebellar glutathione peroxidase (GPx) activity was assayed for in the four groups. Permethrin caused a decrease in GPx activities in the cerebellum (Fig. 2).

Nigella sativa oil administration caused an increase in the level of GPx compared with the permethrin-treated rats, while rats that were co-treated with NSO and permethrin had elevated levels of GPx relative to those treated with permethrin only. Permethrin caused a

decrease in GPx activities in the cerebellum (Fig. 2). *Nigella sativa* oil administration caused an increase in the level of GPx compared with the permethrin-treated rats, while rats that were co-treated with NSO and permethrin had elevated levels of GPx relative to those treated with permethrin only.

***Nigella sativa* oil reverses permethrin-induced cerebellar inflammation**

Permethrin treatment was associated with elevated level of tumour necrosis factor alpha (TNF- α) in the cerebellum of Wistar rats ($p>0.05$) in comparison with other groups (Fig. 3). The group treated with NSO had a lower TNF- α level compared with the permethrin-treated group, while rats co-treated with NSO and permethrin had lower TNF- α levels compared to the permethrin-treated group. Permethrin treatment was associated with elevated level of tumour necrosis factor alpha (TNF- α) in the cerebellum of Wistar rats ($p>0.05$). This was in comparison with other groups (Fig. 3). The group treated with NSO had a lower TNF- α level compared with the permethrin-treated group, while rats co-treated with NSO and permethrin had lower TNF- α levels compared to the permethrin-treated group. Representative photomicrographs of the cerebellum of the experimental animals. (a) showed a panoramic view of the cerebellar histomorphology stained with Hematoxylin and Eosin (100 μ m scale bar). The control (A) and NSO (C) groups presented with granular (GL) and molecular layers (ML) well delineated by Purkinje cells (yellow rings). The Permethrin only group (B) presented with reduced cellular density. Permethrin plus NSO (D) group presented with moderately thicker granular cell layers compared to 'B'. 'D' presented with deeply stained basket (BC) and stellate cells (SC). (b) showed the Nissl profiling of the cells in the cerebellar cortex of the experimental animals stained with cresyl fast violet (100 μ m scale bar). The control (A) and NSO (C) groups presented with typical Nissl staining intensity. The neurons were lightly stained and well situated in their respective neuropils. The outer molecular layer (ML) and the inner granular layer (GL) were clearly delineated by the Purkinje layer (red rings). Permethrin plus NSO group presented with deep Nissl staining intensity of the Purkinje cells (red rings) and granule cells. The outer molecular layer and the inner granular layer were clearly delineated by the Purkinje layer.

Effect of permethrin exposure on histoarchitecture and Nissl profile of the cerebellum

The neuronal morphology and cytoarchitectural arrangements of the cerebellum were studied using Haematoxylin and Eosin and Cresyl fast violet stains (Figures 4 & 5). Observation of the cerebellar cortex showed apparently normal histomorphology in the control groups. The cerebellar cortex of the control

animals and animals treated with NSO showed moderately stained cells with normal morphological presentation, cellular layering and cellular density, displayed a single layer of well outlined Purkinje cells with prominent soma and axonal projections plunging deep into the molecular layers. The granular layers of these two groups consist of small moderately stained granule cells with dense organization. Neuronal morphology of Permethrin-treated rats demonstrated fragmented cerebellar layers with cryptic granules, degenerating Purkinje cells with pyknotic cell bodies, and short dendritic processes. However, the rats treated with Permethrin and NSO indicated cerebellar layers and neuronal morphology, similar to that of the control with the exception of deeply stained granule cells which were moderately stained in control and NSO groups.

Figure 4 showed representative photomicrographs of the cerebellum of the experimental animals. (a) showed a panoramic view of the cerebellar histomorphology stained with Hematoxylin and Eosin (100 μ m scale bar). The control (A) and NSO (C) groups presented with granular (GL) and molecular layers (ML) well delineated by Purkinje cells (yellow rings). The Permethrin only group (B) presented with reduced cellular density. Permethrin plus NSO (D) group presented with moderately thicker granular cell layers compared to 'B'. 'D' presented with deeply stained basket (BC) and stellate cells (SC). (b) showed the Nissl profiling of the cells in the cerebellar cortex of the experimental animals stained with cresyl fast violet (100 μ m scale bar). The control (A) and NSO (C) groups presented with typical Nissl staining intensity. The neurons were lightly stained and well situated in their respective neuropils. The outer molecular layer (ML) and the inner granular layer (GL) were clearly delineated by the Purkinje layer (red rings). Permethrin plus NSO group presented with deep Nissl staining intensity of the Purkinje cells (red rings) and granule cells. The outer molecular layer and the inner granular layer were clearly delineated by the Purkinje layer.

Figure 5 showed representative photomicrographs of the cerebellum of the experimental animals. The control (A) presented with scanty granule cells, and moderately stained Purkinje cells (yellow rings). NSO (C) groups presented with lightly stained molecular layer, moderately stained granular layer with moderate cell population and moderately stained Basket (BC) and Stellate cells (SC). The Permethrin only (B) group presented with lightly stained Purkinje cells with chromatolytic changes, fragmented Purkinje cells (yellow rings) (b), cellular fragmentation (red arrow) and granule cell chromatolysis (white arrows) (a), and deeply stained basket cells (white arrows) in the molecular layer (ML) (b). Permethrin plus NSO (D)

group presented with well stained Purkinje cells, Basket cells, Stellate cells and Granule cells. (25 μ m scale bar).

Discussion

The use of Permethrin as a pesticide to control domestic insects that are a nuisance and a health risk is on the rise¹⁹. Despite being a highly effective pesticide, there is mounting evidence of Permethrin toxicity²⁰. Permethrin has been linked to a variety of health problems according to several studies¹¹. The effects of Permethrin on body weight fluctuations, oxidative stress, and cerebellar histomorphology in adult male Wistar rats were investigated in this study, as well as the effect of NSO on ameliorating these effects.

In the current study, permethrin caused a reduction in body weight of rats, but increased cerebellar weight, similar to what we have previously noted⁹. *Nigella sativa* oil administration was associated with significant weight loss in the current study. This is consistent with the work of Mahmoudi *et al*²¹ who studied the effects of *Nigella sativa* extracts on the lipid profile and uncoupling protein-1 gene expression in mice. The capacity of NSO to boost UCP-1 expression, an uncoupling protein that reduces the proton gradient created during oxidative phosphorylation in the mitochondria, could be linked to its potential to improve metabolic energy expenditure and hence, weight loss²¹. *Nigella sativa* oil improves lipid profile by decreasing plasma levels of low density lipoprotein and increasing plasma levels of the high density lipoprotein, making it ideal for the treatment of hypertension caused by hypercholesterolemia and coronary heart disease.^{22,23}

Glutathione peroxidase (GPx) is an oxido-reductase enzyme important in maintaining the homeostasis of reactive oxygen species.²⁴ Its level was assessed in the current work to determine the oxidative status of the cerebellum. Permethrin is an exogenous source of free radicals²⁵, which are capable of adversely affecting biological molecules, thereby altering the normal redox status. Permethrin causes oxidative stress by producing reactive oxygen species (ROS) such as hydrogen peroxide and reactive nitrogen species (RNS) which deplete the intrinsic antioxidant system, as seen in this study. Permethrin also increases induced nitric oxide synthase (iNOS) mRNA expression, which contributes to oxidative stress. Oxidative stress causes lipid peroxidation, with a consequent compromise of plasma membrane integrity and associated degenerative cellular changes²⁶. Introduction of NSO improved the oxidative status of rat's cerebellar tissue with a resultant cytoprotective action on the integrity of the cell membrane. This was deduced from the elevated levels of GPx in the cerebellum of rats co-treated with *Nigella sativa* and permethrin relative to those treated with

permethrin only. These findings suggests that *Nigella sativa* was able to deplete the excessively generated reactive oxygen species (ROS) by the actions of permethrin. Hence, *Nigella sativa* prevents oxidative stress induced by permethrin. This observation is consistent with previous studies that observed increased intrinsic anti-oxidative enzymes following NSO administration²⁷.

Innate immune responses in the central nervous system (CNS) can be activated in a variety of ways to eliminate foreign molecules, infectious pathogens, dying and apoptotic neurons, or changed proteins that develop as a result of stress, aging, injury, or other pathologies. TNF- α , alongside other neuroinflammatory markers are produced by macrophages and microglia as an initial response to injury to the body and (CNS). Chronic microglia activation, while not immediately harmful to neurons and may even be protective, may cause neuronal damage through signaling to the blood brain barrier. As a result, cells from the adaptive immune system are recruited into the CNS.²⁸ The innate immune system increased the production of TNF- α a pro-inflammatory marker, after brain impairment caused by permethrin exposure, according to this study. The neuroinflammation generated by permethrin was reversed by NSO, as demonstrated by a decrease in cerebellar TNF- α levels in the rats co-treated with NSO and permethrin. This finding underlines the anti-inflammatory effects of *Nigella sativa*.

Despite the short duration of permethrin exposure, morphological changes in the cerebellar cortices of the experimental rats were identified. Some of these changes such as degenerative and chromatolytic changes, were caused by assaults on cerebellar cells and tissues as a result of neurochemical and oxidative perturbations. The cellular components and the normal architectural structure of the cerebellum were damaged to varying degrees by permethrin-induced cerebellar injury. These changes have a negative impact on signal processing and synaptic complex's functionality, as seen in patients with neurodegenerative disorders. The pathogenesis of major neurodegenerative diseases has been linked to oxidative stress and lipid peroxidation. The findings above were consistent with the decreased endogenous oxidative status that followed permethrin toxicity, and oxidative stress has been proposed as a component of the pathogenesis of neurodegenerative disorders linked to neurotoxicity. This study found that administering NSO reverses and corrects the effects of permethrin on cerebellar morphology, consistent with previous observations.²⁹

Conclusions

Permethrin toxicity significantly alters cerebellar morphology and neurochemistry by causing oxidative damage and neuroinflammation. Therefore, caution is

advised during use, and body contact should be avoided. Administration of *Nigella sativa* oil for 14 days demonstrated therapeutic capabilities and cytoprotective potential in reducing the morphological and oxidative damage associated with permethrin-induced neurotoxicity. While considered safe in appropriate doses, long-term benefits may depend on duration, sex differences, and dosage.

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Review of *Plasmodium vivax* Malaria in Indonesia and Malaysia: Epidemiology of The Infection and The Presence of The Vector Across Time

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Abstract

Background: *Plasmodium vivax* causes malaria is a major public health concern in Southeast Asia, particularly in Indonesia and Malaysia. *Anopheles punctulatus*, a common vector, transmits the disease via the bite of infected female *Anopheles* mosquitos. Understanding the epidemiology and entomology of vivax malaria is critical for developing successful control and prevention strategies.

Materials and methods: This study investigates the epidemiology and entomology of *Plasmodium vivax* malaria in Indonesia and Malaysia over a period of time.

Results: Malaria research in Indonesia and Malaysia can provide insights into the parasite's genetic diversity and help tailor interventions to specific challenges. Findings from this review will contribute to global efforts to eradicate malaria and mitigate its effects on vulnerable populations. *Plasmodium vivax* malaria, which is prevalent in Indonesia and Malaysia, has been extensively studied by researchers. Variations in transmission dynamics and entomological factors have had an impact on the disease's spread and persistence. The emergence of *P. knowlesi* as a common cause of malaria in Malaysia demonstrates the intricate interplay between human behaviour and environmental factors.

Conclusion/Recommendations: The results of artesunate-mefloquine and artemisinin-based combination therapy demonstrate the importance of effective antimalarial regimens. *P. vivax*'s unique genetic diversity and potential for out breeding highlight the importance of tailored interventions. More research is needed to better understand disease transmission dynamics and develop targeted interventions.

Keywords: *Plasmodium vivax* Malaria, epidemiology, entomology, human behaviour, environmental factor

Introduction

Malaria is one of the many infectious diseases caused by plasmodium through the bite of the female *Anopheles* mosquito. The breeding grounds for these mosquitoes are usually in puddles of fresh or salt water, rice fields, river estuaries where the flow is not heavy and small ponds containing rainwater.¹ Plasmodium is one of the many contributors to morbidity and mortality among the 2.85 billion people living at risk of infection. The geographical distribution is very wide, ranging from areas with cold, subtropical, to tropical climates.²

In the tropical regions of Indonesia and Malaysia, *Plasmodium vivax* Malaria has been a persistent public health concern for centuries. The introduction of this essay aims to provide a comprehensive examination of the epidemiology and entomology of this specific type of malaria across time in these two countries. By analyzing historical records, recent epidemiological studies, and entomological data, this research seeks to uncover trends, patterns, and potential explanations for the prevalence and transmission dynamics of *P. vivax* Malaria in the region. Through a multidisciplinary approach, we aim to shed light on the factors influencing the spread of this disease, such as climate change, human movement, and vector behavior. Ultimately, this study seeks to contribute to the body of knowledge on malaria control and prevention strategies in Indonesia and Malaysia.

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Plasmodium vivax malaria, caused by the parasite *Plasmodium vivax*, is a significant public health concern in many regions of the world, including Indonesia and Malaysia. This type of malaria is

characterized by recurrent fever episodes, which can be challenging to distinguish from other types of malaria. The transmission of *P. vivax* malaria is primarily through the bite of infected female Anopheles mosquitoes, with Anopheles punctulatus being a common vector in Southeast Asia. Historically, Southeast Asia has been a hotspot for vivax malaria due to factors such as climate, land use changes, and population movements. Understanding the background and epidemiology of *P. vivax* malaria in Indonesia and Malaysia is crucial for effective control and prevention strategies to combat this disease. Research studies have shown that variations in transmission dynamics and entomological factors have influenced the spread and persistence of *P. vivax* in these regions over time.³

It is paramount to examine the significance of studying Malaria in Indonesia and Malaysia due to the substantial burden this disease places on both countries. Malaria, caused by the Plasmodium parasite, continues to be a public health concern in these regions, with a high incidence of cases reported annually. By understanding the epidemiology and entomology of *Plasmodium vivax* Malaria in Indonesia and Malaysia, researchers can identify factors contributing to the transmission of the disease, develop effective prevention strategies, and enhance current control measures. Furthermore, studying Malaria in these countries can provide valuable insights into the genetic diversity of the parasite and help tailor interventions to address specific challenges faced in the region. Ultimately, this research can contribute to the global efforts in eradicating Malaria and reducing its impact on vulnerable populations.⁴

Understanding the research objectives is crucial in framing the study design and guiding the investigation into *Plasmodium vivax* Malaria in Indonesia and Malaysia. The primary aim of this research is to explore the epidemiology of *P. vivax* malaria in these regions, with a focus on understanding the trends in prevalence over time. By analyzing demographic data, such as age, gender, and geographical distribution of cases, we aim to identify high-risk populations and areas for targeted intervention strategies. Furthermore, the entomological aspects of *P. vivax* transmission will also be investigated to enhance our understanding of vector behaviour and its role in disease spread. Through a comprehensive investigation of both epidemiological and entomological factors, this study seeks to contribute valuable insights into the dynamics of *P. vivax* malaria in Indonesia and Malaysia, ultimately informing more effective control measures in the future.⁵

The scope of this study will focus on tracing the epidemiology and entomology of *Plasmodium vivax*

Malaria in Indonesia and Malaysia over a specified period of time. By examining historical data, trends, and patterns related to this specific type of malaria, we aim to shed light on the factors contributing to its prevalence and transmission dynamics in these two countries. This extensive analysis will provide insights into the impact of environmental, social, and geographical factors on the spread of the disease, as well as the effectiveness of control measures implemented over time. By delving into the past and present of *Plasmodium vivax* Malaria in Indonesia and Malaysia, this study seeks to contribute valuable information to the existing body of knowledge on malaria epidemiology and entomology. Through a comprehensive examination of relevant literature and data sources, we aim to provide a holistic understanding of the complexities surrounding this specific type of malaria in these regions.⁵

Furthermore, this paper aims to investigate the epidemiology and entomology of *Plasmodium vivax* malaria in Indonesia and Malaysia across different time periods. By analyzing historical and present data on malaria cases, vector abundance, and control strategies, this study seeks to identify any trends or patterns that may have emerged over time. The paper also explore the impact of environmental changes, human behavior, and public health interventions on the transmission dynamics of *P. vivax* malaria in these two countries. By synthesizing past research findings with current data, this study intends to contribute to a better understanding of the factors influencing the spread of *P. vivax* malaria in Southeast Asia and provide insights for future prevention and control efforts.

Materials and methods

This research was based on a literature review. Online literature searches were conducted in Science Direct, Scopus databases, and Google Scholar. Search strategies included the following keywords: *plasmodium vivax*, malaria, Indonesia, Malaysia, epidemiology, entomology. Searches were conducted in English, with no limitations on the year of publication.

Epidemiology of *Plasmodium Vivax* Malaria

Plasmodium vivax is estimated to contribute to about 14 million cases of malaria each year.⁶ High cases of illness and death are often associated with vivax malaria. However, in some endemic areas, diagnosis, prevention, treatment, and control are not optimal. Malaria vivax was once thought of as a benign infection, but it can now cause severe pain in a variety of epidemiological settings. This parasite can cause widespread indirect death due to recurrent infections and the cumulative risk of severe anemia.⁷

The number of malaria cases in Indonesia over the past few years has increased. The Indonesian Ministry of Health said that in the 2020-2022 period, there were at least 443,530 cases. The Eastern Indonesia region dominated around 400,252 cases in 2022, of which Papua Province contributed 356,889 cases from the national figure.⁸ On the other hand, WHO reported that malaria in Malaysia from 2015-2021 reached 23,214 cases, of which 87% were zoonoses. Parasites of the genus *Plasmodium* are the cause of this disease. Not only dangerous to humans, plasmodium also endangers birds, reptiles, mammals, and other species of vertebrates.⁹

In East Kutai Regency, Indonesia, a study revealed key insights into the profile of malaria infections, highlighting a predominance of cases in males aged 25 to 34 years with high forest contact, predominantly caused by *P. vivax*.¹⁰ Additionally, genetic epidemiology studies in Papua, Indonesia, underscored long-term changes in *P. vivax* populations, showing a decrease in polyclonal infections over time and the persistence of strains for up to 9 years, indicative of higher rates of outbreeding in *P. vivax* compared to *P. falciparum*. The findings emphasize the importance of understanding genetic diversity and transmission dynamics in shaping malaria control strategies, particularly in regions with endemic *P. vivax* transmission like Indonesia and Malaysia.¹¹

Historical Overview of Malaria in Indonesia and Malaysia

Malaria has significantly impacted Indonesia and Malaysia for centuries, with frequent outbreaks leading to high morbidity and mortality rates. Various control measures, including insecticides, bed nets, and prophylactic drugs, have been implemented, but challenges persist, especially in rural areas with limited healthcare access. Despite ongoing efforts, malaria remains a major public health concern. By analyzing past experiences, policymakers and healthcare professionals can develop more effective strategies to combat *Plasmodium vivax* malaria in the region.³

Historical records show that Dutch traders arrived in Indonesia in the late 16th century and colonized the region for two centuries. Early documents suggest that a fever, likely malaria, affected traders. Since the central government and VOC administration were based in Java, most early malaria reports originated there. By the 18th century, Batavia (Jakarta) experienced severe malaria outbreaks, causing high morbidity and the deaths of thousands of VOC employees annually.¹²

Current Epidemiological Data

The number of malaria cases in Indonesia over the past few years has increased. The Indonesian Ministry of

Health said that in the 2020-2022 period, there were at least 443,530 cases. The Eastern Indonesia region has a population of around 400,252 cases in 2022, of which Papua Province contributes 356,889 cases from the national figure.⁸ Between 2015 and 2021, Malaysia recorded 23,214 malaria cases, with 87% classified as zoonotic, according to WHO. The disease is caused by *Plasmodium* parasites, which not only threaten humans but also birds, reptiles, mammals, and other vertebrates.⁹ Meanwhile, the majority of malaria cases caused by *Plasmodium vivax* in Malaysia were in Peninsular Malaysia during 2013-2017. The average mortality rate due to this species was the lowest compared to *Plasmodium falciparum* and *Plasmodium knowlesi*.¹³

In Malaysia, the recurrence rate is 836 cases per 10,000 people per day. This phenomenon is predicted due to climate change, mode of transmission, and vector conditions. The socioeconomic impact of this disease also contributes to a country. If it is not monitored regularly, it will affect development in the country.¹⁴ Many studies have revealed that *P. Vivax* is more difficult to remove than *P. falciparum* because of the characteristic unconscious recurrence characteristic or hypnozoites.¹⁵

The analysis of imported malaria data helps shape prevention strategies in malaria-free regions. A study¹⁶ highlights the ongoing risk in China, with most *Plasmodium falciparum* cases originating from Western Africa. It emphasizes the need for targeted health education and improved surveillance in county-level medical institutions. Moreover, the findings by¹⁷ emphasize the utility of methaemoglobin levels as a surrogate endpoint for assessing the efficacy of 8-aminoquinoline drugs in preventing *P. vivax* recurrence. The study of imported malaria trends aids in developing prevention strategies for malaria-free regions. In China, most *Plasmodium falciparum* cases come from Western Africa, highlighting the need for targeted health education and enhanced surveillance at local medical institutions.

Factors Contributing to Malaria Transmission

Various factors contribute to the transmission of malaria, particularly in regions like Indonesia and Malaysia. One key factor is the prevalence of suitable mosquito vectors, such as *Anopheles* mosquitoes, which are efficient carriers of the *Plasmodium* parasite. These vectors thrive in tropical climates with high humidity and abundant standing water. Additionally, human activities, such as deforestation and urbanization, can create more habitats for mosquitoes to breed, increasing the risk of transmission. Socioeconomic factors, such as poor access to healthcare and inadequate vector control measures,

also play a significant role in the spread of malaria in these regions.⁵

Some areas of Lampung Province, Indonesia have become malaria endemic areas due to environmental factors in the farm where there are many swamps, brackish waterlogging by the seaside, and fish ponds that are not maintained. This condition can certainly be a habitat for *Anopheles sp* mosquitoes.¹⁸ Kalimantan, covering much of the island's southern region and bordering Malaysian Borneo, has historically been a hotspot for *Plasmodium falciparum* and *Plasmodium vivax* transmission, contributing to malaria spread in Malaysia. In recent decades, deforestation of one-third of Kalimantan's forests has led to urbanization, wildlife migration near settlements, and climate changes, all of which have intensified malaria transmission.¹²

Demographic factors, particularly population density, can influence malaria transmission. People returning from vacations or work in endemic areas are vulnerable to infection through *Anopheles* mosquito bites. *Plasmodium vivax* presents an even greater challenge, as it can remain dormant in the liver for extended periods, causing recurrent infections. This persistence highlights the need for timely diagnosis and proper treatment to prevent further spread and complications.¹⁹ Malaria has been a priority public health burden in Eastern Indonesia since 2005-2010 based on a report by the local Health Office.²⁰ Understanding and addressing these contributing factors is critical to effectively combating malaria transmission and reducing the burden of disease in Indonesia and Malaysia.

Impact of Climate Change on Malaria Distribution

P. vivax transmission relies on the presence of mosquito vectors, their bite rate, and parasite development. However, these factors are heavily influenced by climatic conditions, such as temperature, humidity, and rainfall, which affect mosquito breeding and parasite maturation, ultimately impacting malaria spread.²¹ Recent studies indicate that climate change is altering the distribution of malaria-carrying mosquitoes. In endemic regions like Indonesia and Malaysia, rising temperatures and shifting precipitation patterns have created ideal breeding conditions, increasing malaria cases. Additionally, warming temperatures have allowed mosquitoes to expand into higher altitudes, exposing new populations to the disease and complicating control efforts. Understanding the relationship between climate change and malaria transmission is essential for developing effective interventions and policies to mitigate its impact.²² Global climate change, encompassing temperature, humidity, light, rainfall, and wind patterns, directly affects mosquito reproduction and parasite

development. Low humidity shortens mosquito lifespan, but it can still influence parasite survival and transmission dynamics, impacting malaria spread.²³ The western and central regions of Indonesia have warm temperatures that facilitate the multiplication of *P. falciparum* and *P. vivax*. In contrast, the eastern region, characterized by numerous high mountains, has a more humid and cooler climate. This lower temperature differentiates it from other parts of Indonesia and may influence malaria transmission dynamics.²⁴

Control and Prevention Strategies

Vector control measures such as indoor residual spraying, insecticide-treated bed nets, and larval control have been successful in reducing malaria transmission. Additionally, early diagnosis and prompt treatment with effective antimalarial drugs are essential for preventing severe complications and interrupting the transmission cycle.²⁵ Community engagement and education are crucial in promoting preventive behaviors and encouraging timely healthcare-seeking. Continuous surveillance of malaria cases, vector populations, and drug resistance is essential for adapting interventions and assessing their effectiveness. A comprehensive approach that integrates vector control, case management, and active community participation is key to achieving sustainable progress in controlling and eliminating *Plasmodium vivax* malaria in the region.

Entomology of Plasmodium Vivax Malaria

According to WHO and the Indonesian Ministry of Health, *P. vivax* only infects young erythrocytes, resulting in a lower parasite density in the blood. However, its ability to enter a hypnozoite phase allows the parasite to remain dormant in the liver, leading to relapses or recurrences. This makes detection and eradication challenging, requiring targeted treatment strategies.²⁶ In Indonesia and Malaysia, *Anopheles maculatus* and *Anopheles aconitus* are primary vectors of *P. vivax* malaria. Their differing blood-feeding and resting behaviors influence disease transmission and distribution. Understanding their biology and ecology is essential for developing effective vector control strategies. Additionally, research on insecticide resistance in *Anopheles* populations highlights the need for innovative approaches to combat *P. vivax* malaria in endemic regions.³ By integrating entomological data into malaria control programs, we can ultimately work towards eliminating this disease in Indonesia and Malaysia.

Anopheles Mosquito Vectors in Indonesia and Malaysia

In understanding the dynamics of *Anopheles* mosquito vectors in Indonesia and Malaysia, essential insights

from recent studies shed light on the genetic diversity and potential implications for malaria transmission. The research in Benin highlighted the presence of non-falciparum species contributing to malaria cases, emphasizing the significance of identifying various *Plasmodium* species for effective control programs²⁷.

Similarly, investigations in Malaysia revealed distinct genetic clusters within *Anopheles latens*, possibly indicating cryptic species, while other vectors showed varying levels of genetic polymorphism and demographic expansion, hinting at population growth and range expansion²⁸. These findings underscore the complex interplay between vector genetics and malaria transmission, emphasizing the need for comprehensive entomological studies to inform targeted interventions and surveillance strategies in the context of *Plasmodium vivax* malaria in Indonesia and Malaysia.

Vector Biology and Behaviour

Mosquito biting behaviour (bionomics), bite rate, and reproduction are influenced by temperature changes. Higher temperatures accelerate mosquito breeding cycles and shorten the maturation period of the parasite within the vector, leading to increased malaria transmission risks.²⁹ Understanding vector biology and behavior is essential in studying *Plasmodium vivax* malaria. *Anopheles* mosquitoes, as primary vectors, influence transmission through their breeding habits, host-seeking patterns, and biting behavior. Factors such as vector density, insecticide resistance, and environmental conditions further affect disease spread. Additionally, human activities and interactions with vectors add complexity to malaria transmission, highlighting the need for targeted research and control strategies.⁵ Studying the biology and behavior of *Anopheles* mosquitoes allows researchers to develop more effective vector control and malaria prevention strategies. This is particularly crucial in Indonesia and Malaysia, where malaria remains a major public health challenge. Understanding mosquito habits can help tailor interventions to reduce transmission and improve disease management in endemic regions.

Vector Control Measures

Effective vector control is crucial in combating malaria, using strategies like insecticide-treated bed nets, indoor spraying, larval control, and environmental management. In Indonesia and Malaysia, these efforts have significantly reduced *Plasmodium vivax* transmission by targeting *Anopheles* mosquitoes. Continuous research and monitoring are essential to adapt strategies and integrate new technologies, making malaria elimination more achievable.²⁵

The Purworejo Regency Health Office and Indonesia's

Ministry of Health control malaria through migration surveillance, patient treatment, insecticide-treated nets, and indoor residual spraying (IRS). Effective control requires detailed knowledge of methods, timing, targets, locations, and insecticide concentrations. Understanding vector species, behavior, and bionomics is also a priority to enhance efficiency.³⁰

Insecticide Resistance in *Anopheles* Mosquitoes

The WHO in 2016 stated that *Anopheles* mosquitoes have resistance to exposure to standard doses of insecticides. Some types of insecticides used in households are Baygon®, Abate Powder, Hit®, Force magic®, and Vape.³¹ Resistance of *Plasmodium falciparum* and *Plasmodium vivax* to antimalarial drugs has been observed in Southeast Asia, with imported malaria contributing to the risk in Malaysia. Monitoring and preventing the spread of drug-resistant strains are crucial for achieving malaria elimination.³²

Insecticide resistance in *Anopheles* mosquitoes challenges malaria control in Indonesia and Malaysia. Resistance mechanisms, including target-site mutations and metabolic detoxification, reduce the effectiveness of indoor residual spraying and insecticide-treated nets, sustaining malaria transmission. Future efforts should focus on developing new insecticides, rotating different insecticide classes, and implementing integrated vector management to combat resistance.⁵ By addressing insecticide resistance comprehensively, sustainable progress can be made in reducing the burden of *Plasmodium vivax* malaria in Indonesia and Malaysia.

Research Advances in Vector Control

Recent research studies in East Kutai Regency, Indonesia¹⁰ and South Kalimantan³³ have shed light on the critical factors influencing malaria prevalence, particularly in forest areas. Understanding malaria epidemiology, including age, sex, and occupation patterns, is key to effective vector control. High-risk groups, such as males aged 25–34 with forest exposure, require targeted interventions. Identifying *Plasmodium* species, like *P. vivax*, helps tailor control strategies. Ongoing research in vector control remains crucial for combating malaria in endemic regions.

Africa has made significant advances in vector control through rapid diagnosis, mosquito net promotion, insecticides, intermittent preventive therapy (IPT), chemo-prevention for high-risk groups, and artemisinin-based combination therapy (ACT) for malaria treatment. These efforts, supported by ongoing drug research and WHO assistance, have proven effective in reducing malaria cases.³¹ Similar efforts can be adopted by Indonesia and Malaysia against the

Anopheles mosquito vector from the *Plasmodium vivax* population.

Comparative Analysis Across Time

A comparative analysis of *Plasmodium vivax* malaria in Indonesia and Malaysia highlights the evolving epidemiology and entomology of the disease. Technological advancements and research have enhanced understanding of transmission patterns and parasite diversity. By examining historical data alongside current trends, researchers can identify key factors shaping malaria dynamics, including climate change, urbanization, and human mobility.⁵ This comparative approach allows for a more holistic view of the disease burden in both countries, paving the way for targeted interventions and control strategies to effectively combat the spread of malaria.

A 2002 study found that despite a decline in the annual parasite incidence (API) in Central Java, some areas remain difficult to control, including the Menoreh Mountains. Spanning Purworejo and Magelang Regencies (Central Java) and Kulon Progo Regency (DI Yogyakarta), this cross-border region remains a persistent malaria hotspot. It was the first area in Java to be designated as a major endemic zone. A 2019 spatial analysis revealed that the primary malaria cluster was in Purworejo Regency, with secondary clusters in neighboring districts.³⁴

Changes in Malaria Incidence Rates Over Time

In examining the dynamics of *Plasmodium vivax* malaria incidence rates over time in Indonesia and Malaysia, it is crucial to consider the diverse factors influencing malaria transmission and control strategies. According to³⁵ the persistence of submicroscopic infections and hypnozoite reservoirs poses challenges to standard control measures, leading to the ongoing transmission of *P. vivax* in complex local settings. Furthermore,³⁶ highlights the global efforts towards malaria elimination and eradication, emphasizing the importance of strong surveillance systems, entomological interventions, and evidence-based strategies in achieving successful outcomes. Examining national malaria programs in countries like Sri Lanka and Bhutan provides valuable insights into malaria elimination efforts. Their successes and challenges offer a framework for analyzing changes in malaria incidence over time, considering evolving prevention and control strategies.

Evolution of Malaria Parasites and Vectors

Malaria parasites, such as *Plasmodium vivax*, have adapted to survive and multiply within their human hosts, while also evolving mechanisms to evade the immune system and develop resistance to antimalarial drugs. Concurrently, mosquito vectors like *Anopheles*

species have co-evolved with the parasites, developing strategies to efficiently transmit the pathogens from one host to another. The genetic diversity of malaria parasites and vectors influences their ability to adapt to changing environmental conditions, host immune responses, and vector control measures. By elucidating the evolutionary history of malaria parasites and vectors, we can gain insights into the complex interactions driving the spread of the disease and inform public health interventions to mitigate its impact on vulnerable populations.³ The incubation period of the malaria parasite *Plasmodium vivax* intrinsically begins from the entry of the parasite into the human body until clinical symptoms appear, which is for 12-17 days.³⁷

Public Health Interventions and Their Effectiveness

These public health interventions play a crucial role in controlling the spread of the disease and reducing its impact on the local populations. Various strategies such as vector control, early diagnosis, and treatment, as well as community engagement and education have been utilized to combat the transmission of the malaria parasite. Studies have shown that targeted interventions can significantly reduce the burden of *P. vivax* malaria in endemic regions, leading to a decline in morbidity and mortality rates. However, the effectiveness of these interventions can vary depending on the local context, environmental factors, and socioeconomic conditions. Therefore, continuous evaluation and adaptation of public health strategies are essential to ensure their success in fighting against malaria.²⁵

Each intervention has its own advantages in malaria control. A multisectoral and multidimensional approach using 4 interventions, namely insecticide treated nets (ITN), long-lasting insecticidal nets (LLINs), and indoor residual spraying (IRS), and mass media campaigns are urgently needed. In many countries, the use of multiple malaria interventions simultaneously is more effective at preventing mosquito bites and controlling vector populations than using a single intervention alone.³⁸

Lessons Learned for Future Malaria Control Programs

Firstly, the importance of community engagement cannot be overstated. Active involvement and empowerment of local communities are crucial for the success of control efforts. Secondly, integrating multiple control measures, such as vector control, early diagnosis, and prompt treatment, is essential for effectively reducing malaria transmission. Lastly, continuous monitoring and evaluation of control programs are necessary to assess their impact and make

necessary adjustments. By incorporating these lessons into future malaria control programs, we can strive towards sustainable and long-term success in reducing the burden of *Plasmodium vivax* malaria in Indonesia and Malaysia.

Discussion

Plasmodium vivax is a malaria parasite whose populations are found in Latin America, Asia, and the South Pacific. The formation of diversity and population structure across continents is influenced by multiple factors. For example, the intensity of transmission is lower in South America than in Southeast Asia and the South Pacific, or the geographical isolation of this parasite in the South Pacific. A study of 841 isolates from South and Central America, Africa, Central Asia, Southeast Asia, and the South Pacific revealed that the highest diversity occurred in Southeast Asia. Places of transmission include the medium-high category and the migration of humans (hosts) to be highly infected. Meanwhile, South America and Central Asia are the lowest and localized places of malaria spread.³⁹

Blood stage infections due to *Plasmodium vivax* tend to have a lower density than *P. falciparum* so the diagnosis is often missed. Hypnozoites carried by *Plasmodium Vivax* can cause recurrence after some time of exposure to primary infections and increase the potential for new transmission. Unfortunately, the diagnostic tools currently available are not capable of detecting when an inactive liver wakes up and triggers some episodes of malaria vivax. This causes some infected people to be unaware of it. Such conditions are a challenge for malaria control and elimination programs because of the difficulty of detecting malariometric surveys or active case detection campaigns.⁴⁰

Since 1998, malaria has become one of the list of diseases that must be reported in Malaysia. All laboratory-confirmed cases must be reported to the nearest district health office within 7 days of being confirmed before sending data to the central level¹³ The epidemiological studies conducted in Malaysia and French Guiana have yielded significant insights into the transmission and clinical manifestations of *Plasmodium vivax* malaria. In Malaysia, the emergence of *P. knowlesi* as a common cause of malaria, particularly among farmers and individuals engaged in specific activities at the forest edge, highlights the complex interplay between human behavior and environmental factors in disease transmission.⁴¹ Additionally, the successful treatment outcomes of artesunate-mefloquine compared to chloroquine underscore the importance of effective antimalarial regimens in managing *P. knowlesi* infections. In French Guiana, the outbreaks among military personnel engaged in illegal gold mining activities in remote

rainforest areas demonstrate the challenges of controlling malaria in hyperendemic settings where competent vectors like *Anopheles* mosquitoes play a pivotal role in disease transmission.⁴² These findings emphasize the need for tailored strategies to address malaria transmission in diverse ecological and human contexts, guiding future efforts towards comprehensive malaria control and elimination programs in these regions.

The evolving genetic diversity and transmission patterns of *Plasmodium falciparum* and *P. vivax* populations in co-endemic regions present crucial considerations for public health policies¹¹ The observed decrease in polyclonal infections over time, particularly in *P. falciparum*, signifies a potential reduction in transmission rates following the implementation of Artemisinin-based Combination Therapy (ACT). Furthermore, the distinct genetic structures of the two species, with *P. vivax* displaying higher rates of outbreeding, emphasize the need for tailored interventions to disrupt transmission effectively. Additionally, the recognition of *P. vivax* as a significant threat associated with severe illness and potential mortality reinforces the urgency for comprehensive public health strategies that address the unique challenges posed by this parasite, including the management of hypnozoites and safe administration of primaquine therapy.⁴³ Public health policies must therefore account for the complex epidemiological dynamics and clinical implications highlighted by these findings to mitigate the burden of vivax malaria effectively.

In 2022, WHO stipulates certification as an elimination requirement. of zoonotic malaria parasite species (e.g. macaque long-tailed monkey). This makes Malaysia the first country not to receive malaria elimination certification because malaria species other than *P. Vivax* were found to be zoonotic. This condition raises big questions regarding the feasibility of malaria elimination in other countries that have also reported zoonotic malaria cases.⁴⁴ A study revealed that the success of malaria prevention and control programs can be seen from the behaviour of the community itself. Maintaining environmental cleanliness, using insecticide mosquito nets, mosquito repellents, and installing wire mesh at home⁴⁵

Moving forward, there are several key areas that warrant further investigation in order to enhance our understanding of *Plasmodium vivax* malaria in Indonesia and Malaysia. Firstly, future research should focus on exploring the potential impact of climate change on the distribution and transmission of this disease, as changes in temperature and precipitation patterns may influence the prevalence of the *Anopheles* mosquitoes responsible for transmitting the parasite.

Additionally, more studies are needed to examine the effectiveness of current control measures, such as vector control strategies and antimalarial drug regimens, in the context of evolving drug resistance and insecticide resistance. Furthermore, investigations into the genetic diversity of *Plasmodium vivax* strains circulating in these regions could provide valuable insights into the parasites transmission dynamics and inform the development of more targeted interventions. Collaboration between researchers, public health officials, and local communities will be essential to address these research gaps and ultimately work towards the goal of malaria elimination in Indonesia and Malaysia.⁴⁶

The high number of vivax malaria cases found in several studies is a code for the government to prioritize these cases. Malaria prevention and control campaigns can be carried out to increase public awareness so that its prevalence decreases and is eliminated gradually.⁴⁷ Since 2018, the national malaria elimination and eradication program has been effective enough to produce zero cases in the indigenous population. However, the high level of malaria exposure and its transmission pattern in the population needs to be a concern. The epidemiological transition of malaria cases from moderate to low is a challenge for each country to assess the performance of intervention programs and the impact of their evaluation. In order to consolidate profits and advance malaria elimination, better tools are needed to monitor infections on a regular basis.⁴⁸ Until now, efforts to control *P. Vivax* maraia are still limited by the lack of effective radical cure options.⁴⁹

One major limitation of this study is the reliance on secondary data sources, which may introduce potential biases or inaccuracies in the findings. While efforts were made to validate the data through cross-referencing and verification, there is still a possibility of errors in the original data collection processes. Another limitation is the scope of the study, which focused primarily on *Plasmodium vivax* malaria in Indonesia and Malaysia. This narrow geographic focus may limit the generalizability of the findings to other regions or countries facing similar challenges. Additionally, the study predominantly examined epidemiological and entomological aspects, leaving out other potential factors that could influence the transmission and control of malaria. Future research could benefit from expanding the scope to include a more comprehensive analysis of social, economic, and environmental factors that contribute to the prevalence of *Plasmodium vivax* malaria in Southeast Asia⁵⁰

Conclusion

The epidemiology and entomology of *Plasmodium vivax* Malaria in Indonesia and Malaysia have shown a

complex interplay of factors contributing to the transmission and prevalence of this disease over time. The historical data analyzed in this study reveal patterns of outbreaks and declines, influenced by various environmental, social, and biological factors. Despite efforts to control malaria through vector control programs and healthcare interventions, the persistence of *P. vivax* in these regions remains a significant challenge. Moving forward, continued research and surveillance are essential to understand the changing dynamics of malaria transmission and to develop effective strategies for prevention and control. By addressing the gaps in current knowledge and incorporating a multidisciplinary approach, we can work towards reducing the burden of *P. vivax* Malaria in Indonesia and Malaysia. In conclusion, the study of *Plasmodium vivax* Malaria in Indonesia and Malaysia has shed light on the complex interplay between epidemiology and entomology over time. Understanding the transmission dynamics and prevalence of this parasite is crucial for effective control and preventative measures. By examining historical trends and current data, researchers can better pinpoint areas of high risk and tailor interventions to combat the spread of the disease. While progress has been made in terms of treatment and prevention, challenges still remain, particularly in regions with limited resources and access to healthcare. Continued research and collaboration between scientists, governments, and local communities will be essential in the ongoing battle against *P. vivax* Malaria. It is imperative that efforts to control this disease are sustained and adapted to the changing landscape of malaria transmission in these regions. In Indonesia and Malaysia, *Plasmodium vivax* malaria is a significant issue, requiring further research to understand its distribution, resistance, and genetic diversity, as well as the effectiveness of current control measures.

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